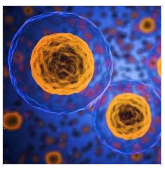

Feb 05, 2021

10X-CITEseq protocol (COVID-19 patient samples +/- tetramer stain)

Forked from 10X-CITEseq protocol

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

Alyssa Ward

ABSTRACT

Purpose

To detail the workflow for scCITE-Seq of 24 PBMC samples with 2 technicians. This is based on internal 10X CITEseq protocol v9.

ATTACHMENTS

10X-CITEseq protocol v9.docx

DOI

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PROTOCOL CITATION

Yang Sun, David Lee, George Hartoularos, Jimmie Ye 2021. 10X-CITEseq protocol (COVID-19 patient samples +/- tetramer stain). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqnqmvdw

FORK NOTE

FORK FROM

Forked from 10X-CITEseq protocol, Alyssa Ward

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LAST MODIFIED

Feb 05, 2021

PROTOCOL INTEGER ID

45488

MATERIALS TEXT

MATERIALS

BSA (DNAse, RNAse and protease free)

Vwr Catalog #0332-25G

RPMI-1640 medium supplemented with 10% FBS **Contributed by users**

Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #422301, 422302**

TotalSeq-C Human Panel 1 Test qty 3 **BioLegend Catalog #99088**

DPBS **Invitrogen Catalog #14190-144**

Penicillin/Streptomycin **Invitrogen**

Glutamax **Invitrogen**

DNAse I (10000U/mL) optional **Contributed by users**

Flowmi strainer

Sigma Catalog #BAH136800040

Trypan blue **Invitrogen**

5 mL Polystyrene tubes **Contributed by users**

RNeasy Plus Mini Kit **Qiagen**

Antibody reconstitution materials:

-TotalSeq-C Human Panel 1 Test (BioLegend, part# 99088), qty 3
-Cell Staining Buffer- 2% BSA in PBS, keep at 4°C
  o5mL of 4% BSA stock (made from VWR #0332-25G and DPBS)
  o5mL of DPBS (Invitrogen, catalog#14190-144)
-Centrifuge at 4°C

Cell thawing materials:

-Samples to be thawed (20 total) requested from BIOS and Ye lab
-Complete RPMI1640 (cRPMI; 10% FBS, 1% Pen/Strep, 1% Glutamax; all from Invitrogen)
-50mL falcon tubes (qty 20)
-15mL falcon tubes (qty 20)
-Vicell sample tubes (qty 20)
-40µm strainer (optional)
-DPBS (Invitrogen, catalog#14190-144)
-Printed labels for 20 samples
-DNAse I (10,000U/mL, optional)

CITE-Seq materials:

-Antibody pool from Antibody reconstitution
-Cells from Cell Thawing
-Cell Staining Buffer- 2% BSA in PBS, keep at 4°C
  o5mL of 4% BSA stock (made from VWR #0332-25G and DPBS)
  o5mL of DPBS (Invitrogen, catalog#)
-Resuspension buffer- 0.04% BSA in PBS, keep at 4°C
  o10mL of 4% BSA stock
  o1mL of DPBS
-5mL polystyrene tubes
-Human TruStain FcX (BioLegend catalot# 422302)
-cRPMI
-Flowmi strainer (**BAH136800040** Sigma)
-Trypan blue for Countess (Invitrogen)

SAFETY WARNINGS

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Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Antibody Reconstitution

1 Take two TotalSeq-C Human Panel vials from **4 °C** fridge.

> Briefly centrifuge each tube before opening.

2 

Reconstitute each vial in **50 µl cell staining buffer** .

3 Vortex for **00:00:05** - **00:00:10** or until the solution visually looks resuspended.

4 

Centrifuge at **10000 x g, 4°C, 00:05:00** .

5 

Combine antibody pool from two vials and keep at **4 °C** until ready for staining.

Cell thawing

6 Turn water bath on to **37 °C** , pre-warm **500 mL cRPMI media** .

7 While cRPMI warms:

7.1 Get a bucket with dry ice and a large container with regular ice.

7.2 Label 24 of each of the following tube/vials:
a. 15 mL falcon tubes
b. Vicell sample tubes

8 Bring all the vials from LN_2 tank and keep on dry ice until needed.

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9

Aliquot **5 mL warm cRPMI media** into each 15mL tube.

10 Thaw four vials at a time in **37 °C** water bath for **00:01:00** until only a small ice crystal remains and immediately hand over to partner to unscrew the vial aseptically. 1m

11

Aliquot **100 µl** - **125 µl** (5-6x10^5 cells) of thawed cells to its respective 15mL falcon tube drop by drop and mix gently twice with a P1000 pipette.

12

Centrifuge at **300 x g, Room temperature , 00:05:00** .

During this centrifugation step, add 570µL of dPBS to each ViCell sample tube in preparation.

13 Cap the cryovial and transfer to a Mr. Frosty and move to -80°C freezer for **24:00:00** before moving to LN_2.

Count Cells

14 Add **570 µl dPBS** to each ViCell sample tube if not performed previously (step 12).

15 Aspirate supernatant and resuspend in **200 µl cRPMI media** , keep cells **On ice** from this step.

16

Count cells on Vicell (**30 µl sample** diluted in **570 µl PBS**).

Record total cell count and viability, using template below:

i. If the total cell count is above 1x10^6, the remainder of the cells can be saved for bulk RNA-seq
ii. In ViCell counting, use cell-type PBMC and dilution factor 1:20

17 Calculate volume needed to collect total 1.5 x 10^6 cells from all samples using the template below:

i. Calculate volume of each sample needed to get equal number of cells from each sample
ii. For samples that don't have enough cells, thaw more cells from the same vial in -80°C freezer
iii. Make note of cell viability and also include dead cells in the total cell count. Do not use samples that have low cell viability (< 85%), or enrich live cell with dead cell removal kit

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Bulk RNA Seq Sample Prep (optional)

18

Take remaining cells from each sample after multiplexing.

19

Spin at **300 x g, Room temperature , 00:05:00** .

20

Aspirate supernatant.

21

Either freeze the pellets in the -80°C or proceed to RNA extraction.

22

Perform RNA Extraction as according to manufacturer's protocol: Extract RNA with Qiagen RNeasy Plus Mini Kit (elute in 30µL) and quantify RNA with Qubit (Use 1µL for Qubit concentration).

23

Use SmartSeq-2 protocol for bulk RNA library preparation.

CITE-Seq protocol 1w 3d 1h 15m

24 Use cell counts from ViCell to pool each sample into 5mL round bottom polystyrene tubes to get 1.5 x 10^6 cells in total.

25

Centrifuge at **300 x g, 4°C, 00:05:00** .

26 Aspirate supernatant, taking care not to disturb the cell pellet
Step 26 includes a Step case.
Typical case
Tetramer stain

step case

Typical case

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27

Resuspend cells in **67.5 µl staining buffer**

28 Add **7.5 µl Fc block reagent**

29

Incubate for **00:10:00** at **4 °C** .

30

Add **75 µl antibody pool** to pooled sample in FcBlock/staining buffer.

31

Incubate for **00:45:00** **On ice** (30-60 min recommended).

32

Add **2 mL staining buffer** .

33

Centrifuge at **300 x g, 4°C, 00:05:00** .

34 Carefully remove supernatant and save in 15mL falcon tube, taking care not to disturb the pellet.

35

Use wide-bore pipette tip to resuspend cell pellet in **2 mL staining buffer** .

36

Centrifuge at **300 x g, 4°C, 00:05:00** .

37 Carefully remove supernatant and save in the same 15mL falcon tube.

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38 Repeat (step 35-37) for one more wash, saving the supernatant:

38.1 

Use wide-bore pipette tip to resuspend cell pellet in **2 mL staining buffer** .

38.2 

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

38.3 Carefully remove supernatant and save in the same 15mL falcon tube.

39 

Resuspend pellet in a small volume of cRPMI (ie. **100 µl cRPMI** ; if the concentration is too high, cells can be diluted), then pass through a Flowmi strainer.

If using Flowmi strainer, be careful not to push the tip too hard as it is fragile.

Note that ~ 30% of the cell suspension is lost when using a flowmi strainer.

If final volume is very small (less than **50 µl**), use conventional cell strainers

40 

Take **6 µl filtered cell suspension** + **6 µl trypan blue** for counting with Countess and check for count and viability. (**Vicell can be used here instead**).

a. The desired cell density is 2.5 x 10^6 cell/mL (or 2,500 cell/µ).
b. If cells are too dense, use staining buffer to dilute cells to final density.
c. Use 30.8 µL (or appropriate amount if concentration different) for each well of 10x library preparation to ***ensure 77,000 cells from each sample are added to the master mix***.
d. Note in 10X VDJ protocol, only 90µL of 100µL cell suspension+master mix is loaded to each well, so ***the actual cells loaded are 2,500 cell/µL*28µL*(90/100) = 50,000.***
e. Maximum volume of cell suspension can be loaded to 10x VDJ run is **31.7 µl** ,make up extra volume using staining buffer.

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41 Load 70,000 cells/well into 6 wells of VDJ kits and perform GEM-RT.

42 Save GEM-RT at **4 °C** for up to 3 days or **-20 °C** for up to 1 week before 10X library preparation

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