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Processing fallopian tube tissue for single cell sequencing analysis

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ABSTRACT

This protocol explains the steps used to isolate cells for single cell sequencing from freshly processed fallopian tube tissue or cells that have been frozen. **Step 1** outlines steps for digesting fallopian tube tissue. This is an overnight process using pronase which must not exceed 72 hours. **Step 2** outlines steps to process cells from **Step 1** for single cell sequencing. **Step 3** parallels **Step 2**, but details processing cryopreserved cells prepared in **Step 1.13**. **Step 4** details steps for assessing viability and cell numbers, which will subsequently be used for single cell sequencing. In **Step 5**, remaining cells from single cell sequencing can be plated and grown for downstream experiments or cryopreservation.

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GUIDELINES

Primary cells may take many days to weeks to grow. During this time, avoid disturbing cells. Change media after 1 week if there is a change in media color. Sometimes cells remain suspended in cell culture media because they have not attached. When changing cell culture media, the existing media with cells can be removed using a pipette and added to another collagen coated well for cell growth. If this is performed, add an additional 250 uL of additional USG cell culture media to the well with the transferred cells.

MATERIALS TEXT

Reagents

UltroserG (USG) (Pall Life Sciences; **15950-017**)

Antibiotic/Antimycotic (100x)(Gibco; **15240062**)

Collagen 1 – Rat tail (Gibco; **A1048301**)

Phosphate buffered saline (PBS) (Lonza; **17-516F**)

Fetal Bovine Serum (FBS) (Sigma Aldrich; **F2442**)

Penicillin/Streptomycin (P/S) (Sigma Aldrich; **P4333)**

Dulbecco's Modified Eagle's Medium (DMEM) **(**Corning; **10-013-CV**)

DMEM/F12 (50/50) (Corning; **10-092-CV**)

DMSO (Sigma Aldrich; **D2650**)

RPMI 1640 (Sigma Aldrich; **R7388**)

Pronase (Sigma-Aldrich, **10165921001**)

Deoxyribonuclease I (DNase) (Sigma-Aldrich, **DN25**)

Solutions

Cell Culture Media

DMEM/F12 (50/50) + 2% USG + 1% Antibiotic/Antimycotic

RPMI Solution

RPMI 1640 + 10% FBS + 2%P/S

Pronase solution

Make 50 mL of pronase solution: Add DMEM + 2% P/S to pronase powder (1.4 mg/mL)*
*must filter solution using 0.2 uM syringe filter

Collagen

1 mL of collagen to 200 mL of PBS + 1% P/S

Freezing medium

10% DMSO + 90% FBS

Wash solution

PBS + 1% P/S

Transport media

DMEM + 10% FBS

Supplies

Blade

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5 mL polystyrene round-bottom tube with cell strainer cap (Falcon; **352235**)

50 mL conical tube

15 mL conical tube

Cell culture dish

SAFETY WARNINGS

Refer to SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

- Obtain approval from Research Ethics Board (REB) or Institutional Review Board (IRB) and patient for tissue donation.
- All reagents should be at room temperature (20-22°C, 69-72°F). Do not warm using water bath.
- Use a solution of 70% EtOH (70% ethanol + 30% MilliQ H_2O) to disinfect surface and hands prior to working with tissue.
- Personal protective equipment (PPE) should be used

Tissue Dissociation Protocol

1

Step 1.1 to 1.13 details steps for digesting human fallopian tube tissue using pronase.

Fallopian tube and fimbria tissue samples

1.1 Collect the fallopian tube tissue within two hours of removal. Samples are transported from the operating room/hospital in DMEM medium with 10% FBS (transport media) on ice

In a biological safety cabinet with appropriate PPE, aspirate the transport medium leaving behind 5 mL

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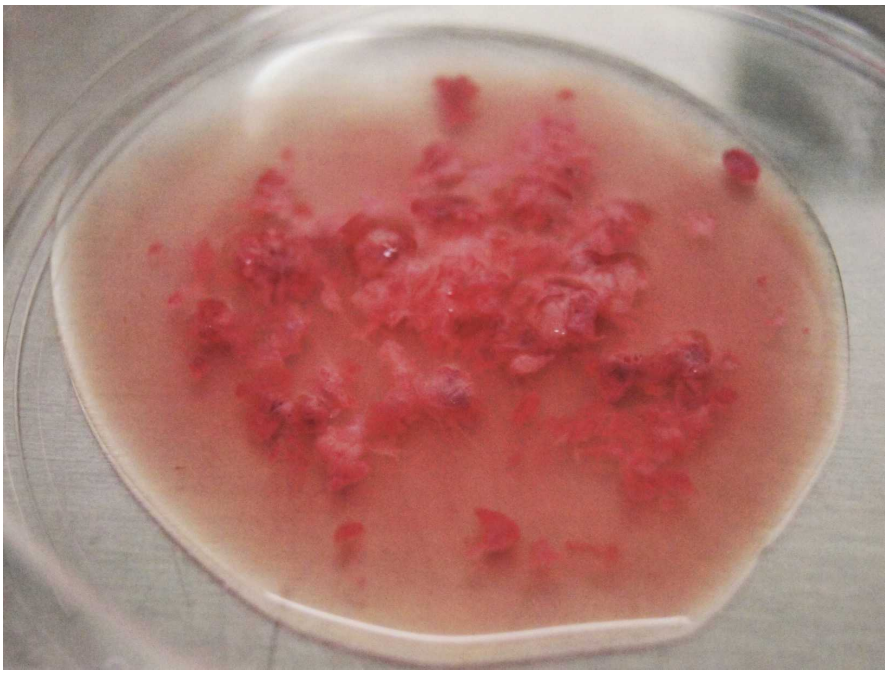
1.2 of media.

1.3 ⚠

Place tissue into cell culture dish and use blade to finely mince tissue. DO NOT USE MORTAR AND PESTLE. Meanwhile label 50 mL conical tube with identifying information.



Minced fallopian tube tissue

1.4 Using a 25 mL pipette, transfer minced tissue to 50 mL conical tube.

1.5 Add 15 mL of pronase solution to 50 mL tube. Add 150 uL of DNase to 15 mL solution.

1.6 Place 50 mL tube containing minced tissue on an orbital shaker at 4 °C and allow to digest for a minimum 24 hours and maximum 72 hours.

1.7 ⚠

Proceed to step 1.8 after 24 - 72 hours. DO NOT LEAVE TISSUE IN PRONASE FOR MORE THAN 72 hours.

Post tissue digestion processing

1.8 Remove 50 mL tube from 4 °C

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Fallopian tube tissue sample after 48 hour pronase digestion at 4°C.

1.9 Add approximately 20 mL of RPMI solution to tube which should amount to no more than 40 mL of total media.

1.10 Gently shake 50 mL tube back and forth to mix media

1.11 Place 50 mL tube into a rack and allow the tissue to settle to the bottom of the tube.

1.12

Spin both tubes in centrifuge at 1500 rpm for 15 minutes at 4°C

1.13 If you would like to proceed with storing the live cells, carefully remove the supernatant and re-suspend the cell pellet in 1 mL of freezing media and cryopreserve cells in a -80°C freezer (transfer to liquid nitrogen within 48 hours). The number of cryovials used for cell cryopreservation depends on the size of the tissue. Otherwise gently aspirate supernatant and proceed to Step 2.

Isolating cells from dissociated fallopian tube tissue

2 In this step, cells isolated from fallopian tube tissues in Step 1.13 are in the form of a pellet within a 15 mL conical tube and are ready to proceed onto the following steps.

2.1 Gently re-suspend pellet with 500 uL of cell culture media to dissociate pellet

2.2 Pre-wet a 5 mL polystyrene round-bottom tube with cell strainer cap with 50 uL of cell culture media

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2.3

Allow liquid to flow through by gravity (if liquid does not flow through, either pipette liquid up and down to allow liquid to flow through or seal cap tube with parafilm and centrifuge tube at 300 x g for 1 min)

2.4 Add 500 uL of resuspended cells from Step 2.1 to pre-wetted cell strainer cap and allow cells to flow through by gravity into 5 mL tube

2.5 Place parafilm on cap to prevent contamination when taken out of the biological safety cabinet

2.6 Place tubes with cells in an incubator (37°C and 5% CO_2) for 15 minutes

2.7 Proceed to Step 4

Isolating cells from cryopreserved fallopian tube cells

3 In this step, cells previously frozen and kept at -80°C or liquid nitrogen from Step 1.13 can be thawed and processed

3.1

Thaw cryo-vial rapidly into 5 mL pre-warmed cell culture USG media.

3.2

Centrifuge cells at 300 x g for 5 minutes

3.3 Aspirate supernatant

3.4 Re-suspend cells gently in 500 uL of cell culture media

3.5 Pre-wet a 5 mL polystyrene round-bottom tube with cell strainer cap with 50uL of cell culture media

3.6 Allow liquid to flow through by gravity (if liquid does not flow through, either pipette liquid up and down to allow liquid to flow through or seal cap tube with parafilm and centrifuge tube at 300 x g for 1 min)

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3.7 Add 500 uL of resuspended cells from Step 3.4 to pre-wetted cell strainer cap and allow cells to flow through by gravity into 5 mL tube

3.8 Place parafilm on cap to prevent contamination when taken out of the biological safety cabinet

3.9 Place tubes with cells in an incubator (37°C and 5% CO_2) for 15 minutes

3.10 Proceed to Step 4.

Analyzing cell viability and quantifying cell numbers

4 This step is used for assessing cell viability and counting the number of cells prior to sequencing

4.1 Count cells using the Nexcelom Cellometer K2 or equivalent cell counter

4.2 Assess viability and cell numbers before proceeding to sequencing

4.3 ⚠

Place remaining cells in 37°C incubator until ready to proceed to Step 5. Do not leave cells in incubator for more than 10 minutes.

Plating cells for growth

5 At this step, the required volume of sample has been used for sequencing. If cell sample remains after single cell sequencing, proceed with the following steps.

5.1 Prepare a 24 well Primaria plate for remaining cells by coating with 250 uL of collagen

5.2 Aspirate excess collagen

5.3 Add 250 uL of cell culture media to each well

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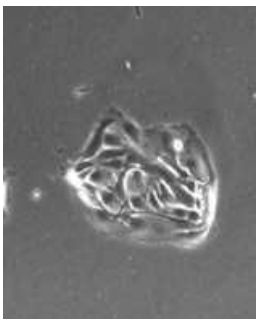
5.4 Remove cells from incubator (placed in incubator from Step 4.3) and very gently re-suspend liquid containing cells

5.5 Add cells to a labelled well of the 24 well collagen coated Primaria plate using a pipette

5.6 Gently move plate back and forth to evenly distribute cells

5.7 Place cells in incubator (37°C and 5% CO_2) and allow to grow.



Primary fallopian tube cells

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