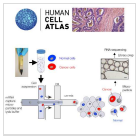

Apr 16, 2021

Single-nuclei suspensions from primary human esophagus tissue

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1 *Works for me* dx.doi.org/10.17504/protocols.io.t9wer7e

CZI START Project

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ABSTRACT

A protocol to dissociate frozen esophagus tissue specimens for single-nuclei transcriptomics.

EXTERNAL LINK

https://www.southampton.ac.uk/medicine/about/staff/tju.page

DOI

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KEYWORDS

esophagus, esophageal, oesophageal, cancer, tissue, dissociation, single-nuclei, single-nucleus, RNA, sequencing

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MATERIALS TEXT

MATERIALS

BSA-Molecular Biology Grade - 12 mg **New England Biolabs Catalog #B9000S**

tweezers **Contributed by users**

Proteases inhibitor Complete **Roche Catalog #11 697 498**

4,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) **Thermo Fisher Scientific Catalog #D1306**

Nuclei EZ lysis buffer **Sigma Catalog #EZ PREP NUC-101**

Dounce homogenizers **Sigma Catalog #D8938-1SET**

Falcon 15 mL Polystyrene Conical Tube **Fisher Scientific Catalog #352095**

NxGen RNAse Inhibitor **Lucigen Catalog #30281-2**

Corning® 40µm Cell Strainer **Corning Catalog #431750**

DNA LoBind Tube 1.5ml **Eppendorf Catalog #022431021**

DTT **Sigma Aldrich Catalog #43816-10ML**

Trypan Blue Solution 0.4% Sterile-filtered **Sigma Aldrich Catalog #T8154**

Aluminum Foil **Contributed by users**

Surgical scissors or scalpel **Contributed by users**

RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #AM9780**

Dulbecco's Phosphate Buffered Saline (PBS) **Contributed by users Catalog #D8537**

Polypropylene Centrifuge Tubes, 50mL Conical bottom tube; Max RCF: 15500xG; High-density polyethylene cap; No rack **Thermo Fisher Catalog #0553868**

Sterilin™ 30 to 140mm Petri Dishes, 60mm **Thermo Fisher Catalog #123-17**

Benchtop Dewar Flasks, 1L **Thermo Fisher Catalog #4150-1000PK**

RNAlater™-ICE Frozen Tissue Transition Solution **Thermo Fisher Catalog #4427575**

Ambion™ RNase Inhibitor, cloned, 40 U/µL **Thermo Fisher Catalog #AM2684**

30 uM Cell Strainer **Miltenyi Biotec Catalog #130-098-458**

BEFORE STARTING

RNALater-ICE pre-treatment of frozen tissue (**Important**: perform on at least the day before nuclei isolation):

1. Pre-chill a minimum of 10 volumes (compared to the sample mass/ volume) of RNAlater-ICE at 70 or ¯80°C in a polypropylene tube.
2. Place frozen tissue (from LN2 or ¯80°C), no larger than 0.5 cm in the smallest dimension, into the RNAlater-ICE. It is important that the RNAlater-ICE stays cold and the tissue remains frozen, so work quickly. Set up the tubes

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of RNAlater-ICE and tissue samples in a container of dry ice. For the frozen tissue, simply pipet at least 10 volumes of pre-chilled RNAlater-ICE into the tube. Tightly cap the tube and invert several times to mix.

3. Soak the tissue in RNAlater-ICE at ¯20°C for at least 16 hours. Samples transitioned to ¯20°C in RNAlater-ICE should be left in the RNAlater-ICE and maintain at ¯20°C or colder.

Reagents to prepare immediately before use:

- Prepare 6 mL of Nuclei Suspension Buffer (NSB; consisting of 1x PBS, 0.01% BSA and 0.2% v/v RNAse inhibitor, 1 mM DTT). Keep cold on ice.
- Prepare 7 mL of Nuclei EZ lysis buffer (0.2% v/v RNAse inhibitor, 0.2% v/v SUPERasin, 1x Protease inhibitor and 4 mM DTT). Keep cold on ice.
- Preparing the 300 nM DAPI solution:
  i) Add 2 mL of ddH20 to the DAPI vial to make a 14.3 mM (5 mg/mL) stock solution. Store at -20C.
  ii) Add 2.1 uL of the 14.3 mM DAPI stock solution to 100 uL PBS to make a 300 µM DAPI intermediate dilution.
  iii) Dilute the 300 µM DAPI intermediate dilution 1:1000 in PBS as needed to make a 300 nM DAPI solution.

NB: Clean benchtop area with RNA-Zap and wipe down pipettes. Keep all reagents on ice at all times.

1 Pre-chill Dounce homogenizer with pestle B, containing 2 ml of ice-cold Nuclei EZ lysis buffer. 10m

2 Remove tissue from storage (-20°C in RNALater-ICE) and hold in a petri dish on wet ice. If not utilizing all of the frozen tissue, sever off the amount of tissue required (using a cold scalpel) and return the remainder to original storage site. 1m

3 Using a cold scalpel cut tissue into very small pieces and transfer to Dounce homogeniser. Do not overload homogeniser with tissue (~30 mg per Dounce at one time). 5m

4 Homogenize for 30 strokes with pestle B (pre-chilled in ice-cold in Nuclei EZ lysis buffer. Tissue should be reduced to ~90% homogenous small particles. [Perform the homogenization on ice, occasionally removing from the ice to check the homogenate]. 5m

5 With a 1 mL pipet, transfer homogenate to 15 mL conical centrifuge tube (on ice) and rinse the Dounce homogenizer out with a further 1 mL of ice-cold Nuclei EZ lysis buffer. Take care not to transfer any large particulates of unhomogenized tissue. This is a potential source of activated RNAses that have undergone renaturation. Incubate on ice for 5 minutes. 6m

6 Filter suspension through a 40 µM cell strainer in to a 50 ml conical centrifuge tube. 1m

7 Centrifuge nuclei/tissue suspension at 700 x g at 4°C for 5 minutes. 5m

8 Re-suspend isolated nuclei in 4 ml of ice-cold Nuclei EZ lysis buffer and incubate on ice for a further 5 minutes. 5m

9 Centrifuge nuclei suspension at 500 x g at 4°C for 5 minutes. 5m

10 Re-suspend nuclei in 3 mL NSB and filter through a 30 µM strainer in to a 50 mL conical centrifuge tube. 1m

11 Centrifuge nuclei suspension at 500 x g at 4°C for 5 minutes. 5m

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12 Re-suspend nuclei in 1-2 mL NSB and hold on wet ice (4°C). 1m

13 Dilute 10 µL of nuclei suspension 1 in 10 with NSB in to a micro-centrifuge tube and count nuclei using 1:2 dilution with dye (either 10 µl Trypan blue or 10 µl DAPI stain (300 nM in PBS; incubate for 2 minutes with nuclei before counting) added to 10 µl of nuclei suspension) and a haemocytometer (Improved Neubauer). 5m

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