

Aug 28, 2020

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

DOI

<https://dx.doi.org/10.17504/protocols.io.98ah9se>

Anand Patel¹, Asa Karlstrom¹, Brittney Gordon¹, Elizabeth Stewart¹, Michael Dyer¹

¹St. Jude Children's Research Hospital

NCIHTAN



Anand Patel

St. Jude Children's Research Hospital

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://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>



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: December 07, 2019

Last Modified: August 28, 2020

Protocol Integer ID: 30690

Keywords: Neuroblastoma, single-cell RNA-sequencing, dissociation of fresh pediatric neuroblastoma sample, fresh pediatric neuroblastoma sample, htapp-dissociation of human neuroblastoma tumor, human neuroblastoma tumor, neuroblastoma resection, processing other tumor, human tumor atlas pilot project, cell suspensions compatible with droplet, cell rna, cell suspension, rna

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](#)**.

Guidelines

Store papain kit at 4° C.



Materials

MATERIALS

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

Safety warnings

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

Before start

- 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 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 
 - 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 μ m 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.

- 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 μ L Albumin-ovomucoid inhibitor solution (vial #4) 150 μ L 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 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 μ m cell strainer into a sterile 50 mL Falcon conical tube.

Room temperature

- 17 Mix 10 μ L of single-cell suspension with 10 μ L of Trypan blue solution, and load 10 μ L 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/ μ L.





- 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