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KAPP-Sen TMC: Nuclei Suspension Preparation for snPATHO-seq

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Cellular Senescence Net… KAPP-Sen TMC

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We use this protocol and it's working

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Abstract

This protocol for nuclei suspension preparation was adapted from Vallejo et al. (https://doi.org/10.1101/2022.08.23.505054) with minor changes. Once the suspension was prepared we proceeded to the Chromium X run using Chromium Fix RNA Profiling by 10x Genomics.

Reagents and Consumables

1 Hyaluronidase: 0.5mg/ml
Liberase TM: 1mg/ml
Collagenase D: 1mg/ml

Nuclei isolation

1.1 Cut up to 2 ~25 µm-thick sections and place it in 1.5 mL Eppendorf tube. Store dry at 4°C if not used immediately. To keep it dry, you may use the cylinder containing silica beads that comes with 10x Genomics chips.

1.2 Wash sections three times with 1 mL Xylene (add to the tube with curls) for 10’ to remove the paraffin, rehydrate in sequential 1’ of 1 mL ethanol immersions (2× 100%, followed by 1× 70%, 50% and 30% ethanol). IMPORTANT: make sure paraffin is fully removed or digestion will be suboptimal.

1.3 Wash 3 times for 1' (2x1 ml wash and 1x800 ul final wash) with 1x PBS + 0.5 mM CaCl2

1.4 Remove as much volume as possible, and digest tissue for 45-60’(*) at 37°C(**) in 1 mL of RPMI1640 supplemented with 0.25-1 mg/ml Liberase TM(***) + 0.25-1 mg/ml Collagenase D(***) + 0.25-1 mg/ml Hyaluronidase + 1 U/ul RNAse Inhibitor.(*) NOTE: some blocks require longer digestion time, so inspect visually and help dissociation by pipetting up and down with a P1000 pipette (pipette up/down every 10-15 mins). (**) Incubation is done in a Thermomixer 800 rpm. IMPORTANT: dissociation does not need to be complete; the objective here is to loosen up the material to facilitate the nuclei release. Dissociation completeness varies from block to block. Tissue does not need to be fully digested.

1.5 After digestion, add 400 uL of Ez Lysis Buffer to the sample, mix by inverting 5× and centrifuge for 5’ at 850xRCF at 4°C.

1.6 Resuspend the pellets (released nucs and undigested tissue) in 250 uL Ez Lysis buffer + 2% BSA + 1 U/uL RNAse Inhibitor and homogenize the sample using a douncer/pestle by stroking 10-20 times.

1.7 After homogenization add 750 uL Ez Lysis buffer + 2% BSA + 1 U/uL RNAse Inhibitor and continue disaggregating by pipetting using a P1000 pipette (10 times). Incubate on ice for 15’. At 5’ mark pipette up and down using a P1000 pipette (10 times).

1.8 Pass sample through a 70 µm PluriStrainer filter (not Flowmi!) and centrifuge the flowthrough for 5’ at 850xRCF at 4°C. This is to remove big chunky, indigested tissue.

1.9 Wash nuclei suspensions once more with 800 uL of EzLysis buffer + 2% BSA + 1 U/uL RNAse Inhibitor (very gentle).

1.10 Pellet the nuclei for 5’ at 850xRCF at 4°C and wash nuclei twice PBS 0.5x + 0.02% BSA and resuspend in 300 uL of PBS 0.5x + 0.02% BSA (resuspension volume can vary depending on pellet size). Pass sample through a 50 µm PluriStrainer filter (not Flowmi!).

1.11 Count using Luna-FX7 or similar based on dual-fluorescence such asAO/PI.

Storage

2 If not proceeding to the Chromium X run using Chromium Fix RNA Profiling (10x Genomics), the user could supplement the sample with 0.1x volume of Enhancer solution (10x Genomics) + 10% Glycerol, rest on ice for 10’ and cryopreserve at −80°C.