Aug 27, 2020

HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain

Anand Patel[1], Asa Karlstrom[1], Brittney Gordon[1], Elizabeth Stewart[1], Michael Dyer[1]

[1]St. Jude Children's Research Hospital

1 Works for me dx.doi.org/10.17504/protocols.io.98ah9se

NCIHTAN

Anand Patel

ABSTRACT

This protocol was used for the dissociation of fresh pediatric neuroblastoma samples from patient tumors or orthotopic patient derived xenografts. Using this method, we were able to prepare highly viable (>90%) single-cell suspensions compatible with droplet-based single-cell RNA-Seq technology (Slyper et al).

For the Human Tumor Atlas Pilot Project (HTAPP), this protocol has been successfully applied to neuroblastoma resections collected from abdominal and paraspinal sites.

Description of this protocol and guidance for testing and selecting methods for processing other tumor and sample types can be found in Slyper et al.

DOI

dx.doi.org/10.17504/protocols.io.98ah9se

PROTOCOL CITATION

Anand Patel, Asa Karlstrom, Brittney Gordon, Elizabeth Stewart, Michael Dyer 2020. HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain. **protocols.io** https://dx.doi.org/10.17504/protocols.io.98ah9se

KEYWORDS

Neuroblastoma, single-cell RNA-sequencing

CREATED

Dec 06, 2019

LAST MODIFIED

Aug 27, 2020

PROTOCOL INTEGER ID

30690

GUIDELINES

Store papain kit at 4° C.

MATERIALS

NAME	CATALOG #	VENDOR
PDS Kit, Papain Vial	LK003178	Worthington Biochemical Corporation
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
Aspen Surgical™ Bard-Parker™ Protected Disposable Scalpel No. 10	02-688-78	Fisher Scientific
A1000 Pipettor	A-1000	MIDSCI
Pipet-aid pipette controller	4-000-110	Drummond Scientific
Falcon 50mL Conical Centrifuge Tubes	14-959-49A	Fisher Scientific
AvantGuard 1250 microliter filter tips	AV1250-H	MIDSCI
Falcon 5mL sterile serological pipets	13-675-22	Fisher Scientific
Falcon 10mL sterile serological pipets	13-675-20	Fisher Scientific
Sterile 40 micron nylon strainer	08-771-1	Fisher Scientific
Trypan blue 0.4% solution	15-250-061	Fisher Scientific
1.5 mL microcentrifuge tubes	05-408-129	Fisher Scientific
Thermo Scientific Sorvall Legend XTR Centrifuge	75-217-420	Fisher Scientific
Bright-Line Hemacytometer	02-671-5	Fisher Scientific
A100 pipetter	A-100	
AvantGuard 100 microliter filter tips	AV1000	MIDSCI
Falcon 60 mm tissue-culture treated dishes	08-772B	Fisher Scientific

SAFETY WARNINGS

Follow general lab safety and institutional guidelines for working with human samples and sharps.

BEFORE STARTING

- Warm water bath to 37° C.
- Allow EBSS (vial #1), albumin-ovomucoid inhibitor mixture (vial #4), papain (vial #2) and DNase I (vial #3) to warm to room temperature for 15 min.
- If you are starting with a new kit, add 32 ml of Earle's balanced salt solution (EBSS; PDS kit vial #1) to albumin-ovomucoid inhibitor (vial #4).

Reagent Preparation

1 Prepare reagents (1 set for each sample):

1.1 15m

Add 5 mL of Earle's Balanced Salt Solution (vial #1) to papain (vial #2). Allow papain to dissolve for 15 min at 37° C. **37 °C**

00:15:00

1.2

Add 500 µL of Earle's Balanced Salt Solution (vial #1) to DNase I (vial #3). Mix using micropipette.

Room temperature

Citation: Anand Patel, Asa Karlstrom, Brittney Gordon, Elizabeth Stewart, Michael Dyer (08/27/2020). HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain. https://dx.doi.org/10.17504/protocols.io.98ah9se

1.3

Add 250 µL from DNase I solution (vial #3) to papain solution (vial #2).

Room temperature

Tissue dissociation

2

Obtain tumor tissue. Use 100-500 mg of tissue per papain vial. Use a disposable scalpel to mince the sample into small pieces (approximately 1 mm fragments).

Room temperature **Process tissue quickly to avoid viability loss (mincing should take less than 5 min)**

Note: We typically receive tissue on ice from the repository. Tissue is processed immediately upon receipt. Once dissociation has started, we do not recommend putting tissue back onto ice (temperature fluctuations lead to tissue degradation).

Note: We typically mince tissue on a 60 mm sterile tissue-culture treated plate.

3

Move minced tissue into a sterile 15 mL Falcon conical tube, and transfer papain-DNase mixture from vial #2 to the conical tube. Incubate conical tube in water bath at 37° C for 15 min.

37 °C Water bath

00:15:00

4

Triturate tissue by pipetting up and down 10-15 times using a 10 mL sterile serologic pipette.

Room temperature

5

Continue dissociation by incubating tube in the water bath for another 15 min.

37 °C Water bath

00:15:00

6

Repeat trituration by pipetting up and down 10-15 times using a 10 mL sterile serologic pipette.

Room temperature

Note: The cell suspension should look cloudy with chunks of debris.

7

Filter cell suspension through a 40 µm cell strainer into a 50 mL Falcon conical tube.

Room temperature

Note: Debris will be captured on the cell strainer, while the filtrate should appear cloudy.

Citation: Anand Patel, Asa Karlstrom, Brittney Gordon, Elizabeth Stewart, Michael Dyer (08/27/2020). HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain. https://dx.doi.org/10.17504/protocols.io.98ah9se

8

Rinse filter with 5 mL Earle's Balanced Salt Solution (vial #1).

9

Centrifuge cell suspension at 500 g for 5 min at room temperature.

500 x g, 00:05:00

Room temperature

Cleanup

10

During centrifugation, prepare the two 15 mL Falcon conical tubes containing:

10.1

Resuspension buffer:

2.7 mL Earle's Balanced Salt Solution (vial #1)

300 µl Albumin-ovomucoid inhibitor solution (vial #4)

150 µl DNase I solution (vial #3)

Room temperature

10.2

Density gradient:

5 mL Albumin-ovomucoid inhibitor solution (vial #4)

Room temperature

11 After centrifugation (step #9), remove supernatant carefully without disturbing the pellet.
Tip: We typically use suction to aspirate the first 9 mL of supernatant, followed by a micropipette to remove the last 0.5-1 mL of supernatant (to avoid suctioning the pellet). If the pellet is very small or difficult to see, we recommend transferring the top 9.5 mL of supernatant to a tube labeled 'supernatant 1' as a backup.

Room temperature

12

Re-suspend pellet with 3 mL resuspension buffer (from step #10.1), and gently mix using a 5 mL serologic pipette.

Room temperature

13

Carefully layer resuspended cells over density gradient (from step #10.2).

Room temperature

14

Citation: Anand Patel, Asa Karlstrom, Brittney Gordon, Elizabeth Stewart, Michael Dyer (08/27/2020). HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain. https://dx.doi.org/10.17504/protocols.io.98ah9se

Centrifuge at 100 g for 6 min at room temperature.

100 x g, 00:06:00

Room temperature

Cell counting and Quality Control

15

Carefully remove supernatant without disturbing the pellet.
Tip: We typically use suction to aspirate the first 7 mL of supernatant, followed by a micropipette to remove the last 0.5-1 mL of supernatant (to avoid suctioning the pellet). If the pellet is very small or difficult to see, we recommend transferring the top 7.5 mL of supernatant to a tube labeled 'supernatant 2' as a backup.

16 Re-suspend pellet in 10 mL Earle's Balanced Salt Solution and filter through a 40 µm cell strainer into a sterile 50 mL Falcon conical tube.

Room temperature

17

Mix 10 µL of single-cell suspension with 10 µL of Trypan blue solution, and load 10 µL onto hemacytometer.

Room temperature

18

Count and report the number of viable single cells.
Tip: We recommend proceeding only with samples that have >80% viability. Low viability samples will have higher cell clumping, and ambient RNA will complicate downstream single-cell RNA-sequencing analysis.

Room temperature

Cell dilution and 10x Genomics single-cell RNA-seq loading

19

If necessary, dilute cells prior to loading onto 10x Genomics 3' single cell RNA-sequencing workflow.
Tip: We aim to load 10,000 cells per channel. We recommend trying to dilute cells to a concentration 1000 cells/µL.

20 Keep diluted cell suspension on ice, and proceed immediately to 10x Genomics single-cell RNA-sequencing workflow.

On ice Proceed immediately to 10x Genomics RNA-seq workflow

Citation: Anand Patel, Asa Karlstrom, Brittney Gordon, Elizabeth Stewart, Michael Dyer (08/27/2020). HTAPP_Dissociation of human neuroblastoma tumors to a single-cell suspension for single-cell RNA-seq using Papain. https://dx.doi.org/10.17504/protocols.io.98ah9se