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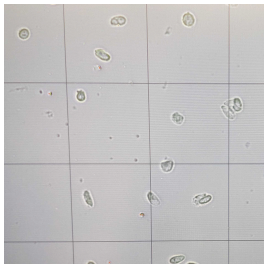
Nuclear extraction from endometrial tumors for single nuclei sequencing



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Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: nuclear extraction from endometrial tumor, endometrial tumor, endometrial adenocarcinoma, protocol for nuclear extraction, nuclear extraction, endometrioid type, single nuclei, nucleus rna, rna, frozen human tumor, sequencing

Abstract

Modified from: Slyper, M. *et al.* A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors. *Nat. Med. 2020 265***26**, 792–802 (2020).

A optimized protocol for nuclear extraction from endometrial tumors. Performed with endometrial adenocarcinoma, endometrioid type, FIGO grade 1.

FILE Nuclear extraction optimization for ...

Materials

Buffers:

2X ST Buffer

	Stock	Final	Volume for 10 mL
NaCl	5 M	292 mM	584 uL
Tris-HCl (pH 7.5)	1 M	20 mM	200 uL
CaCl2	1M	2 mM	20 uL
MgCl2	100 mM	42 mM	4.2 mL
Ultrapure Water	-	-	5 mL

NST Buffer

	Stock	Final	Volume for 10 mL
Nonidet P40 Substitute	10%		200 ul
BSA	5%		20 ul
ST Buffer	2X	1X	5 ml
Nuclease Free Water	-	-	4.75 mL

Nuclei Resuspension Buffer

	Stock concentration	Final	Volume Needed for 50 mL
BSA solution	5%	1%	10 ml
RNase Inhibitor	40 U/ul	0.2 U/ul	0.25 ml
1X PBS	-	-	39.75 ml

1 Place frozen tissue in a well of a 6 well plate containing 1 mL of cold NST disassociation buffer.

1.1 Ensure plate is on ice

2 Chop tissue using a scalpel until mostly homogenized 2m

3 Incubate solution on ice for 5 minutes 5m

4 Filter the homogenized solution through a 40 uM falcon cell strainer into a 50 mL falcon tube

5 Use an additional 1 mL of NST buffer to rinse the well and filter

6 Count nuclei

7 Centrifuge nuclei at 300 g for 10 minutes and aspirate supernatant completely 10m

8 Resuspend pellet and bring the volume up to 5 mL using ST buffer

9 Transfer solution to 15 mL conical tube and centrifuge for 10 minutes at 300 g at 4oC 10m

10 10. Resuspend pellets on ice in 1 ml of the ST buffer

11 11. Filter through 35 um falcon strainer

12 12. Count nuclei

13 Centrifuge nuclei at 300 g for 10 minutes and aspirate supernatant completely 10m

14 13. Resuspend nuclei pellet in resuspension buffer to get a concentration of 1,000 nuclei per uL

14.1 Start with .5 mL per 0.2 grams of endometrial tumor tissue

14.2 Look at nuclear membranes at 40x-60x to ensure high nuclear membrane quality with minimal blebbing