Oct 17, 2019

Nuclei-seq

Mandy Xu[1], Troy Ketela[1]

[1]Princess Margaret Genomic Centre

1 Works for me dx.doi.org/10.17504/protocols.io.8b8hsrw

Human Cell Atlas Method Development Community CZI START Project

Samah El Ghamrasni
Princess Margaret Cancer Centre Research

ABSTRACT

This protocol has been developed by the Princess Margaret Genomics Centre specifically for Nuc-Seq, both at the bulk and single-cell levels.

MATERIALS

NAME	CATALOG #	VENDOR
Triton X-100	T8787-50ML	Sigma Aldrich
Razor blades	12-640	Fisher Scientific
Sucrose	S7903	Sigma Aldrich
DNAse/RNAse free distilled water	10977023	Thermo Fisher Scientific
Magnesium chloride solution for molecular biology (1.00 M)	M1028	Sigma – Aldrich
Protector RNase Inhibitor	3335402001	Sigma – Aldrich
Calcium chloride solution	21115-100ML	Sigma – Aldrich
autoMACS Rinsing Solution	130-091-222	Miltenyi Biotec
DAPI	D3571	Invitrogen - Thermo Fisher
UltraPure 0.5M EDTA, pH 8.0	15575-038	Thermo Fisher Scientific
1M Tris-HCl pH=7.5	15567-027	Invitrogen - Thermo Fisher
Flowmi™ Cell Strainer 40 µm	H13680-0040	Bel-Art
PBS - Phosphate-Buffered Saline (10X) pH 7.4	AM9625	Invitrogen - Thermo Fisher
C-Chip™ Disposable Hemacytometers, Improved Neubauer; 50/Pk.	22600100	Thermo Fisher
Kimble™ Kontes™ Dounce Tissue Grinders	K8853000040	Thermo Fisher
SYBR™ Green II RNA Gel Stain, 10,000X concentrate in DMSO	S7586	Thermo Fisher

MATERIALS TEXT

Component	Vol	Final Conc.
1M sucrose	2560ul	0.32mM
1M $CaCl_2$	40ul	5mM
1M $Mg(Ac)_2$	24ul	3mM
1M Tris-HCl 7.5	160ul	20mM
Triton X-100	8ul	0.1%
0.5M EDTA 8.0	1.5ul	0.1mM
RNase Inhib (40U/ul)	10 ul	40U/ml
H_2O	5196.5ul	-

Lysis Buffer

Nuclei-seq: Preparation

1 Rack 4 falcon tubes (lysis buff, wash buff, nuclei, intermediate) and 1 FACS tube on ice

2 Chill centrifuges (4°C 800 g 10 min), clean hood, fill Styrofoam boxes: one with dry ice (petri dish and razor blade); one with ice packed and slanted for petri dish away from the user; one with ice with falcon tubes (above), 1 weighed epp tube and douncer with A pestle; Clean douncer, pestle and forceps with ethanol, wrap forceps and ethanol again; Pestle B and forceps on kimwipe beside ice in hood; Ranin wide bore tips and p1000;

3 Prep 5 epi tubes on a rack by scope: Three for checking the completion of lysis; One for final resuspension for FACs " leave on ice; One for counting.

Nuclei-seq: Nuclei Isolation from Tissue

4 Weigh tissue (30-50mg ideal, save rest) and cut tissue into 1-2mm3 using a chilled razor on dry ice

5 Add lysis buffer (enough to cover) and transfer to the ice to continue chopping, using a pipette to rinse off blade using the lysis buffer of the sample

5.1 Transfer to nuclei tube and lysis for 5 min

6 Dounce supernatant first (1-2 strokes) and transfer to 4th tube

7 Dounce pellet in thirds (2 strokes)

7.1 Add enough lysis buffer to resuspend pellet in douncer, dounce and transfer to 4th tube

7.2 Douncing is done when solution appears milky

8 Resuspend 4th tube with wide bore tip and check with residual on tip and tip of p20 in SYBR using countess chips

9 Transfer super to nuclei tube, leaving behind thepellet

9.1 Transfer pellet back to douncer and dounce with pestle B (1/3-1/2 of pellet depending on toughness) " 2-4 strokes

9.2 Rinse out douncer with lysis buffer

9.3 Transfer to nuclei tube

10 Resuspend nuclei and count again with leftover in p1000 tip " dounce again based on results

11 Spin at 800g for 10 min

12 Wash with 2ml of wash buffer

13 Spin at 800g for 10 min

14 Wash again with 2ml of wash buffer

15 Spin for 10 min at 800g and resuspend in 1ml

16 Filter with 40um Flowmi cell strainer, transfer to 1.5ml LoBind tube on ice

17 Count nucleiSpin at 800g for 10 min

18 Wash again with 2ml of wash buffer

19 Spin for 10 min at 800g and resuspend in 1ml

20 Filter with 40um Flowmi cell strainer, transfer to 1.5ml LoBind tube on ice

21 Count nuclei

21.1 40ul buff + 5ul nuclei
If >1 million use 10ul of DAPI
If <1 million use 6-8ul of DAPI
DAPI requires 5 min incubation

22 Make note of nuclei condition, number of strokes, nuclei size etc after loading

23 DO NOT DISCARD THE WASH AND RESUSPENSION BUFFER, KEEP IT ON ICE.

24 After FACS, Spin at 800g for 10 min, resuspend the pellet in cold wash and resuspension buffer (volume calculated so that the final conc is around 1000nuclei/ul)

25 
Count and take images

26 Make note of nuclei condition, number of strokes, nuclei size etc after loading

Flow Sorting

27 Stain the nuclei with DAPI at the concentration suggested by the manufacturer.

28 Cell sorter: Influx BRV (TMDT, LAB 2-504), sorting for 1-1.5hrs.

29 Gating: DAPI positive, and exclude any debris/ nuclei aggregates within the DAPI positive gating.

30 Collect nuclei in the Wash and Resuspension Buffer.

31 Wash the nuclei with the Wash and Resuspension Buffer.

32 Count the nuclei and proceed to 10X 3' RNA seq directly.

33 After FACS, Spin at 800g for 10 min, remove the supernatant (sheath buffer+ wash and resuspension buffer), resuspend the pellet in a fresh, cold wash and resuspension buffer (volume calculated so that the final conc is around 1000nuclei/ul)Stain the nuclei with DAPI at concentration suggested by the manufacturer.