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Human breast cancer patient tissue dissociation for single-cell RNA sequencing

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Single-cell RNA sequencing reveals different cellular states in malignant cells and the tumor microenvironment in primary and metastatic ER-positive breast cancer. Furkan Ozmen, Tugba Y. Ozmen, Aysegul Ors, Mahnaz Janghorban, Matthew J. Rames, Xi Li, Aaron Reid Doe, Fariba Behbod, Gordon B. Mills, Hisham Mohammed NPJ Breast Cancer (2025)

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We use this protocol and it's working

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Abstract

Efficient and gentle dissociation of solid tumors is essential for preserving cell viability and transcriptomic integrity in single-cell RNA sequencing workflows. This protocol outlines an optimized approach for processing fresh human breast cancer tissue, designed to minimize cell stress while maximizing viable cell recovery. By combining enzymatic digestion with mechanical dissociation, the method yields high-quality single-cell suspensions with consistently high viability and strong transcript capture, enabling reliable downstream analysis of the tumor microenvironment at single-cell resolution.

Guidelines

This protocol is optimized for the dissociation of **fresh human breast cancer tissue** to generate high-quality single-cell suspensions suitable for RNA sequencing. When performed promptly and under proper conditions, the method yields high cell viability and robust transcript capture, resulting in high UMI and gene counts per cell. Breast tumor specimens are collected fresh from the operating room and immediately submerged in **MACS Tissue Storage Solution** to preserve cell integrity during transport on ice. Timely processing upon arrival in the laboratory is critical to maintaining viability and ensuring high-quality single-cell RNA sequencing results.

Manuscript citation
Please remember to cite the following publication along with this protocol
Single-cell RNA sequencing reveals different cellular states in malignant cells and the tumor microenvironment in primary and metastatic ER-positive breast cancer. Furkan Ozmen, Tugba Y. Ozmen, Aysegul Ors, Mahnaz Janghorban, Matthew J. Rames, Xi Li, Aaron Reid Doe, Fariba Behbod, Gordon B. Mills, Hisham Mohammed NPJ Breast Cancer (2025)

Materials

CONSUMABLES

- Falcon 5 mL Polystyrene Round-Bottom Tube with Cell-Strainer Cap

Fisher Scientific — Catalog #352235

- Protein LoBind® Tubes 5 mL

Eppendorf — Catalog #0030108302

Fisher Scientific — Catalog #14-282-304

- Falcon 15 mL Polystyrene Conical Tube

Fisher Scientific — Catalog #352095

- Falcon 50 mL Conical Centrifuge Tubes

Fisher Scientific — Catalog #14-959-49A

- Nalgene Rapid-Flow Sterile Single Use Vacuum Filter Units

Thermo Fisher Scientific — Catalog #568-0020

- Tissue Culture Dish 60 × 15 mm / Petri Dish

Fisher Scientific — Catalog #08772B

- Micro Dissecting Scissors, 3.5″ Straight Sharp

Roboz — Catalog #RS-5910

- Tweezer (standard sterile forceps)
- gentleMACS C-Tube

Miltenyi Biotec — Catalog #130-093-334

- MACS SmartStrainers (70µm)

Miltenyi Biotec — Catalog #130-098-462

- 40µm Cell Strainer

Fisher Scientific — Catalog #07-201-430

- FACS Tubes with Cell Strainer Cap

Fisher Scientific — Catalog #08-771-23

- 5 mL Eppendorf Tubes

Fisher Scientific — Catalog #14-282-301

- Aspen Surgical™ Bard-Parker™ Protected Disposable Scalpel No. 10

Fisher Scientific — Catalog #02-688-78

- 1 mL and 5 mL syringe plungers (for mashing tissue on strainers)
- Low-retention, filtered pipette tips

(1000 µL, 1000 µL wide bore, 200 µL, 20 µL)

- Serological Pipettes

(5 mL, 10 mL, 50 mL)

- Kimwipes

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REAGENTS

- Phenol Red-Free RPMI, Thermo Fisher cat# 11835030

- 1X Dulbecco's Phosphate Buffered Saline (DPBS), no calcium, no magnesium, Thermo Fisher Scientific — Catalog #14190094
- Fetal Bovine Serum (FBS), Certified, Heat Inactivated, U.S. Origin Thermo Fisher — Catalog #10082139
- Trypan Blue Dye, Gibco cat#15250061
- Y-27632 (ROCK Inhibitor), Selleck Chemicals — Catalog #S1049 (Resuspend in water to 10mM (10µM final), aliquot, and store at −80°C or −20°C short term.)
- gentleMACS Human Tumor Dissociation Enzymes, Miltenyi Biotec — Catalog #130-095-929

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MAGNETIC REMOVAL REAGENTS

- EasySep Dead Cell Removal (Annexin V) Kit, STEMCELL Technologies — Catalog #17899
- EasySep RBC Depletion Reagent, STEMCELL Technologies — Catalog #18170
- EasySep Magnet, STEMCELL Technologies — Catalog #18000
- Biotin Selection Cocktail
- RapidSpheres (vortex before use)

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10X GENOMICS REAGENTS

- Chromium Next GEM Chip G Kit

10x Genomics — Catalog #1000120

- Chromium Next GEM Single Cell 3′ Kit v3.1

10x Genomics — Catalog #1000268

- Dual Index Kit TT Set A

10x Genomics — Catalog #1000215

- Dynabeads MyOne Silane

10x Genomics — Catalog #2000048

- 0.2 mL PCR 8-Strip Magnetic Separator (optional)

Permagen — Catalog #MSRLV08

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CRITICAL EQUIPMENT

- gentleMACS Dissociator
- Centrifuge with temperature control
- Cell counter (e.g., Countess, Thermo Fisher)
- Benchtop incubator with rocker
- Standard pipettes and multichannel pipettes
- 10X Genomics Chromium Controller

Protocol materials

- Phenol red free RPMI, +/- 5% CS-FBS (charcoal stripped) **Gibco - Thermo Fisher Scientific** **Catalog #** A338210
- Dulbeccos Phosphate-Buffered Saline (DPBS), No Calcium, No Magnesium **Gibco - Thermo Fisher Scientific** **Catalog #**14190144
- gentleMACs human dissociation enzymes (130-095-929) **Miltenyi Biotec** **Catalog #**130-095-929
- Y-27632 (ROCK inhibitor) (Selleck Chemicals Catalog # S1049) **Selleckchem** **Catalog #**S1049
- EasySep Dead Cell Removal (Annexin V) Kit (17899) **STEMCELL Technologies Inc.** **Catalog #**17899
- EasySep RBC Depletion Reagent (18170) **STEMCELL Technologies Inc.** **Catalog #**18170
- EasySep Magnet (18000) **STEMCELL Technologies Inc.** **Catalog #**18000
- Chromium Next GEM Single Cell 3-prime Kit v3.1 **10x Genomics** **Catalog #**1000268
- Chromium Next GEM Chip G Kit **10x Genomics** **Catalog #**1000120

Before start

Before You Start

This protocol is optimized for single-cell isolation from **fresh human tumor biopsy cores** using **10x Genomics 3′ v3.1 chemistry**. Before beginning, ensure that:

Sample integrity is maintained by keeping tissue **on ice at all times** before dissociation.
Sterile technique is used throughout to prevent contamination and ensure sample quality.
All necessary sterile consumables and reagents prepared in advance.
A ROCK inhibitor stock (10 mM Y-27632) is pre-aliquoted and stored at −80°C.
Pre-aliquated dissociation enzymes and tubes are thawed.
Dissociation media is prepared fresh just prior to the dissociation step.
All centrifuges and incubators are set to appropriate temperatures (e.g., 4°C for centrifuge, 37°C for incubation).
The EasySep magnet and dead cell/RBC removal reagents, sterile scissors/tweezer sets are readily accessible and brought to room temperature as required.
Cell counter (Countess) is calibrated, clean, and functional.

Note

‰ Note: This protocol is optimized for small biopsy cores (≤2 mm). For larger chunks or solid tumors, dissociation time and filtering steps may require adjustments. Refer to notes throughout the procedure for alternate handling of large specimens.

Single cell isolation from fresh human tumor biopsy for 10X scRNA seq

1 This protocol is optimized for small biopsy cores. Adjust for big tumor chunks.

Reagents:

1.1 Phenol red free RPMI, +/- 5% CS-FBS (charcoal stripped) **Gibco - Thermo Fisher Scientific Catalog #** A338210

1.2 Dulbeccos Phosphate-Buffered Saline (DPBS), No Calcium, No Magnesium **Gibco - Thermo Fisher Scientific Catalog #**14190144

1.3 gentleMACs human dissociation enzymes (130-095-929) **Miltenyi Biotec Catalog #**130-095-929

1.4 EasySep Dead Cell Removal (Annexin V) Kit (17899) **STEMCELL Technologies Inc. Catalog #**17899

1.5 Y-27632 (ROCK inhibitor) (Selleck Chemicals Catalog # S1049) **Selleckchem Catalog #**S1049

1.6 EasySep RBC Depletion Reagent (18170) **STEMCELL Technologies Inc. Catalog #**18170

1.7 EasySep Magnet (18000) **STEMCELL Technologies Inc. Catalog #**18000

1.8 Chromium Next GEM Single Cell 3-prime Kit v3.1 **10x Genomics Catalog #**1000268

1.9 Chromium Next GEM Chip G Kit **10x Genomics Catalog #**1000120

Equipments

1.10

Equipment

5ml Eppendorf Tubes	NAME
5ml Eppendorf Tubes	BRAND
14-282-301	SKU

1.11

Equipment

Falcon Round-Bottom (FACS) Tubes with Cell Strainer Cap (08-771-23) or 40µm cell strainer (07-201-430)	NAME
Falcon Round-Bottom (FACS) Tubes with Cell Straine	BRAND
08-771-23	SKU

1.12

Equipment

Falcon™ Standard Tissue Culture Dishes	NAME
Falcon™ Standard Tissue Culture Dishes	BRAND
08-772E	SKU

1.13

Equipment

new equipment NAME

Biology Tweezer 115 mm, Ideal Tak BRAND

7SG.CX.0 SKU

Biology Tweezer 115 mm (Ideal Tak Chiasso, Switzerland). Manufacturer Part Number: 7SG.CX.0 SPECIFICATIONS

1.14

Equipment

Micro Dissecting Scissors 3.5 Straight Sharp (RS-5910) NAME

Roboz Surgical Store BRAND

RS-5910 SKU

1.15

Equipment

Tube C (130-093-237)	NAME
GentleMacs	BRAND
130-093-237	SKU

1.16

Equipment

GentleMACS™ Octo Dissociator with Heaters	NAME
Dissociator with Heaters	TYPE
Miltenyi Biotec	BRAND
130-134-029	SKU
https://www.miltenyibiotec.com/US-en/products/gentlemacs-octo-dissociator-with-heaters.html	LINK

1.17

Equipment	
Chromium Controller	NAME
microfuidic based single-cell capture	TYPE
10x Genomics	BRAND
1000171	SKU
https://www.10xgenomics.com/instruments/chromium-controller	LINK

Tumor dissociation:

2 Keep sample on ice until ready for dissociation.

3 Rinse the tissue in DPBS in a sterile plastic petri dish.

4 Add 2ml of phenol red-free RPMI to a Tube C with the cocktail of enzymes H+A+R plus Rock inhibitor (final 10uM).

5 While holding the Tube C tilted and maintaining media at the bottom of the tube, mince cells with a sharp scissor on the neck of the Tube C. Close the cap and mix well gently.

6 Run h_tumor_02 program on the gentleMACs dissociator. (for very hard tumors use h_tumor_01). Spin for short (5s) to collect media+cell at the bottom of the tube.

7 Incubate at 37°C on a bench incubator with rocker, gently rocking the media (speed 190) for 20-25 min. (Place the tube at a 45-degree angle while rocking).

8 After 20-25 min, gently pipette the tissue (4-5 times). For most biopsy cores, they will be completely dissociated after 20-25 min and with gentle pipetting. If there are still undissociated pieces after pipetting, run h_tumor_02 program on the gentleMACs dissociator. If still see big chunks, incubate for another 5-10 minutes.

8.1 For biopsy cores which are small additional incubation is not required. Mash the chunks on the strainer and they'll dissociate and pass through the strainer.

9 Filter the dissociated cells+dissociation media through a 70µm cell strainer.

9.1 For Big and multiple cores: filter through a 40µm cell strainer in a 50ml tube. Mash the undissociated chunks with a 5ml syringe plunger.

9.2 For small cores, filter through a FACS Tubes with Cell Strainer Cap. Mash the undissociated chunks with a 1ml syringe plunger.

10 Wash the tube C with RPMI+2% CS-FBS to collect any residual cells and pass through the same strainer to wash the strainer as well. Transfer to a FACS tube if performing DEAD/RBC magnetic removal.

11 Spin at 4 degrees 300g for 7 min to collect cells

12 Decant the media gently without disturbing the pellet. While still holding the tube upside down dry the tube rim with a Kimwipe. Add DPBS+2%CS-FBS (total of ~200µl liquid in the tube with the leftover wash media).

13 Depending on tissue quality and appearance, dead cell and RBC removal can be performed in a **combined** or **separate** step. If the sample is particularly **bloody, necrotic, or has low viability**, it is recommended to perform **sequential magnetic binding steps** to improve removal efficiency and downstream cell quality.

Optional Isolate DEAD+RBC in a combined step (or separate if desired- Follow EasySep Protocols)

13.1 Add 100µL/mL of sample volume of Dead Cell Removal (Annexin V) Cocktail to sample (20µL for 200µL of sample).

13.2 Add 100µL/mL of sample volume of Biotin Selection Cocktail to sample.

13.3 Gently mix by tapping the tube, incubate @RT for 3 minutes

13.4 Vortex the RapidSpheres and add 100µL/mL of sample volume of RapidSpheres and mix gently.

13.5 Add DPBS+2%CS-FBS to top up the sample to 2.5ml. Mix by gently pipetting up and down 2-3 times

13.6 Add 100µL/mL of original sample volume of RBC Depletion Reagent to the 2.5ml mix.

13.7 Place the tube containing the DEAD cell removal+ RBC Depletion reagent in a magnet and incubate for 5min.

13.8 Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the cell suspension into a new 5ml tube.

Cell Counting

14 Count 3 times on the countess and average the count.

15 Adjust volume so that there is 700-1,200 cells/µl for 10X Genomics 3’ v3.1 protocol.

16 If there are still RBC or dead cells after the spin and count, repeat DEAD+RBCC removal one more time and incubate in the magnet for 5min to increase the purity.

Cell Capture on 10x Chromium

17 Load 20,000 cells using the Chromium Next GEM Single Cell 3-prime kit v3.1, onto a 10x Chip G and process according to manufacturer's protocol (CG000315 Rev A).

Protocol references

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Chromium Next GEM Single Cell 3-prime kit v3.1, manufacturer's protocol (CG000315 Rev A).