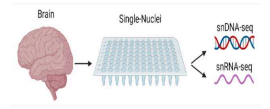


Sep 23, 2024

Human post-mortem nuclei preparation and single-nucleus multiome DNA/RNA sequencing by ResolveOME

DOI

<https://dx.doi.org/10.17504/protocols.io.3byl498bzgo5/v1>



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Protocol Citation: Ester Kalef-Ezra, George Morrow, Yanping Guo, Christos Proukakis 2024. Human post-mortem nuclei preparation and single-nucleus multiome DNA/RNA sequencing by ResolveOME. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.3byl498bzgo5/v1>

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Protocol status: Working

We use this protocol and it's working

Created: August 26, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 106665

Keywords: ASAPCRN, nucleus multiome dna, resolveome protocol, using resolveome, resolveome, nucleus dna, protocol for nuclei isolation, nuclei from different body area, single live mammalian cell, sequencing, nuclei isolation, dna, rna, activated nuclear sorting, nuclei, nuclear sorting

Funders Acknowledgements:

Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: 000430

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Abstract

Here we describe a protocol for nuclei isolation, immunostaining and sorting (FANS: Fluorescence-activated nuclear sorting) for single-nucleus DNA/RNA-seq using ResolveOME (BioSkryB) method from human post-mortem brain samples. We have used it to isolate nuclei from human post-mortem substantia nigra tissue, but it can be adapted for nuclei from different body areas.

The ResolveOME protocol is generally designed to work with single live mammalian cells and here we have done minor modifications in order to use it for human post-mortem brain samples.

Attachments



[2024_06_13_ResolveOM...](#)

3.1MB



[2024_06_14_Best Prac...](#)

587KB



[BN-020_Single Nucleu...](#)

182KB

Image Attribution

Title image was created with BioRender.com (RRID:SCR_018361, agreement LR277KLRDJ).

Guidelines

Critical note: Unless otherwise indicated, keep all the reagents and steps at  4 °C to protect DNA and RNA integrity.

Materials

Table 1. Kits and reagents:

A	B	C	D	E
Item	Supplier	Catalogue Number	Preparation before use	Storage
Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells	Invent Biotechnologies	BN-020	Read kit guidelines	Fridge
UltraPure DNase/RNase-Free Distilled Water	Thermo Fisher Scientific	10977049	Aliquot in 7 ml or 50 ml tubes	RT
10x PBS, RNase free	Invitrogen	AM9625.	Dilute in dH2O to make 1x PBS	Fridge
UltraPure™ BSA (50 mg/mL), tested for DNase, RNase, endonuclease, and protease activity	Thermo Fisher Scientific	AM2616, UltraPure™ BSA (50 mg/mL, 5%)	-	Fridge
Triton-X100	Merck Life Science Limited	T9284-100M	Prepare 10% solution in dH2O	RT
RNasin Plus RNase inhibitor	Promega	N2615	-	Freezer
PI (Propidium iodide)	Thermo Fisher Scientific	P3566	Prepare aliquots	Freezer
ResolveDNA™ Cell Buffer for FACS Kit -	BioSkryB	PN100183	-	Freezer
ResolveOME™ Box 1, 96 Reactions	BioSkryB	PN100768	-	Freezer
ResolveOME™ Box 2, 96 Reactions	BioSkryB	PN100973	-	Freezer
Single Use Library Adapter Set A, 96 Reactions	BioSkryB	PN100940	-	Freezer
Single Use Library Adapter Set B, 96 Reactions	BioSkryB	PN100941	-	Freezer
ResolveOME™ Bead Purification Kit	BioSkryB	PN100772	-	Fridge
High Sensitivity dsDNA Assay kit	ThermoFisher Scientific	Q32854	-	RT & Fridge
HS D5000 Reagent	Agilent	5067-5593	-	Fridge

	A	B	C	D	E
	HS D1000 Reagent	Agilent	5067-5585	-	Fridge
	Ethanol, for molecular biology	Merck	51976-500ML-F	Prepare 80% in the same day needed for bead purification wash	RT
	Optional: Horseradish Peroxidase (HRP)	ThermoFisher Scientific	31490	-	Freezer
	Optional: TMB substrate	BioLegend	421501	-	Fridge

1.  Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells **Invent Biotechnologies inc Catalog #BN-020**
2.  UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Catalog #10977049**
3.  PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Invitrogen - Thermo Fisher Catalog #AM9625**
4.  UltraPure™ BSA (50 mg/mL) **Thermo Fisher Scientific Catalog # AM2616**
5.  Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T9284**
6.  RNasin(R) Plus RNase Inhibitor, 10,000u **Promega Catalog #N2615**
7.  Propidium Iodide - 1.0 mg/mL Solution in Water **Thermo Fisher Catalog #P3566**
8.  Qubit™ dsDNA Quantification Assay Kits **Thermo Fisher Scientific Catalog #Q32854**
9.  High Sensitivity D5000 Reagents **Agilent Technologies Catalog #5067-5593**
10.  High Sensitivity D1000 Reagents **Agilent Technologies Catalog #5067-5585**
11.  Ethanol for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #51976-500ML-F**
12. Optional:  Pierce™ Horseradish Peroxidase **Thermo Fisher Catalog #31490**
13. Optional:  TMB High Sensitivity Substrate Solution **BioLegend Catalog #421501**

Table 2. Antibodies for immunodetection:

	A	B	C	D	E	F
	Antibody	Type	Supplier	Catalogue Number	Stock Concentration	Working Dilution
	Anti-Olig2-AF647	Conjugated	Abcam	ab225100	0.5 mg/ml	1/1000
	IgG-AF647	Isotype Control	Abcam	ab199093		



1.  Recombinant Alexa Fluor® 647 Anti-Olig2 antibody [EPR2673] (ab225100) **Abcam Catalog #ab225100**
2.  Recombinant Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093) **Abcam Catalog #ab199093**

Equipment:

General lab pipettes and low bind filtered pipette tips.

- Pair of forceps for tissue transfer.
- Hood for human sample handling.
- Refrigerated centrifuge for 1.5ml tubes that can achieve at least 13000 x g. e.g. Sigma Aldrich 1 - 14K Refrigerated Micro Centrifuge).
- Gentle plate shaker in a fridge or similar instrument that can allow antibody incubation @  4 °C with gentle mixing by placing the tubes covered in parafilm, inside falcon tubes on the plate shaker. Alternatively, a tube roller in the fridge can be used.
- Lab Fridge and Freezers ( -20 °C &  -80 °C).
- Plate centrifuge.
- BD FACS Aria Fusion sorter (BD Biosciences).
- PCR hood for single cell sample handling.
- PCR-Cooler & PCR-Rack (Eppendorf 10192281).
- Thermal Cycler, e.g. Multigene Optimax Gradient Thermal cycler with 96 well block (Labnet MB052).
- DynaMang-96 Side Magnet (Invitrogen 12331D).
- Qubit Fluorometer.
- Agilent TapeStation.
- PCR Plate Mixer.
- PCR Plate Spinner.
- Benchtop PCR 0.2 ml Strip & 1.5 ml Tube Centrifuge, e.g. Fisherbrand™ Mini-Centrifuge (Fisher Scientific 16617645).
- Vortex Mixer, e.g. iSwix VT Digital Vortex Mixer (Appleton Woods ST6000).
- Optional: 16-Tube SureBeads™ Magnetic Rack (Bio-Rad Laboratories 1614916).
- Optional: Magnet compatible with 0.2 ml strips, e.g. Dual Volume Strip Tube Magnet (BioSkrby 100226).

Consumables:

Low Bind 96-Well PCR Plates HS 39269099 (BioSkrby PN100149) or other low bind 96-well plates compatible with lab PCR.

- PCR Plate Sealing Film, e.g. BioSkrby PN100152 or ThermoFisher Scientific AB0558 .
- Alternatively other DNase/RNase-free caps compatible with the plates to be used.

 VersiCap Mat, 96-well, domed cap strips **Thermo Fisher Catalog #AB1810**

- 1.5 ml Low Bind Microcentrifuge Tubes, e.g. X500 Eppendorf DNA LoBind Tubes, 1.5ml, PCR clean (Fisher Scientific 16628742).
- Parafilm (general supplier).

- 50 ml falcon tubes (Appleton Woods AB036).
- Accudrop beads (Becton Dickinson, 345249).
-  PBS, pH 7.4 (flow cytometry grade) **Thermo Fisher Catalog #A1286301**
-  Qubit™ Assay Tubes **Fisher Scientific Catalog #Q32856**
-  High Sensitivity D5000 ScreenTape **Agilent Technologies Catalog #5067-5592**
-  High Sensitivity D1000 ScreenTape **Agilent Technologies Catalog #5067-5584**
- Ice & dry-ice.

Table 3. Optical Filters used for sorting using BD FACS Aria Fusion:

	A	B	C
	Laser	Diva Parameter Name	Fluorophore(s)
	560 nm Yellow - Green	YG 610/20	PI
	633 nm Red	R 670/30	Olig2-AF647, IgG-AF-647

Surface/pestle cleaning materials:

-  RNase AWAY®; Decontamination Reagent **Thermo Fisher Catalog #10328011**
-  RNaseZap® **Thermo Scientific Catalog #AM9780**
- DNA AWAY Surface Decontaminant (Thermo Fisher Scientific 1022347)
- NaOH 0.2M (any supplier)
- 70% EtOH in dH₂O
- dH₂O
-  Conti Washcloth Patient Cleansing Wipes 75 Wipes **Brosch Direct Catalog #PH5959**

Personal protective equipment: lab coats, googles, gloves

Safety warnings

 Please follow the Safety Data Sheets (SDS) for all reagents for safe handling and safety hazards.



Nuclei extraction from human post-mortem brain tissue

50m



1 Experimental steps:

Note

The Invent Biotech BN-020 kit was initially created for customers to avoid sorting and according to the manufacturer can work with as little as 1 mg of neuronal tissue or cells.

However, in our case we adapted it to isolate nuclei in a faster and more consistent manner compared to the manual method that requires hands-on preparation of the buffers to be used.

Due to our technical needs for cell-type selection and the difficulty to weigh very small amounts of frozen tissue, we use as minimum 10 mg of frozen post-mortem brain tissue.

Furthermore, as our downstream aim is to do single-cell multiome DNA & RNA sequencing of selected nuclei, the use of this kit is followed by nuclei immune-staining and sorting.

- are optional according to Invent Biotech team, but we use them to prepare nuclei population that are as clean possible.

Note

All centrifugation steps can be performed at Room temperature , but we suggest performing them at 4 °C to increase RNA preservation.

Day 1

- 1.1 Clean pestles provided in the Nuclei Isolation Minute kit the day before with 0.2 Mass Percent NAOH, 10% Presept and place them in a falcon tube containing RNase AWAY Decontamination Reagent.

- 1.2 Incubate the pestles in a 50 mL falcon tube containing RNase AWAY O/N @ Room temperature .



2 Day 2



- 2.1 Wash pestles with MiliQ-H₂O and then with dH₂O (RNase-free), air-dry with a dry wipe and pre-cool at  4 °C .



Note

The pestles should be cooled @  4 °C for at least  00:30:00 before use.

- 2.2 Clean carefully the PCR hood with 70% EtOH, dH₂O, DNA AWAY and RNase Zap and place not opened sterile filtered pipette tips.

- 2.3 Wipe the RNA lab pipettes with DNA AWAY and RNase Zap and place in the PCR hood.



- 2.4 Place in the PCR hood Low Bind 96-well plates to be used for nuclei sorting.

- 2.5 UV-treat the Low Bind 96-well plates and pipettes to be used for  00:20:00 -  00:30:00 before use.

50m



- 2.6 Pre-weight 1.5 ml tubes need for tissue scaling.

- 2.7 Prepare PBS (1x, RNase-free).

- 2.8 Clean the human tissue handling hood with  0.2 Molarity (M) NaOH, 10% Presept, 70% EtOH and dH₂O, DNA AWAY and RNase Zap.

- 2.9 Transfer the pipettes, filtered tips in the human tissue handling hood.



- 2.10 Clean a pair of forceps with  0.2 Mass Percent NaOH, 10% Presept, 70% EtOH and dH₂O, DNA AWAY and RNase Zap and transfer it to the human tissue handling hood.

2.11 Place a small beaker with **1M** 0.2 Mass Percent NaOH in the human tissue handling hood in order to use it to decontaminate the forceps and filtered tips after use.

2.12 In the human tissue handling hood Prepare 5% BSA in PBS (1x) and store **On ice** until use,
e.g. for 1 donor:

A	B
1x, RNase-free	2.083.34 µl
BSA stock 30%, Merck 126625	416.66 µl
Total	2.500,00 µl

2.13 Add RNasin Plus Ribonuclease Inhibitor to Buffer A and B prior to use to final concentration of 0.2 U/µl, e.g. for 1 sample:

- Buffer A: **5 µL** RNasin Plus Ribonuclease Inhibitor in **995 µL** Buffer A
- Buffer B: **5 µL** RNasin Plus Ribonuclease Inhibitor in **995 mL** Buffer B

2.14 Remove tissue from **-80 °C** freezer and transfer it to the human tissue handling hood on dry-ice.

2.15 Cut small brain pieces of the tissue of interest in the pre-weighed 1.5 ml Eppendorf tubes.

2.16 Weigh the tissue in a scale aiming for ~ **19 mg** (aim for **15 mg** - **30 mg** of each tissue/donor).

2.17 Add **200 µL** cold Buffer A (containing 0.2 U/µl RNasin Plus Ribonuclease Inhibitor) in each tube containing tissue and place it on wet ice.



Note

Critical Note:

From now on, keep the samples on wet ice, if not otherwise stated.

- 2.18 Homogenize the tissue using a pre-chilled pestle by grinding gently with twisting force for 50-60 times.



Note

Keep the tubes on wet ice while doing this step.

- 2.19 Add  500 μL cold Buffer A (containing 0.2 U/ μL RNasin Plus Ribonuclease Inhibitor) to the tube and continue to grind for 20-30 times.



- 2.20 Incubate the tube  On ice for  00:05:00 and carefully transfer homogenate to a filter (column) in collection tube (avoid larger debris that sink to the bottom of the tube).

5m



- 2.21 Incubate the tube with cap open at  -20 °C for  00:07:00 .

7m



Note

Incubation time can vary between  00:05:00 -  00:10:00 .

- 2.22 Cap the filter and immediately centrifuge at  13000 x g, 4°C, 00:00:30 .

30s



- 2.23 Discard the filter (column) and resuspend the pellet by pipetting up and down gently for 10-20 times.



**Note**

- Try to avoid lipids that attach to the wall of the tube.
- If there is a liquid retention in the filter reduce the amount of starting material by half.

2.24 Centrifuge at  600 x g, 4°C, 00:05:00 .

5m



2.25 Pour out the supernatant and resuspend the pellet in  200 μ L PBS with 5% BSA that will be overlaid on top of Buffer B (containing 0.2 U/ μ L RNasin Plus Ribonuclease Inhibitor) in the next step.

**Note**

The pellet may not be obvious as these are isolated nuclei.

2.26 Add  1 mL cold Buffer B (containing 0.2 U/ μ L RNasin Plus Ribonuclease Inhibitor) to a 1.5 ml Eppendorf tube.

**Note**

Remove bubbles if present.

2.27 Carefully overlay the  200 μ L nuclear suspension from  on top of Buffer B by slowly expelling the nuclear suspension against the wall of the tube.

2.28 Centrifuge the tube at  1000 x g, 4°C, 00:10:00 .

10m



Note

- After centrifugation, cellular debris, oil, and myelin will stay on the top (white-milky layer). The purified nuclei are found in the pellet.
- The nuclei pellet may not be visible. This depends on the brain region used.
- Extending the centrifugation time to a total of 00:15:00 - 00:20:00 may be beneficial to increase nuclei yield.

- 2.29 Carefully remove the milky layer by withdrawing it into a 1 ml pipette tip and discard the rest of the supernatant.
- 2.30 Pour out the remaining Buffer B, leaving 50 μ L in the bottom of the ultracentrifuge tube (as it contains the nuclear fraction).

Nuclei immunostaining

30m

- 3 Prepare Blocking Buffer: 0.8% BSA + 0.2 U/ μ L RNasin in 10X PBS, pH 7.4 (1x), e.g. for 2 mL :

A	B	C
Solution	Volume	Final
dH ₂ O (DNase/RNase-free)	1.470 μ	
10X PBS (RNase-free) pH 7.4	200 μ l	1x
5% BSA (freshly made)	320 μ l	
Vortex briefly to mix		
RNasin Plus RNase inhibitor (40 U/ μ l)	10 μ l	

Mix gently by inverting the tube 5-10 times and store it on ice until use.

**Note****Critical Notes:**

- When discarding the supernatants after each centrifugation step, always leave the last  50 μL at the bottom.
- After each centrifugation of this part of the protocol, resuspend in the same volume as in the prior step to keep the final volume consistent between the steps, but on the last centrifugation step of this section increase the volume to have at least  100 μL in each tube. As an example, after the final centrifugation resuspend, the nuclei treated with the conjugated antibodies in  500 μL and the immunostaining control nuclei in  200 μL .

- 4 Resuspend the pellet in  500 μL (or volume of choice depending on the initial tissue input) of cold Blocking Buffer by pipetting up and down gently 5 times. 

Note

Be sure to rinse the wall of the tube to collect all nuclei.

- 5 Separate the samples into different tubes for nuclei with antibodies and negative control samples, e.g.: 

A. Nuclei with PI and Olig2-AF647s:  500 μL .

B. Nuclei with PI and Isotype Control (IgG-AF647):  50 μL . Then supplement with Blocking Buffer to have final volume  200 μL .

C. Nuclei with PI, but no AF647:  50 μL . Then supplement with Blocking Buffer to have final volume  200 μL .

D. Nuclei without PI or AF647:  50 μL . Then supplement with Blocking Buffer to have final volume  200 μL .

- 6 Incubate the nuclei in Blocking Buffer for  00:30:00 @  4 °C on a plate shaker (tubes coved in parafilm, placed inside a falcon tube and the falcon tube on a plate shaker in the fridge).  

30m



7 Add antibodies directly to the nuclei in Blocking Buffer.

8 Incubate all samples for at least 00:30:00 @ 4 °C on a plate shaker (tubes coved in parafilm and foil, placed inside a falcon tube and the falcon tube on a plate shaker in the fridge).

30m



Note

During this time, place the bottle containing Cell Buffer from BioStryB kit at @ 4 °C for 00:30:00 - 01:00:00 .

9 Pellet nuclei at 800 x g, 4°C, 00:10:00 .

10m



Note

The pellet may not be clearly visible, and this depends on the brain region and the quantity of the nuclei.

10 Carefully discard supernatant leaving ~ 50 µL of buffer above the pellet.

11 Resuspended in pre-chilled Blocking Buffer (same volume as before) by gently pipetting up and down 5 times.



12 Re-pellet nuclei at 800 x g, 4°C, 00:10:00 and discard the supernatant.

10m



13 Wash nuclei again with Blocking Buffer.



14 Pellet nuclei at 800 x g, 4°C, 00:10:00 and carefully discard the supernatant.

10m



**Note****Critical Notes:**

- During this time prepare ice-cold Blocking Buffer with PI (final 1 µg/ml, e.g.  1998 µL Blocking Buffer +  2 µL PI = 2000 µl
- Prepare 96-well low bind plates for sorting by placing  3 µL of the thawed Cell Buffer in each well of the plate, seal the plates carefully and keep at  4 °C until use.

- 15 Resuspend nuclei to sort in  800 µL and the control nuclei in  200 µL Blocking Buffer with PI (or volume of interest depending on the input tissue).

Note

- BioStryB recommends using PI instead of DAPI for nuclear staining, as DAPI interferes with the ResolveOME chemistry in the downstream steps.
- Do not wash the nuclei after the addition of the PI staining solution.

- 16 Re-label clean 1.5 ml low bind Eppendorf tubes.

- 17 Filter the nuclei using Flowmi Cell Strainers and place the filtered nuclei in the pre-labelled clean 1.5 mL low binding Eppendorf tubes.

- 18 Transfer the nuclei to the sorting facility  On ice and perform the sorting as soon as possible.

**Note**

In our case, we use BD FACSaria Fusion Cell Sorter instrument at the UCL Cancer Institute Flow Cytometry facility in London.

Nuclei sorting (FANS)

16m



- 19 Before single-nucleus sorting, use Accudrop beads for a test sort to evaluate the position of the plate and to ensure the sorted cells will be deposited into each well accurately in the middle.
- 20 **Optional:** As an extra layer of assessment for accurate sorting, assess plate positioning with colorimetric method. 

Adapted from: Rodrigues OR, Monard S. A rapid method to verify single-cell deposition setup for cell sorters. Cytometry A. 2016 Jun;89(6):594-600. doi: [10.1002/cyto.a.22865](https://doi.org/10.1002/cyto.a.22865). PubMed PMID: 27144818

- 20.1 Add  1 mL of dH₂O into the vial of powder HRP, dissolve (this stock is 10x concentrated as compared to working solution). 

- 20.2 Make a working solution ( 1 mg/ml), e.g. For  2 mL : 200µl stock HRP ( 10 mg/ml) +  1800 µL diH₂O + 2 drops of Accudrop beads. Store in the fridge. 

- 20.3 To run the test:

1. Aliquot  2 µL of TMB into each well of the test plate.
2. Sort a single bead into a whole plate (or wells needed).
3. Once the sort is completed, immediately seal the plate and centrifuge  500 x g, 00:01:00 .
4. Wait  00:05:00 -  00:10:00 and count the number of wells that have turned blue.  

16m

Note

Critical Note:

We aim for >90% success.

4. If successful deposition is achieved, proceed with sorting cells. If not successful, recalibrate the alignment and try again!

- 21 Acquire small amounts of nuclei from all samples and perform a batch analysis to assess the sample quality and identify the targeted populations for sorting.

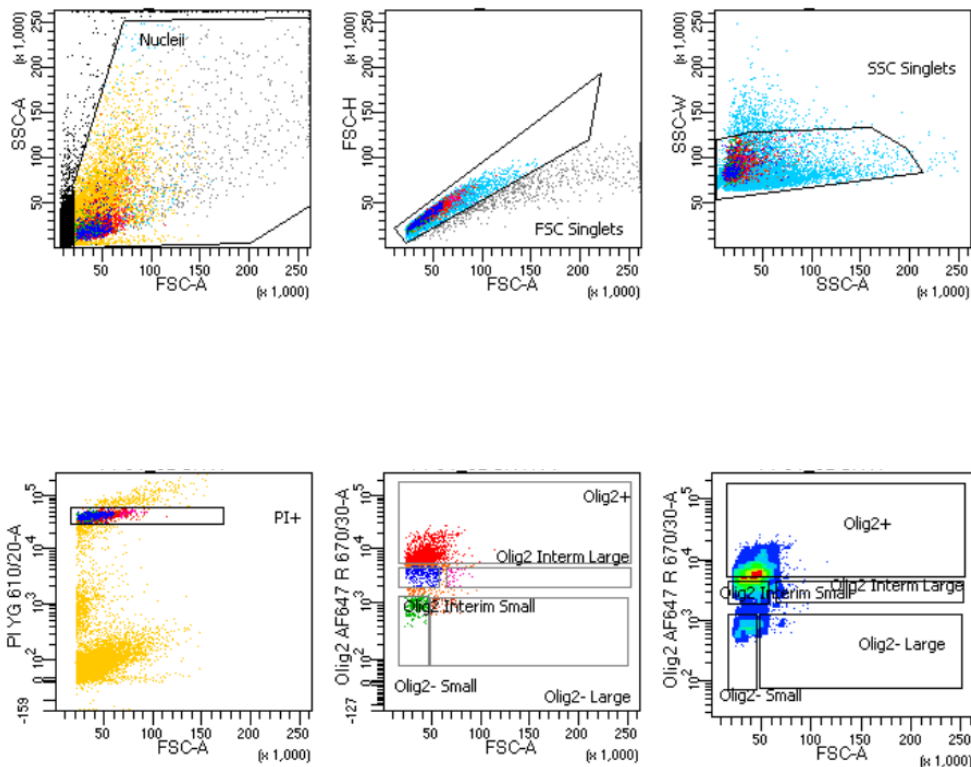
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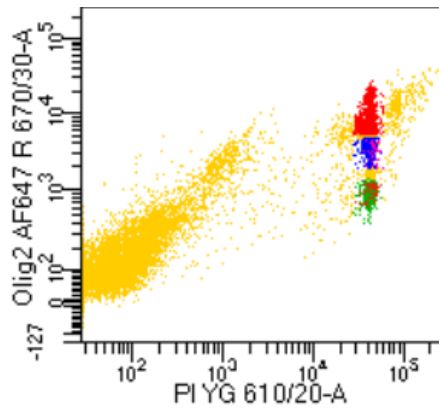
Use negative control samples as the threshold references.

- 22 Select gating parameters to isolate the singlets from the overall detected particles by selecting forward (FSC) and side scatter (SSC), FCS single cell gate and SSC single cell gate.
- 23 Select the human post-mortem nuclei by their PI expression.
- 24 From the nuclei population (PI+), apply further gating parameters based on the antibodies used.

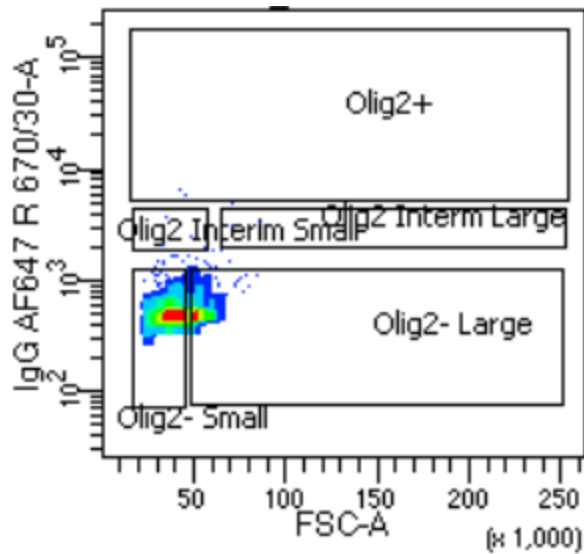
Figure 1. Example of the gating strategy of human post-mortem samples.

A. Nuclei with Olig2-AF647 and PI

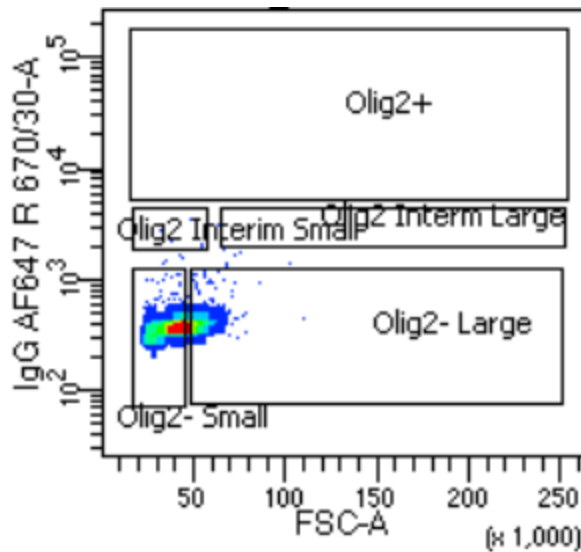




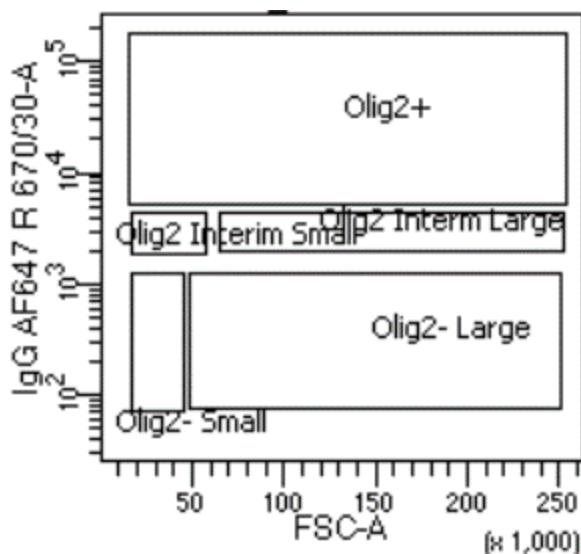
B. Nuclei with isotype AF647 control and PI



C. Nuclei with PI but no AF647



D. Nuclei without PI (no nuclei selection) or AF647



25 Centrifuge for 00:00:10 at low speed the collection plates to ensure reagent at bottom and place them On ice .

10s



26 Sort single-nuclei of interest into the centrifuged 96-well collection plates that are placed in a pre-cooled plate holder.



27 After sorting, seal immediately seal the plates with VersiCap Mat cap strips and pre-labelled plate sealers.

28 Immediately centrifuge briefly at  500 x g, 4°C, 00:01:00 .

1m



29 Place each plate in individually sealed bag.

30 Place immediately on dry-ice and transfer to the lab, if needed.

31 Stored the plate(s)  -70 °C until further use.



Single-nucleus DNA/RNA multiome by ResolveOME kit

32 Follow the ResolveOME kit according to BioSkryB guidelines (BioSkryB TAS-074.1).

Note

As the input material here are nuclei from human post-mortem brain samples instead of cells from cell culture, we suggest the following modifications:

- Dilute the DNA fraction in  30 µL Elution Buffer.
- Dilute the RNA fraction in  5 µL Elution Buffer.



Protocol references

This protocol was adapted from:

Protocols.io

- Ester Kalef-Ezra, Lucia Friscioni, Dominic Horner, Caoimhe Morley, George Morrow, Yanping Guo, Christos Proukakis 2024. Fluorescence-activated nuclei sorting (FANS) for single-cell Whole Genome Sequencing (scWGS). protocols.io <https://dx.doi.org/10.17504/protocols.io.81wgbzybygpk/v1>.
- Ester Kalef-Ezra, Dominic Horner, George Morrow, Vanda Knitthoffer, Andy Goldson, Iain Macaulay, Yanping Guo, Christos Proukakis 2024. Nuclei isolation, immunostaining, and Fluorescence-activated nuclei sorting (FANS) for Smart-Seq2. protocols.io <https://dx.doi.org/10.17504/protocols.io.j8nlk8wjxl5r/v1>.

Manufacturer protocols

- Nuclei isolation using Invent Bioscience kit BN-020 (Invent Biosciences).
- ResolveOME™ Whole Genome and Transcriptome Single-Cell Core Kit, TAS-074.1 | 06/2024 (BioSkryB)
- Best Practices: FACS Plate Alignment Considerations (BioSkryB).

Publication

- Perez-Rodriguez D, Kalyva M, Santucci C, Proukakis C. Somatic CNV Detection by Single-Cell Whole-Genome Sequencing in Postmortem Human Brain. *Methods Mol Biol.* 2023;2561:205-230. doi: [10.1007/978-1-0716-2655-9_11](https://doi.org/10.1007/978-1-0716-2655-9_11). PMID: 36399272.
- Rodrigues OR, Monard S. A rapid method to verify single-cell deposition setup for cell sorters. *Cytometry A.* 2016 Jun;89(6):594-600. doi: [10.1002/cyto.a.22865](https://doi.org/10.1002/cyto.a.22865). PubMed PMID: 27144818

Acknowledgements:

- We thank and BioSkryB for technical support, especially Alexandru Manole and Dr James Freimuller.
- This research was funded as a whole by Aligning Science Across Parkinson's [Grant ID: 000430] through the Michael J. Fox Foundation for Parkinson's Research (MJFF).