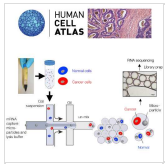

Mar 16, 2021

Single-cell suspensions from primary human esophagus tissue

Annette Hayden[1], Rob Walker[1], Jack Harrington[1], Lucy Kimbley[1], Sara Waise[1], Chris Hanley[1], Gareth Thomas[1], Peter Johnson[1], Jonathan West[1], Matthew JJ Rose Zerilli[2], TIm Underwood[1]

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1 *Works for me* dx.doi.org/10.17504/protocols.io.t9ver66

CZI START Project

Matthew Rose Zerilli
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SUBMIT TO PLOS ONE

ABSTRACT

A protocol to dissociate fresh esophagus tissue specimens for single-cell transcriptomics.

EXTERNAL LINK

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Based on protocols described in: Waise S, Parker R, Rose-Zerilli MJJ, et al. An optimised tissue disaggregation and data processing pipeline for characterising fibroblast phenotypes using single-cell RNA sequencing. Scientific Reports. 2019 Jul;9(1):9580. DOI: 10.1038/s41598-019-45842-4.

DOI

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PROTOCOL CITATION

Annette Hayden, Rob Walker, Jack Harrington, Lucy Kimbley, Sara Waise, Chris Hanley, Gareth Thomas, Peter Johnson, Jonathan West, Matthew JJ Rose Zerilli, TIm Underwood 2021. Single-cell suspensions from primary human esophagus tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.t9ver66

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

esophagus, esophageal, oesophageal, cancer, tissue, dissociation, single-cell, RNA, sequencing

Citation: Annette Hayden, Rob Walker, Jack Harrington, Lucy Kimbley, Sara Waise, Chris Hanley, Gareth Thomas, Peter Johnson, Jonathan West, Matthew JJ Rose Zerilli, TIm Underwood (03/16/2021). Single-cell suspensions from primary human esophagus tissue. https://dx.doi.org/10.17504/protocols.io.t9ver66

CREATED

Oct 03, 2018

LAST MODIFIED

Mar 16, 2021

PROTOCOL INTEGER ID

16405

GUIDELINES

Ensure all instruments needed are available and to hand before you start

MATERIALS TEXT

Equipment / reagents:

- Tubes - 50ml, 1.5ml (not non-stick), 0.5ml
- 40um strainer: ThermoFisher (10737821)
- 70um strainer: EASYstrainer (542070)
- Scalpel: Swann Morton No. 21 Sterile Disposable Scalpel (0507)
- Syringe plungers: Sigma (Z248010-1PAK)
- Centrifuge
- Incubator with shaking
- PBSA: Amphotericin B (ThermoFisher: 15290026) at a working concentration of 2.5 ug/ml in PBS
- 5ml DMEM complete:
  1 aliquot of Collagenase P (100ul at 150U/ml. Sigma: 112138857001) working concentraion 3U/ml
  1 aliquot of DNAse (100ul at 2000U/ml. Sigma: 11284932001) working concentration 40U/ml
  DMEM (Sigma: D5671-500ml)
  10% FBS (Pan Biotech: F40-37500)
  1% LGlut (ThermoFisher: 25030081)
  1% Penstrep (Sigma: P4333-100ml)
- DMEM empty
- RBC lysis solution:
  1ml RBC lysis reagent: ThermoFisher (00-4300-54)
  9ml H_2O
- Trypsin-EDTA solution: Sigma (T3924-100ml)
- Trypan blue: Sigma (T8154-20ml)
- CChip: Labtech (DHC-F01)

SAFETY WARNINGS

Biological Hazard -
All biological samples should be treated as a possible cause of infection. Your departmental guidelines must be adhered to when handling human material. Working with primary cells may put the user at risk of exposure to blood-borne pathogens.

- Physical Hazard -
Scalpes are sharp. Care must be taken when using scalpels.

Always wear a lab coat and gloves

BEFORE STARTING

Work in a containment level 2 facility and safety cabinet

1 Wash tissue in PBSA.

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2 Mince tissue with scalpel.

3 Transfer the minced tissue to the Collagenase/DNAse solution in a 50ml Falcon.

4 Shake the suspension at 110-150 rpm for 60 mins at 37°C (with the tube on its side to increase agitation of the tissue).

5 After 15, 30, and 60 mins – pipette up and down with descending sized pipettes (25, 10 and 5 ml).

6 Pipette thoroughly.

7 Strain with a 70 um cell strainer.

8 Add 10 ml DMEM (empty) to sieve and push through with syringe plunger.

9 Keep on ice from this point.

10 Spin 1500 rpm for 5mins and remove media.

11 Resuspend pellet in RBC lysis buffer and incubate @ 4°C for 10mins.

12 Add 10 ml DMEM (empty) and pipette up and down thoroughly.

13 Pass through 40 um cell strainer – pushing through with plunger.

14 Spin at 1500 rpm for 5 mins and remove media.

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15 Resuspend pellet in 1 ml of cell suspension buffer.

16 In a 0.5ml tube, add 10 ul cell solution with 10 ul trypan blue.

17 Add resultant 20 ul mix onto disposable haemocytometer (C-Chip, FR type).

18 Count the cells and record cell viability

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