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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' (2×1 ml wash and 1×800 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 as AO/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.