Human Kidney / Tumour Tissue Disaggregation for Single Cell RNA Sequencing (10x Genomics platform)

Kevin Loudon,Alexandra Riding,John Ferdinand,Menna Clatworthy

Abstract

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Materials

Percoll 17-0891-01 by Sigma Aldrich

1 Gallon PBS [10X] (Phosphate Buffered Saline) (80mM Na2HPO4, 1.5M NaCl, 20mM KH2PO4, 30mM KCl, pH 7.4) R028 by G-Biosciences

RPMI 1640 Medium G7080 by Promega

Liberase TM 000000005401119001 by Sigma Aldrich

DNase I recombinant, RNase-free 000000004716728001 by Sigma Aldrich

Dead Cell Removal Kit 130-090-101 by Miltenyi Biotec

Protocol

Tissue Preparation

Step 1.

Take dissected tissue (renal cortex, medulla or tumour) and weigh tissue (typical biopsy size used 0.5 - 1 gram)

Tissue Preparation

Step 2.

Pour approximately 2-3 mLs of "Digest Mix" onto sampe in $10cm^3$ petridish

NOTES

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PREPARATION OF DIGEST MIX

Ingredients

(1) RPMI alone

(2) Liberase TM (Sigma Aldrich)

(3) DNAse (Sigma Aldrich)

For 50mLs of RPMI add:

--> 625 microlitres of Liberase (Stock solution 2.5mg/mL)

--> 250 microlitres of DNAse (Stock solution 0.05mg/mL)

Tissue Preparation

Step 3.

Using a razor blade mince into small pieces approximately $2mm^3$.

Tissue Preparation

Step 4.

Transfer tissue into a gentleMACS C tube and add further 3-4 mLs of Digest mix.

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GentleMACS C tube by Miltenyi Biotec (Cat.130-096-334)

Tissue Preparation

Step 5.

Place in shaking incubator at 37°C for 30 minutes.

Tissue Preparation

Step 6.

Homogenise sample in GentleMACS tube using program "Spleen 4" and "Lung 2" on GentleMACS dissociator.

Tissue Preparation

Step 7.

Pass through a 100µm cell strainer with of a 2.5ml syringe plunger and wash through with cold running buffer.

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PREPARATION OF RUNNING BUFFER

Ingredients (for 1 litre)

(1) 1L PBS

(2) 5ml BSA (from reagent diluent kit)

(2) 4ml 0.5M EDTA

Tissue Preparation
Step 8.

Centrifuge in a bench top centrifuge at 2000 RPM for 10 minutes and CAREFULLY remove the supernatant.

Tissue Preparation
Step 9.

If sample is contaminated with red blood cells an additional red cell lysis step can be taken.

Tissue Preparation
Step 10.

To ensure optimal yield for 10X Genomics single cell platform, a live cell enrichment step is required - this was performed using Miltenyi 'Dead Cell Removal Kit' (Please see manufacturers instructions for further details).

REAGENTS
Dead Cell Removal Kit 130-090-101 by Miltenyi Biotec

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LIVE CELL ENRICHMENT (Miltenyi - Dead Cell Removal Kit)

Ingredients

(1) Dead Cell removal Kit - Miltenyi (Order No. 130-090-101)

(2) MACS Column (LS or MS)

In brief for MACS colum LS

(1) Use LS column for 10^8 dead cells or 10^9 total cells.

(2) Remove supernatant completely following previous steps

(3) Resuspend pellet in 100 µL of '*Dead Cell Removal MicroBeads*' per approximately 10^7 total cells.

(4) Incubate 15 minutes at room temperature (20–25 °C).

(5) Rinse column with 1x binding buffer as per manufacturers instructions.

(6) Apply cell suspension in 1-10mLs of binding buffer and collect the effluent as the NEGATIVE cell population (i.e the live cells).

(7) Wash cells with PBS for 5 minutes at 1500rpm.

Tissue Preparation

Step 11.

Count the cells and resuspend the live cell supsension in appropriate volume of PBS for the 10X application.

Tissue Preparation

Step 12.

Resuspend the pellet in 44% percol solution in a 15ml tube and centrifuge for 20 min at 2000 RPM in a bench top centrifuge with the breaks OFF.

****The pellet contains the cells of interest****

NOTES

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PREPARATION OF 44% PERCOLL

Ingredients (50mLs of 44% Percoll)

(1) 10x PBS

(2) Percoll (GE Healthcare Life Sciences)

(3) RPMI with 10% FCS

For 50mLs of 44% Percoll:

(1) 2.5ml 10x PBS

(2) 22.5mLs Percoll (from stock)

(3) 28mLs complete RPMI (RPMI with 10% FCS)