Single nucleus RNAseq, ATAC-seq, and Multiome data generation; Isolation of RNA and determination of RNA Integrity Number (RIN) from frozen human brain tissue

Sample processing:

This sample set includes tissues from Alzheimer's Disease spectrum donors. Postmortem brain tissues were processed using a standard procedure to produce 4mm thick tissue slabs, which were flash frozen in a bath of dry ice and isopentane. To remove a specific region of interest for downstream nuclear sequencing applications, these 4mm thick tissue slabs were removed from storage at –80C, briefly transferred to a –20C freezer to prevent tissue shattering during dissection, and then handled on a custom cold table to maintain tissues at –20C during dissection. Dissections were performed using dry ice cooled razor blades or scalpels to prevent warming of tissues. Dissected tissue samples were transferred to vacuum seal bags, sealed, and stored at –80C until the time of use. To isolate nuclei, tissue blocks were removed from the –80C and placed directly into pre-cooled Dounce homogenizers. Tissue homogenization and nuclei extraction was done following the standard procedure outlined in the protocol linked below. Briefly, after homogenization, isolated nuclei were stained with a primary antibody against NeuN to label neuronal nuclei. Nuclei samples were analyzed using a BD FACS Aria flow cytometer and nuclei were sorted using a standard gating strategy to exclude multiplets. A defined mixture of neuronal (70%) and non-neuronal (30%) nuclei was sorted for each sample. Nuclei isolated for 10x V3.1 single nucleus RNA-sequencing were concentrated by centrifugation after FACS and were frozen and stored at –80C until later chip loading. Nuclei isolated for 10xATACv1.1 or 10x Multiome were concentrated by centrifugation after FACS and were immediately processed for chip loading.

https://www.protocols.io/view/isolation-of-nuclei-from-adult-human-brain-tissue-ewov149p7vr2/v2

Method 1: 10xV3.1

10x processing:

https://www.protocols.io/view/10xv3-1-genomics-sample-processing-dm6gpwd8jlzp/v2

10xV3.1 guidelines: Chromium Next GEM Single Cell 3' Reagents Kits v3.1 User Guide Rev C 191015

10xV3.1 chip loading was performed as per 10x guidelines. Nuclei concentration was calculated either manually or using the NC3000 NucleoCounter. For most loads, 16,000 total nuclei were loaded into each port, typically 4-8 ports per chip, with an expected capture count of ~10,000. As per manufacturer protocol, total volume of

nuclei was brought up to 41.6ul with Nuclease-free water. Regular (non wide-bore) tips were used for nuclei and reagent pipetting. Care was taken to triturate the nuclei suspension 10 times using a P200 with a low-retention tip. Care was taken throughout the loading to not introduce air bubbles. Post RT GEM emulsions were stored at –20C for up to a week before cDNA amplification.

10xV3.1 cDNA amplification was performed manually as per 10x guidelines, with the exception of increasing the number of amplification cycles. For most AD samples, 12-15 cycles were used. Additional cycles were added for any sample that had a partial port clog during loading. Target yield of cDNA after amplification for library input was 100-500ng (10-50ng/ul, using 10ul for library input), but AD samples generally fell into the 50-150ng input range (5-15ng/ul, average 8ng/ul or 82ng input). Low retention tips were used for sample pipetting before PCR. Samples were quantitated using Picogreen Assay. Samples were visualized using Agilent's Fragment Analyzer and were passed if product above 400bp was detected (desired range 400-6000bp). Samples were failed if no product was detected, or if product was degraded and did not proceed to library construction or sequencing. Samples were stored at –20C until library construction.

10xV3.1 Library construction was performed manually as per 10x guidelines using Single Index Plate T Set A. 10ul of unnormalized cDNA was used as input into the library reaction and PCR cycles were determined based on this input. Generally, 11-16 cycles were used for library construction and final sample volume was 35ul. Samples were quantitated using Picogreen Assay. Samples were visualized and sized using Agilent's Fragment Analyzer and were passed if product size was between 400-550bp and concentration was above 10nM. Generally, samples yielded 500-1500ng, resulting in 50-150nM concentration (100nM average) and were on average 457bp. Samples were failed and not sequenced if no product was seen, if sizing was not in desired range, or if concentration was below 10nM. Samples were stored at –20C until normalization and pooling for sequencing.

10xV3.1 Sequencing was performed using Illumina's NovaSeqS4_v1.5 instrument and chemistry. Library samples were normalized individually to 10nM, then pooled to a target of 120,000 reads per nucleus (generally 10-12 libraries depending on cells/library). Sequencing was performed at either Northwest Genomics Center at University of Washington or at SeqMatic. Fastq files were received and aligned using Cell Ranger 3.0. Libraries were assessed for number of nuclei detected, median gene detection for the library, and % doublets. Libraries that had significantly fewer nuclei detected than expected or had low median gene detection (<2000) were failed.

Method 2: 10xATACv1.1

10x processing: https://www.protocols.io/view/10x-atac-genomics-sample-processing-14egn7ndmv5d/v1

10x guidelines: CG000209_Chromium_NextGEM_SingleCell_ATAC_ReagentKits v1.1_UserGuide_RevF.pdf

10xATACv1.1 chip loading was performed as per 10x guidelines. Nuclei concentration was calculated either manually or using the NC3000 NucleoCounter. For most loads, 16,000 total nuclei were loaded into each port, typically 2-4 ports per chip, with an expected capture count of ~10,000. As per manufacturer protocol, total volume of nuclei was brought up to 5.0ul with 1x Nuclei Buffer. Low retention tips were used for pipetting sample. Care was taken to triturate the nuclei suspension 10 times using a P200 with a low-retention tip. Transposition reaction was performed at 37C for 1hr then immediately loaded onto the chip according to 10x guidelines. Care was taken throughout the loading to not introduce air bubbles. Samples were stored at –20C until library construction.

10xATACv1.1 Library construction was performed manually as per 10x guidelines using Single Index Plate N Set A. Following 10x guidelines, 40ul of sample was used as input into the library reaction and PCR cycles were determined based on expected Nuclei recovery. Generally, 9 cycles were used for library construction and final sample volume was 20ul. Samples were quantitated using Picogreen Assay. Samples were visualized using Agilent's Fragment Analyzer and were passed if morphology of nucleosome free, mononucleosome, dinucleosome, and multinucleated peaks were as expected according to 10x guidelines, and concentration was above 10nM. Generally, samples yielded 1000-2000ng, resulting in 100-250nM concentration. Samples were failed and not sequenced if no product was seen, if peak morphology was aberrant, or if concentration was below 10nM.

10x ATACv1.1 Sequencing was performed using NovaSeqS4_v1.5 instrument and chemistry. Library samples were normalized individually to 10nM, then pooled to a target of 85,000-120,000 reads per nucleus (generally 10-16 libraries depending on cells/library). Sequencing was performed at either Northwest Genomics Center at University of Washington or at SeqMatic. Fastq files were received and aligned using Cell Ranger ATAC2.0. Libraries were assessed for number of nuclei detected and TSA enrichment.

Method 3: 10xMultiome

10xMultiome processing: https://www.protocols.io/view/10x-multiome-sample-processing-bp2l61mqrvqe/v1

10x guidelines: CG000338_ChromiumNextGEM_Multiome_ATAC_GEX_User Guide_RevE.pdf

10xMultiome chip loading was performed as per 10x guidelines. Nuclei concentration was calculated either manually or using the NC3000 NucleoCounter. For most loads,

16,000 total nuclei were loaded into each port, typically 2-4 ports per chip, with an expected capture count of ~10,000. As per manufacturer protocol, total volume of nuclei was brought up to 5.0ul with 1x Nuclei Buffer. Low retention tips were used for pipetting sample. Care was taken to triturate the nuclei suspension 10 times using a P200 with a low-retention tip. Transposition reaction was performed at 37C for 1hr then immediately loaded onto the chip according to 10x guidelines. Care was taken throughout the loading to not introduce air bubbles. Samples were stored after quenching at –80C until Pre-PCR.

10xMultiome Pre-PCR was done according to 10x guidelines. Low retention tips were used throughout the process whenever pipetting sample. A standard 7 cycles for PCR was used on all samples regardless of nuclei load count. Typically, samples were processed through cDNA amplification (RNASeq) and ATAC Library the same day as Pre-PCR.

10xMultiome RNASeq cDNA amplification was done according to 10x guidelines. Most samples were amplified with 8 cycles of PCR, resulting in an average of 16ng/ul (for an average input of 160ng into library construction). Low retention tips were used for sample pipetting before PCR. Samples were quantitated using Picogreen Assay. Samples were visualized using Agilent's Fragment Analyzer and were passed if product above 400bp was detected (desired range 400-6000bp). Samples were failed if no product was detected, or if product was degraded and did not proceed to library construction or sequencing. Samples were stored at –20C until library construction.

10xMultiome RNASeq library construction was done according to 10x guidelines, using Dual Index plate TT set A. 10ul of unnormalized cDNA was used as input into the library reaction and PCR cycles were determined based on this input. Generally, 10-14 cycles were used for library construction and final sample volume was 35ul. Samples were quantitated using Picogreen Assay. Samples were visualized and sized using Agilent's Fragment Analyzer and were passed if product size was between 400-550bp and concentration was above 10nM. Generally, samples yielded 500-1500ng, resulting in 60-300nM concentration (140nM average) and were on average 470bp. Samples were failed and not sequenced if no product was seen, if sizing was not in desired range, or if concentration was below 10nM. Samples were stored at –20C until normalization and pooling for sequencing.

10xMultiome ATAC library construction was done according to 10x guidelines, using Single Index Plate N Set A. Following 10x guidelines, 40ul of sample was used as input into the library reaction and PCR cycles were determined based on expected Nuclei recovery. Generally, 7 cycles were used for library construction and final sample volume was 20ul. Samples were quantitated using Picogreen Assay. Samples were visualized using Agilent's Fragment Analyzer and were passed if morphology of nucleosome free, mononucleosome, dinucleosome, and multinucleated peaks were as expected according to 10x guidelines, and concentration was above 10nM. Generally, samples yielded 1000-2000ng, resulting in 80-150nM concentration (average 111nM).

Samples were failed and not sequenced if no product was seen, if peak morphology was aberrant, or if concentration was below 10nM.

10xMultiome RNASeq Sequencing was performed using Illumina's NovaSeqS4_v1.5 instrument and chemistry. Library samples were normalized individually to 10nM, then pooled to a target of 120,000 reads per nucleus (generally 10-12 libraries depending on cells/library). Sequencing was performed at either Northwest Genomics Center at University of Washington or at SeqMatic. Fastq files were received and aligned using ARC2.0. Libraries were assessed for number of nuclei detected, median gene detection for the library, and % doublets. Libraries that had significantly fewer nuclei detected than expected or had low median gene detection (<2000) were failed.

10xMultiome ATAC Sequencing was performed using NovaSeqS4_v1.5 instrument and chemistry. Library samples were normalized individually to 10nM, then pooled to a target of 85,000-120,000 reads per nucleus (generally 10-16 libraries depending on cells/library). Sequencing was performed at either Northwest Genomics Center at University of Washington or at SeqMatic. Fastq files were received and aligned using ARC2.0. Libraries were assessed for number of nuclei detected and TSA enrichment.

Isolation of RNA and determination of RNA Integrity Number (RIN) from frozen human brain tissue

To assess RNA quality, three tissue samples (~50mg each) were collected from the tissue slab corresponding to the frontal pole of each donor brain. Tissue slabs were removed from the -80C freezer and placed in a -20C freezer for at least 30 minutes to avoid tissue shattering during dissection. Tissue slabs were then handled on a custom cold table maintained at -20C for the duration of the dissection procedure. Tissue samples were collected from three different regions of the tissue slab to assess within-slab variability in RNA quality. Dissected tissues were stored in 1.5ml microcentrifuge tubes on dry ice or in the -80C until the time of RNA isolation.

Tissue samples were homogenized using a sterile Takara BioMasher (Takara, 9791A). RNA isolation was performed using either a Qiagen RNeasy Plus Mini Kit (Qiagen, 74134) or a Takara NucleoSpin RNA Plus kit (Takara, 740984) following the manufacturer's protocol. RNA integrity (RIN) values for each sample were determined using the Agilent RNA 6000 Nano chip kit (Agilent, 5067-1511) and an Agilent Bioanalyzer 2100 instrument following the manufacturer's protocol.