Nov 14, 2024

Sample preparation for Single cell RNA sequencing (scRNA-seq)

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpz1wjlzp/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpz1wjlzp/v1

Protocol Citation: Jun B. Ding, Richard Roth, Fuu-Jiun Hwang, Yue Sun 2024. Sample preparation for Single cell RNA sequencing (scRNA-seq). **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpz1wjlzp/v1

Protocol status: Working
We use this protocol and it's working

Created: August 29, 2024

Last Modified: November 14, 2024

Protocol Integer ID: 106637

Keywords: ASAPCRN, RNA, Sequencing, scRNA-seq, Brain, mouse brain tissue sample preparation, single cell rna, scrna, cell rna, rna, sample preparation, sequencing, seq, sample

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020551

Abstract

Mouse brain tissue sample preparation for single cell RNA sequencing (scRNA-seq) that is performing in the Ding lab.

Attachments

DOCX

Ding Lab_scRNA-seq s...

31KB

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.

Solution Preparation (in advance)

1 Solutions recipes with catalog information can be found in attached document

- ACSF 10X
- HBSS
- Isethionate Solution
- Kynurenic Acid Solution
- Nitro-Arginine Solution
- Glutathione Solution
- Sodium Pyruvate Solution
- Albumin Inhibitor (Worthington Cat# LS000290): Add 32 mL of HBSS to the albumin bottle and allow it to dissolve at room temperature. Mix by inversion, aliquot 3 mL per tube, and store at 4 °C .

Solution Preparation (day of processing)

2 Solutions recipes with catalog information can be found in attached document

- 1x ACSF: Check osmolality (~300 mOsm). Bubbling with 95/5 O_2/CO_2
- HBSS with supplements (HBSS/s). Bubbling with pure O_2.

  Isethionate
  Solution with supplements (Ise/s). Bubbling with pure O_2.
- Papain (Worthington LK003176) solution: adding 5 ml oxygenated HBSS/s to the bottle and incubating at 37°C. Transfer the Papain solution into spin flask (Wheaton #3572651). Displace the pure O_2 tube

  in the flask, but **do not bubble gas through the solution**. Check the osmolality every 20 min (~ 320 mOsm).
- Sample buffer: 1X PBS (calcium and magnesium-free) containing 0.04% BSA (400 µg/ml)

Cutting acute slices

3 Perfuse the mouse with 25 ml of cold, freshly prepared, oxygenated 1x ACSF and dissect out the brain.

4 Cut the brain using a PFA-free vibratome (thickness 300 um, speed 1.0 mm/s, amplitude 1.4 mm).

5 Transfer slices to a chamber with bubbling ACSF at 34 °C and incubate for 00:30:00 . 30m

Micro-Dissection

6 Dissect the desired region(s) of interest/tissue in a plate containing oxygenated ACSF.

7 Refresh the solution if the dissection takes more than 00:03:00 . 3m

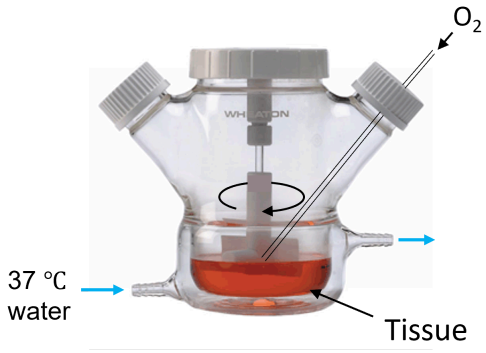
8 Transfer the tissue pieces to HBSS/s bubbling with pure O_2.

Papain incubation 1h

9 Transfer the tissue to a spin flask with Papain solution and allow it to settle at the bottom.

10 Place the O_2 tube on the surface of the solution.

11 Incubate at 37 °C with slow, constant agitation for 01:00:00 using a use a spinner flask (WHEATON #356943) on a magnetic stirrer. The setup looks like this: 1h



Trituration

12 Transfer the tissue to a 15 mL tube and centrifuge at 300 x g for 00:05:00 . 5m

13 Discard the supernatant and add 5 ml HBSS/Proteinase Inhibitor.

14 Triturate the tissue using fine-polished Pasteur pipettes (~600 µm, 10 times; ~300 µm, 10 times; ~100 µm, 5 times).

15 Filter through a 40 µm cell strainer (Falcon Facs tube #352235) and centrifuge at 300 x g for 00:05:00 . Avoid making bubbles. 5m

Debris Removal 6m

16 Discard the supernatant and resuspend the cells in 500 uL sample buffer.

17 Layer the cloudy cell suspension on 5 mL albumin and centrifuge at 70 x g for 00:06:00 6m

Note: A benchtop centrifuge without the brake setting was used and allowed to completely stop; with the very slow spin-down, the gradient should remain undisturbed even with sudden braking.

18 Discard the supernatant and wash once with 500 uL sample buffer.

Dead Cell Removal

19 Follow the protocol for Annexin V-conjugated magnetic beads (Miltenyi Biotec, Cat# 130-090-201).

20 Resuspend the cells in sample buffer (~50uL -100uL).

21 Check viability and density under a microscope and place the cells on ice.

Cell Quality for library prep

22 For prepare single cell library with Chromium (10x Genomics), cell quality should be:

Viability > 70%
700~1200 cells/ml
50~ 100 ul volume