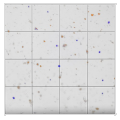

Human Heart Nuclei Isolation for DroNc-seq

Version 2

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ABSTRACT

Recent advances in single cell mRNA sequencing technology such as Drop-seq (*Cell*, 2015) have allowed the investigation of cellular composition and processes within complex tissues. Although some fresh tissue samples are available for prospective studies, there is an abundance of archived clinical samples readily available in biobanks to investigate different biological processes. However, archived tissues are not easily amenable to tissue digestion, yielding cells with compromised integrity. An alternate method to single-cell sequencing called DroNc-seq (*Nature Methods*, 2017) employs the use of single nuclei. DroNc-seq takes advantage of the higher structural integrity of the nuclear membrane as compared to the cellular membrane. This permits exploration of tissues for which a viable single-cell suspension cannot be obtained, such as cardiac tissue. With growing interest in cataloguing cardiac transcriptomic data for the Human Cell Atlas Project, the need for optimized nuclei isolation protocols proves critical. Here, we develop a method for obtaining single-nucleus suspensions from snap-frozen human heart tissue for use in DroNc-seq, expanding the variety of samples from which we can obtain transcriptomic data.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Keep samples on ice throughout procedure to prevent RNA degradation.

MATERIALS TEXT

Materials:

- 2 mL Glass Dounce Tissue Grinder, with two pestles, A and B (such as Sigma, Cat.# D8938)
- Centrifuge with temperature control, rotor for 15 mL/ 50 mL conical tubes
- 20 µm cell strainer (such as pluriStrainer, SKU: 43-50020-03)
- 10 µm cell strainer (pluriStrainer, SKU: 43-50010-03)
- 15 mL conical tubes
- 50 mL conical tubes
- Neubauer Improved (NI) Hemocytometer (Fisher, Cat.#: 22600101)
- Tweezers
- Scalpel

Reagents & Buffers:

- Nuclei EZ-Prep Lysis Buffer (Sigma, Cat.#: N3408)
- Nuclei Suspension Buffer (NSB):
  - 1X PBS
  - 0.01% BSA (5 µL/mL buffer, from 2% stock) (Fisher, Cat.#: NC0506695)
  - 0.1% RNase Inhibitor (1 µL/mL buffer) (Lucigen, Cat.# 30281-1)

For Staining and Counting:

- DAPI, 10 mg/mL stock solution, diluted 1:100 in NSB
- Wheat Germ Agglutinin, Alexa Fluor 594 Conjugate (ThermoFisher, Cat.#: W11262), prepared according to manufacturer instruction, diluted 1:1000 in NSB

BEFORE STARTING

Heart tissue samples were collected in 1.5 mL microfuge tubes and snap-frozen before attempting nuclei isolation.

Reagent Prep

1 For each nuclei preparation, aliquot 20 mL of EZ-Prep Lysis Buffer and prepare 7 mL NSB. Place both on ice to chill while preparing tissue.

Tissue Prep

2 Weigh tissue to be prepared (Usable numbers of nuclei have been retrieved from 30-40 mg of heart tissue). Prior to douncing, mincing tissue with a scalpel or razor blade can be useful.

Nuclei Isolation

3 Place tissue in douncing mortar with 2 mL ice-cold EZ-Prep lysis buffer. Dounce 25 strokes with Pestle A.

4 Transfer from mortar to 15 mL conical tube. Wash mortar into tube with 2 mL additional ice-cold EZ-Prep. Incubate sample on ice 5 minutes.

5 Centrifuge 500 g, 5 minutes, 4ºC.

6 Aspirate supernatant. Wash and resuspend pellet with 2 mL ice-cold EZ-Prep.

7 Transfer back to douncing mortar and dounce 25 strokes with Pestle B.

8 Repeat steps 4 and 5.

9 Wash and resuspend pellet with 4 mL ice-cold EZ-Prep, incubate on ice 5 minutes.

10 At this point, reserve a 10 µL sample to check for nuclei (see below: “Counting Nuclei”).

NOTE

a. If additional douncing is needed, repeat steps 7-9 until an appropriate suspension is achieved. The samples used here required 25-35 additional dounces with Pestle B.

b. If membrane fragments are still present after additional douncing, the suspension may be filtered before transferring to NSB. The suspensions obtained here were sequentially filtered once with a 20 µm filter, then once with a 10 µm filter into fresh 50 mL conical tubes.

11 Repeat step 5.

12 Aspirate supernatant, wash and resuspend pellet in 4mL ice-cold NSB.

13 Repeat step 5.

14 Aspirate supernatant and resuspend pellet in 2 mL ice-cold NSB.

15 Filter nuclei into a fresh 50 mL conical tube using a 10 µm filter. Keep on ice until needed for experiment.

Counting Nuclei

16 For a 10 µL hemocytometer, mix 10 µL nuclei with 10 µL DAPI and 10 µL WGA in a microcentrifuge tube.
Load 10 µL into hemocytometer and count using fluorescent and phase contrast images overlaid, if possible.

This should allow accurate assessment of the number of bare nuclei in suspension in comparison to the amount of membrane fragments or unstained debris.

Diluting Nuclei for DroNc-seq

17 After counting, dilute nuclei to desired concentration using remaining NSB. The tissue samples used here yielded 300-400K nuclei/mL of final suspension.