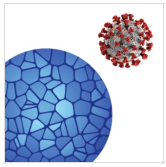

Oct 31, 2020

Human Nasopharyngeal Swab Processing for Viable Single-Cell Suspension

Ying Tang[1], Carly G.K. Ziegler[2], Vincent N. Miao[2], Andrew W. Navia[2], Joshua D. Bromley[2], Kenneth J. Wilson[3], Yilianys Pride[3], Mohammad Hasan[3], Taylor Christian[3], Hannah Laird[3], Anna Owings[3], Meredith Sloan[3], Haley B. Williams[3], Tanya O. Robinson[3], George E. Abraham III[3], Michal Senitko[3], Sarah C. Glover[3], Bruce Horwitz[1], Alex K. Shalek[2], Jose Ordovas-Montanes[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.bjhkkj4w

Human Cell Atlas Method Development Community | Coronavirus Method Development Community

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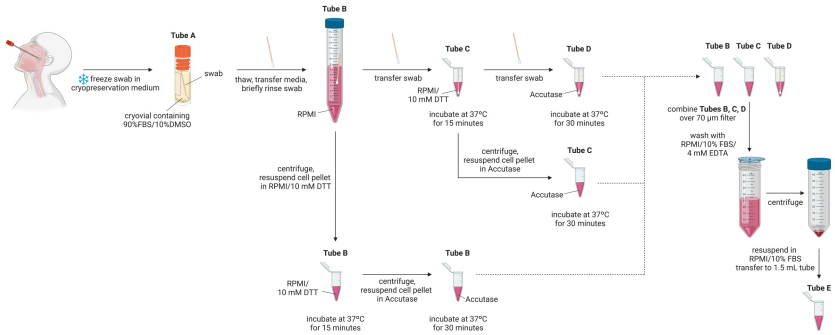
ABSTRACT

A protocol for recovering viable single cell suspensions from cryopreserved human nasopharyngeal swabs for downstream applications, such as single-cell RNA-seq. The illustrated schematic below details the process.



ATTACHMENTS

Human_Nasal_Swab_Dissocation_Ziegler_Tang.docx

DOI

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PROTOCOL CITATION

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KEYWORDS

Nasal Swab, COVID-19, Nasopharyngeal Swab, single-cell RNA-seq, nasal epithelia, nasopharynx, sars-cov-2

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GUIDELINES

Samples should be collected by a trained medical professional using a nasal swab (FLOQSwabs, Copan flocked swabs) in accordance with the manufacturer's instructions. Briefly, the process was performed as follows. First, the patient's head was tilted back slightly, and the swab was inserted along the nasal septum, above the floor of the nasal passage to the nasopharynx until slight resistance was felt. The swab was then left in place for several seconds to absorb secretions and slowly removed while rotating. The swab was placed in a 1.7 mL cryovial containing 90% fetal bovine serum (FBS)/10% dimethyl sulfoxide (DMSO) and frozen using a slow-cooling device (Thermo Scientific Mr. Frosty Freezing Container) at -80 °C, and stored in liquid nitrogen.

MATERIALS TEXT

For processing of 1 cryopreserved nasopharyngeal swab:

- 2.5 mL of RPMI/10 mM Dithiothreitol (DTT) (made fresh)
- 3.5 mL of Accutase
- 6 mL RPMI
- 8 mL quenching media containing RPMI/10% fetal bovine serum (FBS)/4 mM EDTA
- 2 mL RPMI/10% FBS
- 15 mL conical labeled **Tube B** containing 5 mL RPMI
- 1.5 mL tube labeled **Tube B**, empty
- 1.5 mL tube labeled **Tube C**, with 1 mL RPMI/10 mM DTT
- 1.5 mL tube labeled **Tube D**, with 1 mL Accutase
- 50 mL conical
- 70 µm cell strainer that fits 50 mL conical
- 1.5 mL tube labeled **Tube E**
- Forceps and scissors
- Thermomixer set to 37°C, agitating at 300 rpm
- 10 µL trypan blue
- NI hemocytometer
- 96 well plate for cell counting
- RLT buffer (Qiagen)/1% 2-mercaptoethanol (BME)
- Cryovials or snap-top ependorf tubes for population lysates

SAFETY WARNINGS

For hazard information and safety warnings regarding nasopharyngeal swabs or any listed materials, please refer

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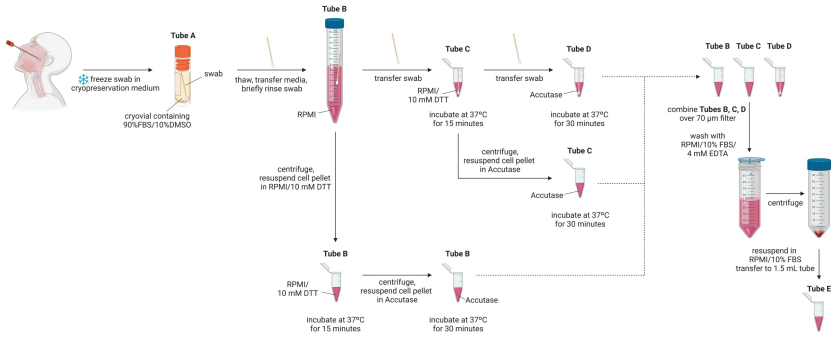
to the SDS (Safety Data Sheet).

For samples obtained from individuals diagnosed with, or at any risk of, an infection (e.g. SARS-CoV-2), additional precautions should be taken in accordance with your institute's regulations on biosafety. These include elimination of aerosol generating steps where possible (e.g., no vacuum aspiration), all steps prior to cell lysis should be carried out in a biosafety cabinet, including thermomixing and centrifugation where possible. When necessary, samples should only be removed from the biosafety cabinet in decontaminated and sealed secondary containment. Personal protective equipment including a gown, two pairs of non-sterile gloves, a protective surgical or N95 mask, and a face shield should be worn during sample processing.

DISCLAIMER:

ABSTRACT

A protocol for recovering viable single cell suspensions from cryopreserved human nasopharyngeal swabs for downstream applications, such as single-cell RNA-seq. The illustrated schematic below details the process.



Before You Start

1 Prepare and label a 15 mL conical as **Tube B** with **5 mL RPMI** .

2 Prepare and label a 1.5 mL tube also as **Tube B**, leave this one empty.

3 Prepare and label a 1.5 mL tube as **Tube C** with **1 mL RPMI/10 mM DTT** .

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4 Prepare and label a 1.5 mL tube as **Tube D** with **1 mL Accutase** .

Tube A

5 Rapidly thaw cryovial (**Tube A**) in hands or thermal block set to **37 °C** .

> Carefully perform in accordance with your institute's safety guidelines. If handling potentially infectious material, inspect for cracks or leaks during warming

6 Remove swab from **Tube A** using clean forceps, trim swab handle using scissors if necessary.

7 Place swab in **Tube B** (15 mL conical), dip briefly to rinse swab.

8 Move swab from **Tube B** (15 mL conical) to **Tube C**. Proceed directly to step 23 for **Tube C**.

9 

Transfer liquid in **Tube A** to **Tube B** (15 mL conical).

10 

Using **1 mL RPMI** from **Tube B** (15 mL conical), wash **Tube A**.

10.1 Collect washing from **Tube A** in **Tube B** (15 mL conical).

11 Discard **Tube A**.

Tube B

12 

Centrifuge **Tube B** (15 mL): **400 x g, 4°C, 00:05:00** .

13 Remove supernatant with serological pipette.

Citation: Ying Tang, Carly G.K. Ziegler, Vincent N. Miao, Andrew W. Navia, Joshua D. Bromley, Kenneth J. Wilson, Yilianys Pride, Mohammad Hasan, Taylor Christian, Hannah Laird, Anna Owings, Meredith Sloan, Haley B. Williams, Tanya O. Robinson, George E. Abraham III, Michal Senitko, Sarah C. Glover, Bruce Horwitz, Alex K. Shalek, Jose Ordovas-Montanes (10/31/2020). Human Nasopharyngeal Swab Processing for Viable Single-Cell Suspension. https://dx.doi.org/10.17504/protocols.io.bjhkkj4w

14 Resuspend pellet in **1 mL RPMI/10mM DTT** .

15 

Transfer suspended cells from **Tube B** (15 mL) to **Tube B** (1.5 mL). Discard empty 15 mL conical.

16 Place **Tube B** (1.5 mL) on thermomixer (37ºC, 300 rpm).

17 

Incubate for **00:15:00** .

18 

Centrifuge **Tube B** (1.5 mL): **400 x g, 4°C, 00:05:00** .

19 

Remove supernatant with P1000 pipette.

20 Resuspend pellet in **1 mL Accutase** .

21 Place **Tube B** (1.5 mL) on thermomixer (37ºC, 300 rpm).

22 

Incubate for **00:30:00** .

Tube C

23 Place **Tube C** on thermomixer (37ºC, 300 rpm).

24 

Incubate for **00:15:00** .

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25 Place swab in **Tube D**. Proceed directly to step 31 for **Tube D**.

26 

Centrifuge remaining liquid at **400 x g, 4°C, 00:05:00** .

27 

Remove supernatant with P1000 pipette.

28 Resuspend pellet in **1 mL Accutase** .

29 Place **Tube C** on thermomixer (37ºC, 300 rpm).

30 

Incubate for **00:30:00** .

Tube D

31 Place **Tube D** on thermomixer (37ºC, 300 rpm).

32 

Incubate for **00:30:00** .

After **Tube B**, **C**, and **D**'s 30 minute Incubations

33 After **Tube B**, **Tube C**, and **Tube D** have each finished their 30 minute incubations:

In practice, we wait until all tubes have finished their 30 minute incubation in Accutase to synchronize. We leave tubes on incubation for a maximum 50 minutes.

34 Place 70 µm cell strainer in a 50 mL conical.

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35 Wet cell strainer with **3 mL quenching buffer (RPMI/10% FBS/4 mM EDTA)** .

36 

Pipette contents of **Tube B**, **Tube C**, and **Tube D** onto cell strainer.

Do not discard tubes.

37 

Use **1 mL quenching buffer** to wash each **Tube B**, **Tube C**, and **Tube D**.

37.1 Manually agitate the swab in **Tube D** in the quenching buffer to ensure full rinse.

38 

Add quenching buffer from Tube washes to cell strainer. Discard **Tubes B**, **C**, and **D**.

39 

Wash cell strainer with additional **2 mL quenching buffer** .

40 Discard cell strainer, cap 50 mL conical.

41 

Centrifuge 50 mL conical **400 x g, 4°C, 00:10:00** .

42 Remove supernatant with serological pipette.

43 

Resuspend cell pellet in residual volume (often ~500 µL).

Transfer resuspended cells from 50 mL conical to **Tube E** (1.5 mL tube).

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44

45

Wash 50 mL conical with **500 µl RPMI/10% FBS** .

45.1

Transfer washing from 50 mL conical to **Tube E**.

46

Centrifuge **Tube E** **400 x g, 4°C, 00:05:00** .

47

Remove supernatant with P1000 pipette.

48 Resuspend pellet in **200 µl RPMI/10% FBS** .

Count cells from **Tube E**

49

In 96 well plate, add **10 µl trypan blue** .

50

Add **10 µl cells from Tube E** to well containing trypan blue.

51

Pipette to mix cells in trypan blue, transfer **10 µl** to hemocytometer port.

52

Count viable cells across 4 quadrants.

53

Record total cell number and calculate cell concentration.

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54 

Take photo of cells at 20x.

55 Load 20,000 viable cells on Seq-Well array.

Or load total volume of **Tube E** if total cell count is lower than 20,000.

Population Lysates

56 Label cryovials and add **100 µl RLT/1% BME** to each tube.

57 Add 20,000 viable cells to each tube. Tap or pipette to mix cell suspension with RLT/1% BME

58 Snap freeze on dry ice for at least **00:10:00** .

59 Transfer to **-80 °C** .

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