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Nuclei-seq

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Protocol status: Working

We use this protocol and it's working



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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

MATERIALS

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

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 ₂ O	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 the pellet
- 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.