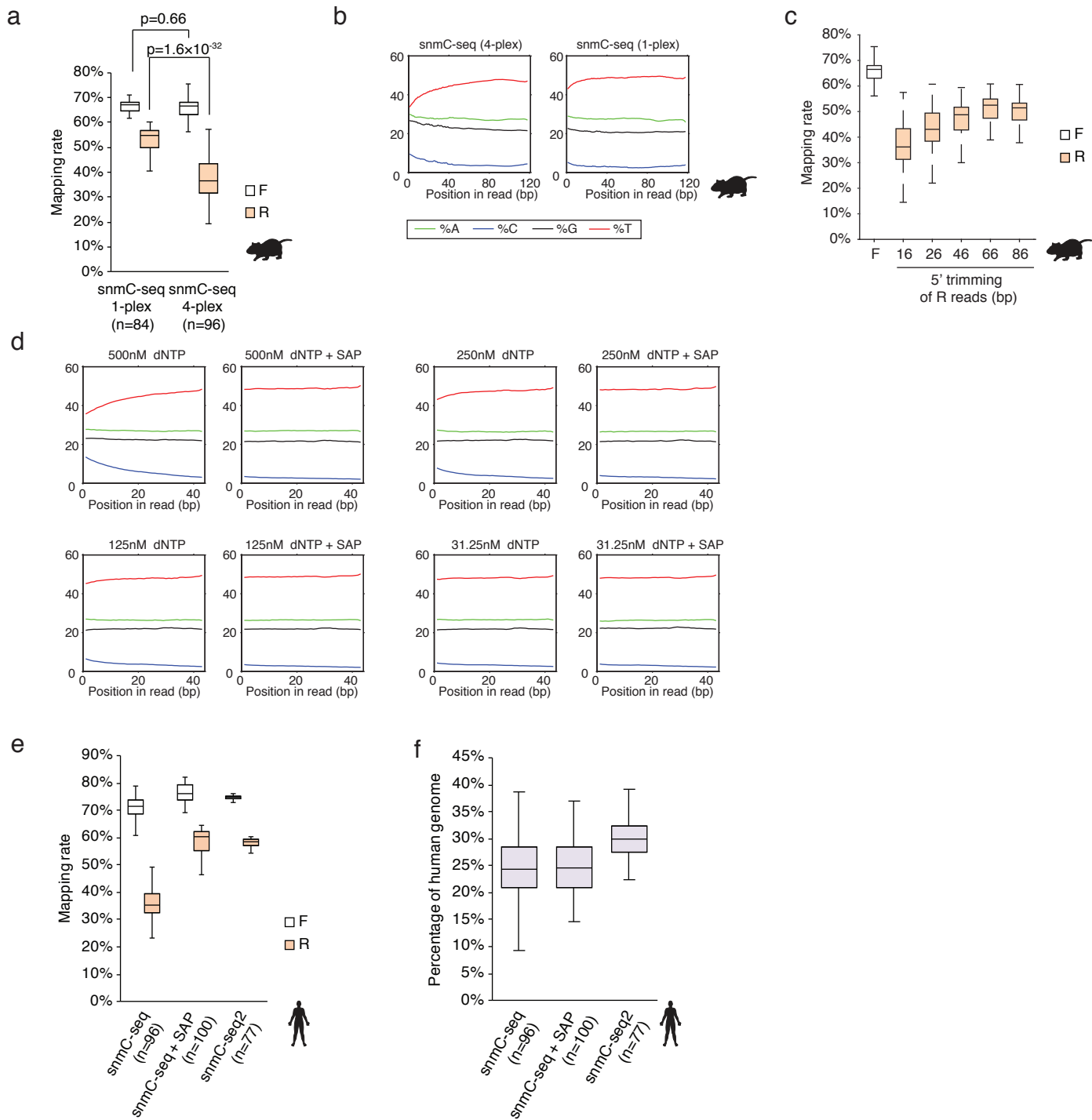


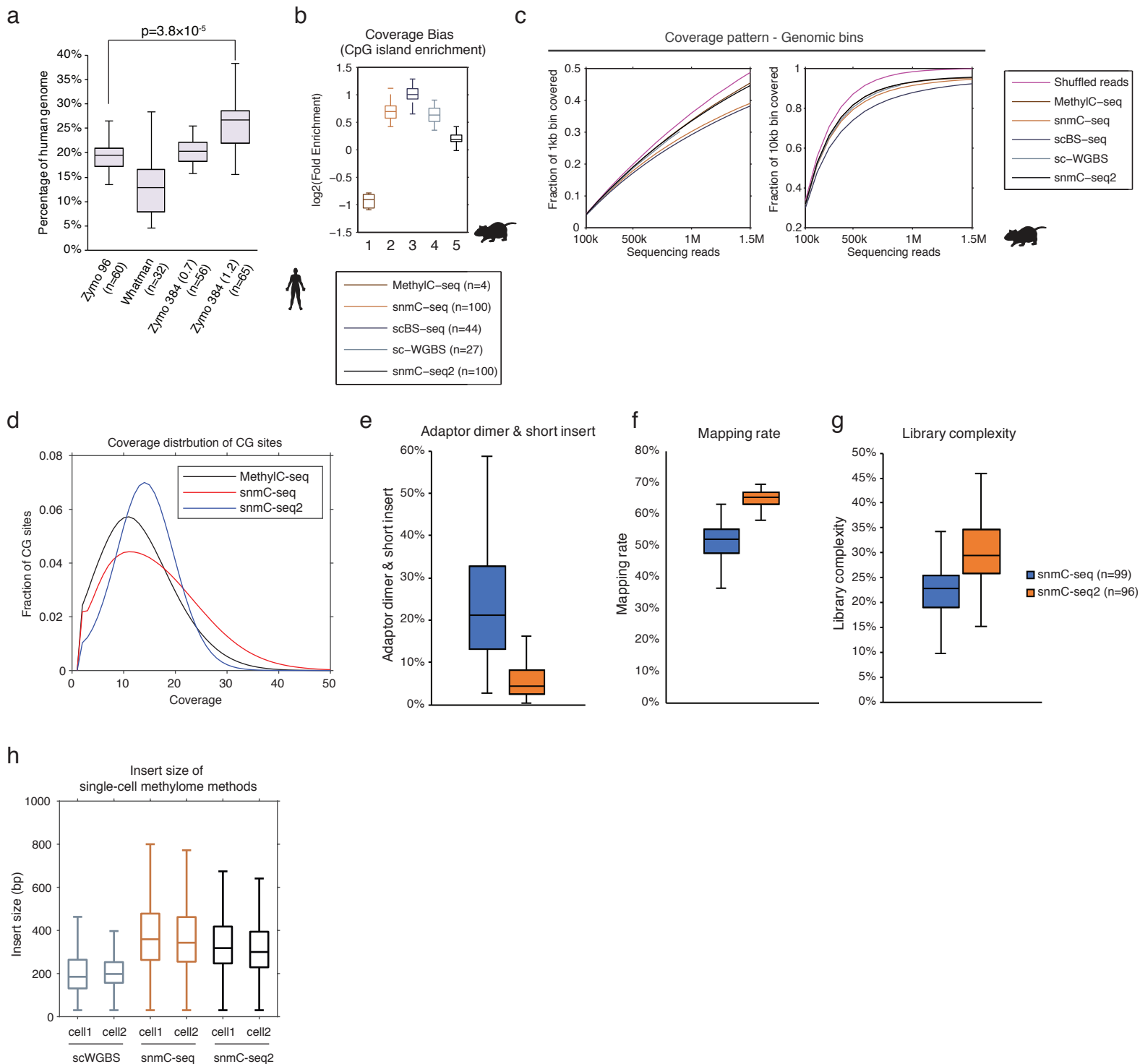
Supplementary Information for

Robust single-cell DNA methylome profiling with snmC-seq2

Luo et al.

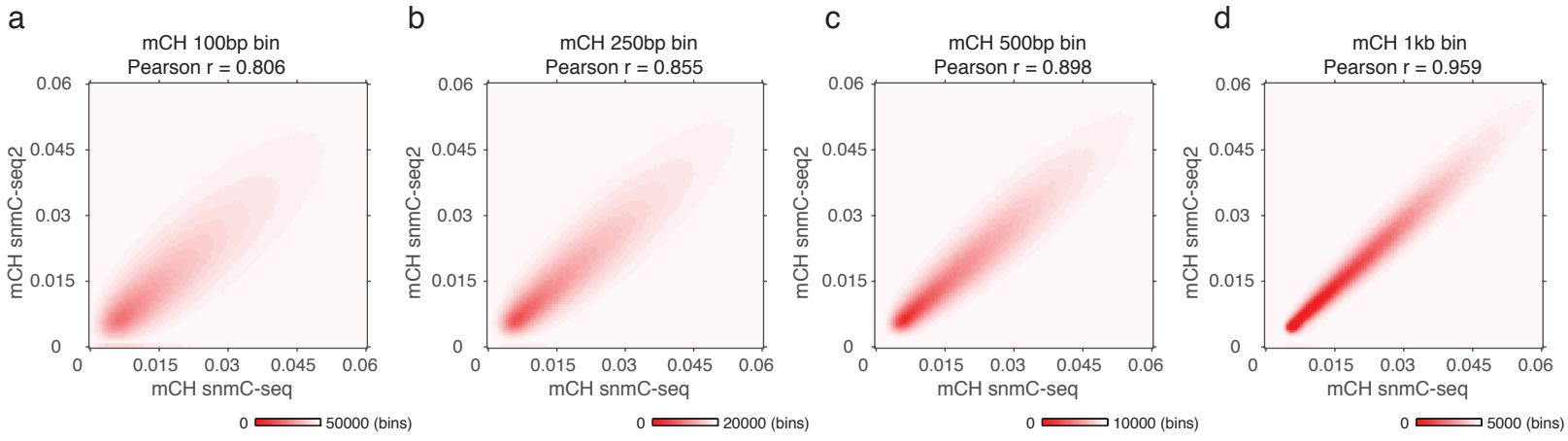


Supplementary Figure 1. (a) Multiplexed (4-plex) smnC-seq shows reduced reverse reads (R) mapping compared to non-multiplexed smnC-seq (1-plex). (b) Multiplexed smnC-seq shows aberrant reverse reads base composition. (c) Trimming of 5' end increases the mapping rate of reverse reads (n=94). (d) Reducing dNTP concentration in random-primed DNA synthesis or treatment with Shrimp Alkaline Phosphatase (SAP) suppresses the aberrant reverse reads base composition. (e) SAP treatment increases the mapping rate of reverse reads for single-cell methylomes generated from human cortical tissues. (f) Comparison of library complexity for libraries generated with smnC-seq, smnC-seq with SAP treatment (snmC-seq + SAP) and smnC-seq2 using single nuclei isolated from human cortical tissues. The elements of all box-plots are defined as following - center line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range.

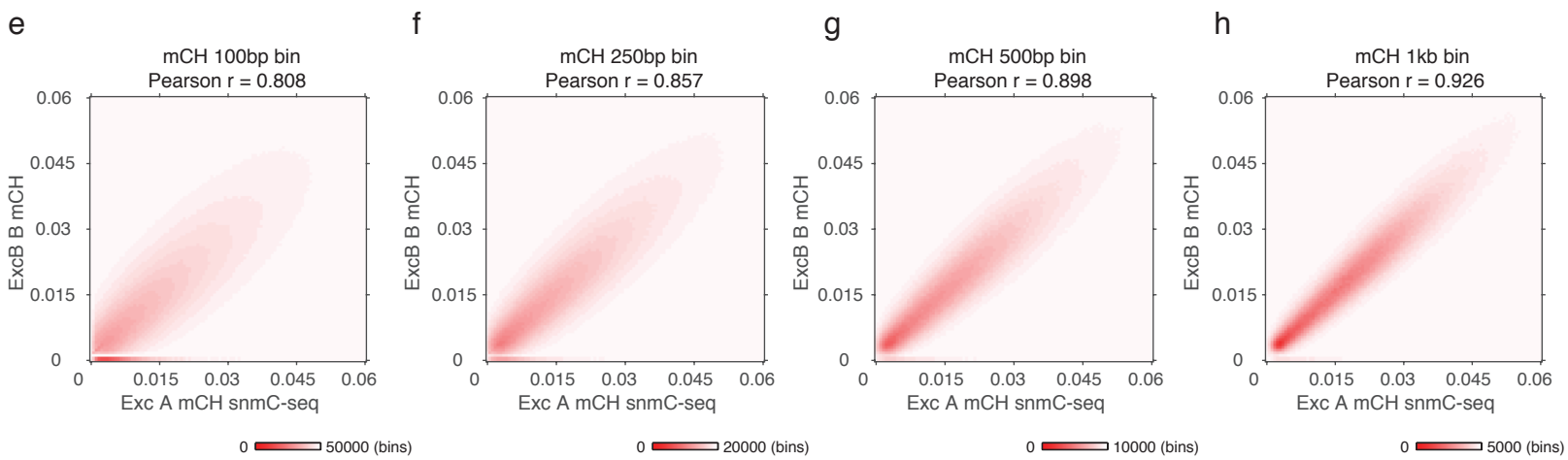


Supplementary Figure 2. (a) Library complexity for single cell methylomes generated using different DNA binding column plates. Zymo 96 - Zymo-Spin I-96 Binding Plates (Zymo C2004). Whatman - 384 Wells Unifilter Microplate (Whatman 7700-2110). Zymo 384 (0.7) - 384-well DNA binding column plates with glass fiber pore size  $0.7 \mu\text{m}$ . Zymo 384 (1.2) - 384-well DNA binding column plates with glass fiber pore size  $1.2 \mu\text{m}$ .

(b) Enrichment of CpG islands in DNA methylome generated by traditional MethylC-seq1, snmC-seq2, scBS-seq3, sc-WGBS4 and snmC-seq2. (c) Fraction of 1kb and 10kb non-overlapping bins covered by single cell methylome reads as a function of the sequencing depth. (d) Coverage distribution of genome-wide CG sites shows snmC-seq2 has more uniform coverage than MethylC-seq and snmC-seq. (e-g) Comparison of the quality of methylome libraries generated by snmC-seq, snmC-seq2. (h) Library insert size distribution for scWGBS, snmC-seq and snmC-seq2. The elements of all box-plots are defined as following - center line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range.



Comaprison of biological replicates of excitatory neuron (Camk2a+) methylomes



Supplementary Figure 3. (a-d) Correlation of CH methylation by snmC-seq2 and snmC-seq were compared for 100bp, 250bp, 500bp and 1kb genomic bins. (e-h) Correlation of biological replicates of excitatory neuron methylomes (Exc) were compared for 100bp, 250bp, 500bp and 1kb genomic bins.

## Supplementary Methods - snmC-seq2 protocol

Protocol to bisulfite convert and generate 3,072 single-cell methylome libraries in one experiment (8x 384-well plates) from mouse or human frozen brain tissue.

### Materials

#### General reagents

- FACS sorted single-nuclei into 384 well PCR plate
- HyClone™ HyPure™ Molecular Biology Grade (MB) Water (GE Life Sci. cat. no. SH30538.03)
- 200-Proof (100%) Ethanol (Koptec cat. no. V1001)

#### Collection of single nuclei by Fluorescence-activated Cell Sorting (FACS)

- M-Digestion Buffer (Zymo cat. no. D5021-9)
- Proteinase K (Zymo cat. no. D3001-2-D)
- Proteinase K Storage Buffer (Zymo cat. no. D3001-2-B)
- Unmethylated Lambda DNA (Promega cat. no. D1521, 100pg/μL)

#### Bisulfite conversion

- CT Conversion Reagent (Zymo cat. no. D5003-1)
- M-Solubilization Buffer (Zymo cat. no. D5021-7)
- M-Dilution Buffer-Gold (Zymo cat. no. D5006-2)
- M-Reaction Buffer (Zymo cat. no. D5021-8)
- M-Binding Buffer (Zymo cat. no. D5021-7)
- M-Wash Buffer (Zymo cat. no. D5040-4)
- M-Desulphonation Buffer (Zymo cat. no. D5040-5)
- M-Elution Buffer (Zymo cat. no. D5007-6)

#### Random primers

- HPLC purified Random primers were ordered from Integrated DNA Technologies (IDT)  
P5L\_AD002\_H  
/5SpC3/TTCCCTACACGACGCTCTTCCGATCTCGATGT(H1:33340033)(H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)  
P5L\_AD006\_H  
/5SpC3/TTCCCTACACGACGCTCTTCCGATCTGCCAAT(H1:33340033)(H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)  
P5L\_AD008\_H  
/5SpC3/TTCCCTACACGACGCTCTTCCGATCTACTTGA(H1:33340033)(H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

P5L\_AD010\_H

/5SpC3/TTCCCTACACGACGCTCTTCCGATCTTAGCTT(H1:33340033)(  
H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

P5L\_AD001\_H

/5SpC3/TTCCCTACACGACGCTCTTCCGATCTATCACG(H1:33340033)(  
H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

P5L\_AD004\_H

/5SpC3/TTCCCTACACGACGCTCTTCCGATCTTGACCA(H1:33340033)(  
H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

P5L\_AD007\_H

/5SpC3/TTCCCTACACGACGCTCTTCCGATCTCAGATC(H1:33340033)(  
H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

P5L\_AD012\_H

/5SpC3/TTCCCTACACGACGCTCTTCCGATCTTGTGA(H1:33340033)(  
H1)(H1)(H1)(H1)(H1)(H1)(H1)(H1)

#### Sera-Mag Solid Phase Reversible Immobilization (SPRI) beads

- Sera-Mag SpeedBeads Magnetic Carboxylate Modified (GE Healthcare cat. no. 45152105050250)
- Poly(ethylene glycol) PEG 8000 (Sigma cat no. 89510-250G-F)
- TE buffer pH=8.0 (Ambion cat. no. AM9858)
- 5M NaCl
- 1M Tris-HCl pH=8.0
- 0.5M EDTA pH=8.0
- AMPure XP beads (Beckman Coulter cat no. A63881)
- 100 bp DNA ladder (New England Biolabs cat no. N3231L)

#### snmC-seq2 library preparation

- Blue Buffer (10x) (Enzymatics cat. no. P7010-HC-L)
- Klenow Exo- (50U/ $\mu$ L) (Enzymatics cat. no. P7010-HC-L)
- Deoxynucleotide Solution Mix (10mM each dNTP) (NEB cat. no. N0447L)
- Exonuclease I (20U/ $\mu$ L) (Enzymatics cat. no. X8010L)
- Shrimp Alkaline Phosphatase (rSAP) (1U/ $\mu$ L) (NEB cat. no. M0371L)
- Sera-Mag SPRI beads
- M-Elution Buffer (Zymo cat. no. 5007-6)
- EB buffer (Qiagen cat. no. 19086)
- Accel-NGS<sup>®</sup> Adaptase<sup>™</sup> Module (Swift Bio cat. no. 330384)
- P5 Indexing Primer (IDT custom DNA oligo, standard desalted)
- P7 Indexing Primer (IDT custom DNA oligo, standard desalted)

- KAPA HiFi HS RM (Kapa cat. no. KK2602)
- Qubit dsDNA BR Assay Kit (Thermo Fisher cat. no. Q32850)

### Equipment

- 384-Well Hardshell PCR Plate Clear, 20 Pcs/pk (Thermo Fisher cat. no. 4483285)
- 96-Well Hardshell PCR Plate GPLE, 20 Pcs/pk (Thermo Fisher cat. no. 4483348)
- Zymo-Spin 384 Well Plate, 2 pack (Zymo cat. no. C2012)
- 2.0mL 96-well Deep Well Polypropylene Plate, Sterilized (USA-SCI. cat. no. 1896-2110)
- Reservoir Single Well 96 Bottom High Profile, Clear (Axygen cat. no. RES-SW96-HP)
- Reservoir Single Well 96 Bottom Low Profile, Clear (Axygen cat. no. RES-SW96-LP)
- 15mL Centrifuge Tubes (Olympus cat. no. 28-103)
- 50mL Centrifuge Tubes (Olympus cat. no. 28-106)
- 1.5µL Eppendorf Tubes (Thermo Fisher cat. no. 02-681-320)
- 300µL 8-Strip Tubes
- Microamp Clear Adhesive Film, 100pc (Thermo Fisher cat. no. 4306311)
- Microporous Film, -20C to 80C, 50p, sterile (USA-SCI. cat. no. 2920-1010)
- Speedball Deluxe Soft Rubber Brayer, 4 inches (Statesville N.C.)
- 37°C Incubator
- 384-well and 96-well Compatible Thermocycler
- DynaMag™-96 Side Magnet (Thermo Fisher cat. no. 12331D)
- DynaMag™-2 Magnet (Thermo Fisher cat. no. 12321D)
- Allegra 25R Centrifuge with S5700 2x96 Swinging Bucket Rotor (Beckman Coulter cat. no. 368954)
  - All centrifuge steps at 5000xg for 5 minutes at room temperature unless otherwise specified
- (Optional) Axxygen DITI 50µL STE.FIL. Robotics Tips (Thermo Fisher cat. no. EVF-50-RS)
- (Optional) Axxygen DITI 180µL STE.FIL. Robotics Tips (Thermo Fisher cat. no. EVF-180-RS)
- (Optional) TECAN Freedom Evo 100 Base Unit with MultiChannel™ Arm MCA 96
- (Optional/Required for TECAN use) Collection Spacer/Adapter, Type 2, 26.5mm, Te-VacS (Tecan US Inc. cat. no. 10760662)

### **Reagent Setup**

### Ethanol, 80%

To 40mL of 200-proof Ethanol, add 10 $\mu$ L MB water in a 50mL tube. Keep solution sealed when not in use. Prepare ~6 tubes fresh before each library preparation.

### Digestion Buffer Plates (for FACS sorting)

In a 50mL tube, combine 15mL M-Digestion Buffer (incubate for 10 minutes at 37°C to dissolve precipitate) with 14mL MB water. Resuspend one tube Proteinase K with 1mL Proteinase K Resuspension Buffer and add 1mL to the digestion buffer mix. Add 10 $\mu$ L  $\lambda$  DNA to aid determination of bisulfite conversion efficiency. Vortex to mix. Aliquot 2 $\mu$ L per well to 20 384-well plates to use for FACS sorting of stained nuclei (TECAN script ). Plates can be stored at 4C.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *2uL\_Digestion\_Buffer*.

### Sera-Mag Solid Phase Reversible Immobilization (SPRI) Beads

1. Mix Sera-Mag SpeedBeads and transfer 1mL to a 1.5ml tube.
2. Place SpeedBeads on a magnetic stand until clears and carefully remove the supernatant. Wash the beads twice with 1ml of TE. For each wash, remove the tube from the magnet and mix by inversions. Resuspend the washed beads in 1ml of TE.
3. Add 9g of PEG 8000 to a new 50ml sterile conical tube.
4. Add 10ml of 5M NaCl to the 50ml tube.
5. Add 500 $\mu$ l of 1M Tris-HCl pH=8.0 and 100ul of 0.5M EDTA pH=8.0 to the 50ml tube.
6. Mix until all dissolves into solution.
7. Add 1ml of resuspended SpeedBeads to the 50ml tube and fill the volume with MB water.
8. Test against AMPure XP beads using 100bp DNA ladder.

### CT Conversion Reagent

Add 7.9 mL M-Solubilization Buffer and 3 mL M-Dilution Buffer to a bottle of CT Conversion Reagent. Shake vigorously at room temperature to fully dissolve before adding 1.6 mL M-Reaction Buffer.

### M-Wash Buffer

Add 288mL 200-proof Ethanol to four bottles of M-Wash buffer. Invert to mix. Make fresh each time. Extra buffer can be stored at room temperature.



### Random Primer solution

35µL random primer stock (100µM) in 7mL M-Elution buffer (500nM final primer concentration). Aliquot to eight 96-well plates.

### Random Priming Master Mix

To have sufficient reagents for robotic preparation of 3,072 reactions, prepare 3600 reactions of Random Priming Master Mix in a 15mL tube and vortex to mix. Aliquot 180µL of master mix into each well of one 96-well plate. Keep sealed on ice until use. Do not store.

Reagent	Vol. per reaction	Vol. for 3,600 reactions
Blue Buffer (10x)	1 µL	3600 µL
Klenow exo- (50U/µL)	0.25 µL	900 µL
dNTP (10mM each)	0.5 µL	1800 µL
MB water	3.25 µL	11700 µL

### Exo/rSAP Master Mix

To have sufficient reagents for robotic preparation of 3,072 reactions, prepare 3600 reactions of Exo/SAP Master Mix in a 15mL tube and vortex to mix. Aliquot 53µL master mix to each well of one 96-well plate. Keep sealed on ice until use. Do not store.

Reagent	Vol. per reaction	Vol. for 3,600 reactions
Exonuclease 1 (20U/uL)	1 uL	3600 uL
rSAP (1U/uL)	0.5 uL	1800 uL

### Adaptase Master Mix (using Accel-NGS® Adaptase™ Module)

To have sufficient reagents for robotic preparation of 3,072 reactions, prepare 3600 reactions of Adaptase Master Mix in a 15mL tube and vortex to mix. Aliquot 48 uL master mix to each well of on 96-well plate. Keep sealed on ice until use. Do not store.

Reagent	Vol. per reaction	Vol. for 3,600 reactions
EB	4.25 uL	1912.5 uL
Buffer G1	2 uL	900 uL
Reagent G2	2 uL	900 uL
Reagent G3	1.25 uL	562.5 uL
Enzyme G4	0.5 uL	225 uL
Enzyme G5	0.5 uL	225 uL

### PCR Primer Mix

Sequences of indexing primers with unique dual barcodes are provided in Supplementary Table 2. Each PCR Primer Mix contains a P5L indexing primer (600 nM) and a P7L indexing primer (1  $\mu$ M).

### **Procedure**

#### Bisulfite Conversion

Timing ~ 5h

1. Add 15 $\mu$ L CT conversion reagent to each well of 384-well plate. Pipette up and down for 8 times to mix the sample.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *15uL\_Conversion\_Buffer*

2. Seal the plate with adhesive film and quick spin for 10s at 2,000 $\times$ g at room temperature. Place the plate in a thermocycler and run the following program:

98°C	8 min
64°C	3.5hrs
4°C	Hold

3. Load 80 $\mu$ L M-Binding buffer to each well of 384-Well DNA Binding Plate.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *80uL\_Binding\_Buffer*

4. Transfer bisulfite conversion reactions to Zymo-Spin 384 Well DNA Binding Plate. Pipette up and down for 8 times to mix the sample. Place the 384-Well DNA Binding Plate on a 2.0mL 96-well Deep Well Plate and centrifuge for 5 min at 5,000g. Discard the flow through by decanting.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *17uL\_Conversion\_Rxn\_To\_Binding\_Buffer*.

5. Add 100 $\mu$ L M-Wash Buffer to each well of 384-Well DNA Binding Plate. Centrifuge for 5 min at 5,000g and discard the flow through by decanting.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *100uL\_M\_Wash\_Buffer*.

6. Add 50 $\mu$ L M-Desulphonation Buffer to each well of 384-Well DNA Binding Plate. Incubate at room temperature for 15 min. Centrifuge for 5 min at 5,000g and discard the flow through by decanting.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *50uL\_Desulphonation\_Buffer*.

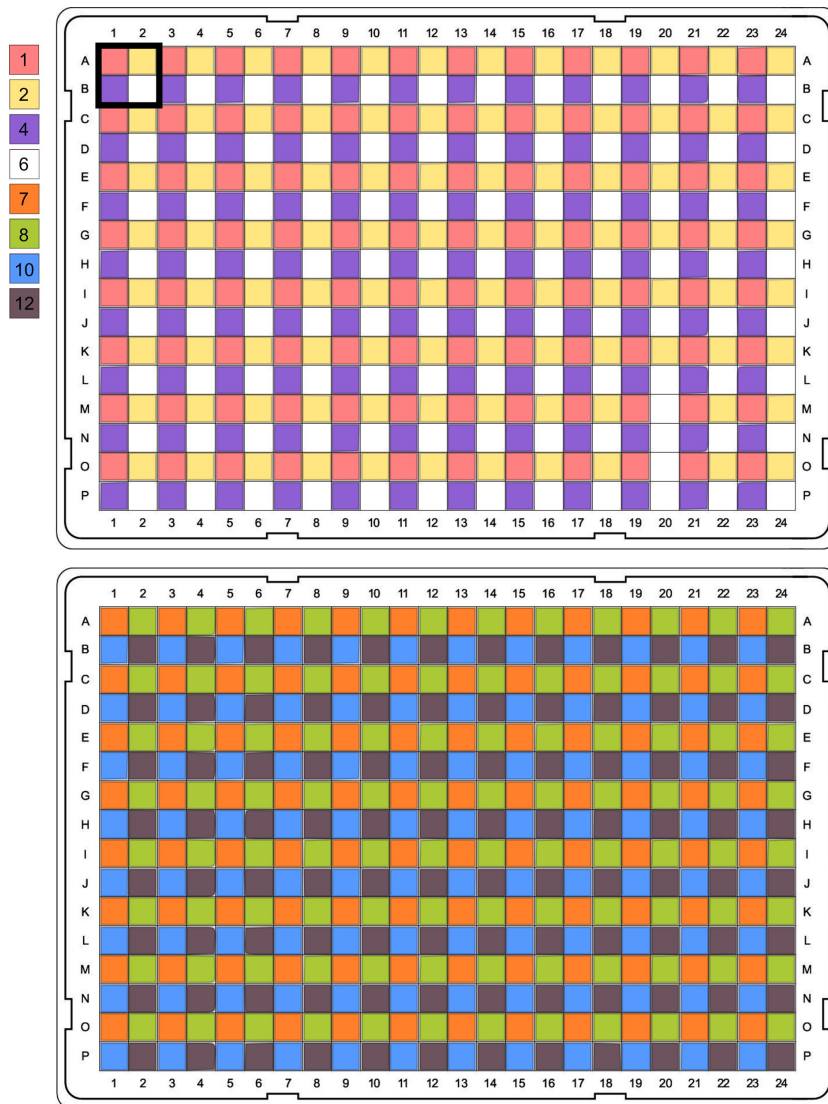
7. Add 100 $\mu$ L M-Wash Buffer to each well of 384-Well DNA Binding Plate. Centrifuge for 5 min at 5,000g and discard the flow through by decanting.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *100uL\_M\_Wash\_Buffer*.

8. Repeat step 7.

9. Place 384-Well DNA Binding Plate on new 384-well PCR plate. Add 7 $\mu$ L Random Primer Solution. Each quadrant of 384-well plate is barcoded with a distinct indexed random primer (Fig. 1). Every two 384-well plates receive a complete set of all eight indexed random primers. Incubate for 5 min at room temperature. Centrifuge for 5 min at 5,000g and discard the 384-Well DNA Binding Plate.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *7uL\_Primer\_Elution*.



**Figure 1.** Schematics of single-cell barcoding with indexed random primers. Using a TECAN Freedom Evo 100 with MultiChannel Arm MCA 96, each indexed random primer is added into a quadrant of 384-well DNA Binding Plate. The color of each quadrant indicates the random primer barcode.

10. Seal the plate with adhesive film and store at  $-20^{\circ}\text{C}$  for up to 1 week.

### Random-primed DNA synthesis

Timing ~2h

1. Denature the samples by placing 384-well PCR plate on a thermocycler and run the following program.

95°C            3 min

Immediately place the plate on ice for 2 minutes.

2. Add 5 $\mu$ L Random Priming Master Mix to each well of the 384-well PCR plate. Vortex and quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *5uL\_Random\_Priming\_Mix*.

3. Place the plate in a thermocycler and run the following program:

4°C	5 min
25°C	5 min
37°C	60 min
4°C	Hold

#### *Inactivation of free primers & dNTP*

4. Add 1.5 $\mu$ L Exo/rSAP Master Mix to each well of the 384-well PCR plate. Vortex to mix the samples and quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *1uL\_Exo\_rSAP\_MM*.

5. Place the plate in a thermocycler and run the following program

37°C	30 min
4°C	Hold

#### *Sample clean-up*

6. Add 73.6 $\mu$ L (0.8x) SPRI Beads to each well of four clean 96-well PCR plates.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_Add\_To\_Clean\_96well*.

7. Transfer samples from two 384-well plates to each 96-well plate. The eight quadrants (from two 384-well plates) barcoded with distinct indexed random primers are combined. Mix the samples by vortexing and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_Transfer\_Lib\_384well\_To\_96well*.

- Place 96-well plates on DynaMag™-96 Side Magnet, let stand until solution in wells is clear of beads (~5 minutes). Remove supernatant and wash beads 3x with 150µL fresh 80% EtOH. Remove all EtOH, remove plates from magnet, and let beads dry at room temperature. DO NOT overdry beads.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_2Plate\_150uL\_EtOH\_Wash*.

- Add 10µL EB buffer and resuspend beads by pipet. Vortex and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg. Place back on magnet and let stand until solution is clear of beads (~5 minutes).

- Remove 10uL supernatant to a clean 96-well PCR plate.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *10uL\_96Well\_Elution*.

### Adaptase reaction

- Denature the samples by placing 96-well plates on a thermocycler and run the following program.

95°C            3 min

Immediately place the plate on ice for 2 minutes.

- Add 10.5µL Adaptase Master Mix to each well of the 96-well PCR plate. Vortex and quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *10uL\_25uL\_Adaptase\_KAPA*. Enter 10.5uL for the volume transferred.

- Place the plate in a thermocycler and run the following program:

37°C            30 min  
95°C            2 min  
4°C              Hold

### Library amplification

14. Add 5 $\mu$ L PCR Primer Mix.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *5uL\_PCR\_Primer*.

15. Add 25 $\mu$ L 2x KAPA HiFi Mix. Vortex and quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *10uL\_25uL\_Adaptase\_KAPA*. Enter 25uL for the volume transferred.

16. Place the plate in a thermocycler and run the following program:

- a. 95°C            2 min
- b. 98°C            30s
- c. 98°C            15s
- d. 64°C            30s
- e. 72°C            2 min
- Go to step c 14 times
- f. 72°C            5 min
- g. 4°C             Hold

#### Library clean-up

17. *Sample cleanup*. Add 40 $\mu$ L (0.8x) SPRI Beads to each well of four 96-well PCR plates. Transfer contents of one 96-well plate to another to combine four 96-well plates to two 96-well plates. Vortex and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_Add\_Beads\_And\_Combine\_96well*.

18. Place 96-well plates on DynaMag™-96 Side Magnet, let stand until solution in wells is clear of beads (~5 minutes). Remove supernatant and wash beads 2x with 150 $\mu$ L fresh 80% EtOH. Remove all EtOH, remove plate from magnet and let beads dry at room temperature. DO NOT overdry beads.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_2Plate\_150uL\_EtOH\_Wash* (stop script after two wash steps).

19. Add 25 $\mu$ L EB and resuspend beads by pipet. Vortex and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg. Place back on magnet and let stand until solution is clear of beads (~5 minutes). Remove 25 $\mu$ L supernatant to a clean 96-well PCR plate.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *20uL\_96Well\_Elution*.

20. Add 20 $\mu$ L (0.8x) SPRI Beads to each well of two 96-well PCR plates. Transfer contents of one 96-well plate to another to combine two 96-well plates to one 96-well plate. Vortex and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_Add\_Beads\_And\_Combine\_96well*.

21. Place 96-well plate on DynaMag™-96 Side Magnet, let stand until solution in wells is clear of beads (~5 minutes). Remove supernatant and wash beads 2x with 150 $\mu$ L fresh 80% EtOH. Remove all EtOH, remove plate from magnet, and let beads dry at room temperature. DO NOT overdry beads.

(Optional) This step can be automated using TECAN Freedom Evo 100 with script - *BP\_1Plate\_150uL\_EtOH\_Wash*.

22. Add 20 $\mu$ L EB buffer and resuspend beads by pipet. Vortex and incubate for 5 minutes at room temperature, then quick spin for 10s at 2,000xg. Place back on magnet and let stand until solution is clear of beads (~5 minutes). Combine 20 $\mu$ L eluent from all wells in each column of the 96-well plate (8 wells per column, 12 columns) into 12 1.5 $\mu$ L Eppendorf tubes.

23. Add 128 $\mu$ L (0.8x) SPRI Beads to each 1.5 $\mu$ L Eppendorf tube. Pipette to mix and incubate for 5 minutes at room temperature.

24. Place 1.5 $\mu$ L tubes on DynaMag™-2 Magnet, let stand until solution in tubes is clear of beads (~5 minutes). Remove supernatant and wash beads 2x with 200 $\mu$ L fresh 80% EtOH. Remove all EtOH, remove tubes from magnet, and let beads dry at room temperature. DO NOT overdry beads.

25. Add 40 $\mu$ L EB and resuspend beads by pipet. Incubate for 5 minutes at room temperature. Place tubes back on magnet and let stand until solution is clear of beads (~5 minutes). Remove 40 $\mu$ L supernatant to 12 clean 1.5 $\mu$ L Eppendorf tubes.

26. Measure concentration of each 1.5 $\mu$ L Eppendorf tube with Qubit dsDNA BR Assay Kit. Normalize library concentrations and pool for sequencing.



Supplementary Table 1. Metadata for single-cell methylomes generated with snmC-seq + SAP and snmC-seq2

Sample	Species	Brain area	Labeling	Bisulfite conversion method	Library type	Sequencer	Total reads	Mapped reads	% Mapped reads	% Mapped forward reads	% Mapped reverse reads	Non-clonal reads	% Non-clonal reads	Filtred reads (MAPQ > 10)	% Filtred reads (MAPQ > 10)	mCCODG	mCGD	mCHC	Estimated mCGD	Estimated mCHC	Coverage (%)
Pool_1801_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	15092372	15092372	100.00	50.00	50.00	50.00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	3.73
Pool_1698_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	2441886	2441886	100.00	62.1%	62.0%	56.9%	1654010	67.7%	1341801	54.9%	0.01461	0.8088	0.06231	0.79996	0.04841	4.29
Pool_1693_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5763206	3878896	67.3%	78.2%	78.2%	59.6%	2641130	64.0%	2057507	82.9%	0.00996	0.7821	0.02088	0.76662	0.01501	6.57
Pool_1646_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4522641	4522641	100.00	68.8%	68.8%	58.8%	2589777	74.8%	2156463	81.3%	0.00663	0.7914	0.01417	0.78599	0.03363	6.43
Pool_1698_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5296394	3540255	66.8%	74.8%	74.8%	58.9%	2140229	60.5%	1477331	81.7%	0.02239	0.81934	0.02919	0.81213	0.0714	5.59
Pool_1699_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5060676	3572701	68.6%	74.2%	74.2%	56.7%	2307725	70.0%	1989374	82.1%	0.00903	0.78567	0.02479	0.78420	0.03407	6.28
Pool_1710_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5095954	3540255	69.5%	74.2%	74.2%	56.7%	2307725	70.0%	1989374	82.1%	0.00903	0.78567	0.02479	0.78420	0.03407	6.28
Pool_1718_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5132044	3322656	64.7%	74.2%	74.2%	55.5%	2462086	74.1%	2007775	81.3%	0.00744	0.79399	0.01861	0.76222	0.01125	6.73
Pool_1719_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	3784830	2307725	61.0%	73.2%	73.2%	55.5%	2307725	70.0%	1989374	82.1%	0.00903	0.78567	0.02479	0.78420	0.03407	6.28
Pool_1729_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5423486	3347614	61.7%	70.0%	70.0%	53.2%	2380172	71.1%	1928528	81.0%	0.00661	0.78758	0.02477	0.74725	0.01828	6.31
Pool_1732_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4042382	2520120	62.5%	73.2%	73.2%	51.5%	1724093	68.4%	1400285	81.2%	0.01376	0.78568	0.02477	0.72623	0.05001	4.61
Pool_1747_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4579560	2520120	55.1%	73.2%	73.2%	51.5%	1724093	68.4%	1400285	81.2%	0.01376	0.78568	0.02477	0.72623	0.05001	4.61
Pool_1750_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4850370	3064384	63.2%	73.2%	73.2%	53.8%	2355150	74.2%	1903913	80.4%	0.01784	0.77557	0.04482	0.73545	0.05041	6.29
Pool_1751_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	3793830	6311661	65.0%	74.2%	74.2%	56.5%	3654450	62.5%	3229990	81.5%	0.01158	0.82473	0.04498	0.80448	0.02378	6.61
Pool_1758_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4244840	3898862	91.8%	72.2%	72.2%	51.9%	2520234	64.8%	2048665	81.3%	0.00553	0.77378	0.02465	0.73966	0.00363	8.4
Pool_1769_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	7199966	4621405	64.2%	70.0%	70.0%	57.7%	3043871	62.5%	2524257	82.9%	0.00851	0.76633	0.02197	0.74232	0.01358	7.98
Pool_1775_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4479595	4368150	76.2%	78.2%	78.2%	58.5%	2362208	65.6%	2221124	81.1%	0.01137	0.78972	0.0164	0.76641	0.04312	7.51
Pool_1787_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	10421900	7210031	69.2%	78.2%	78.2%	61.6%	4244437	58.9%	3483859	82.1%	0.00384	0.79195	0.00683	0.75822	0.00302	11.46
Pool_1788_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	7627984	5319148	69.7%	77.8%	77.8%	61.6%	2965413	56.1%	2463998	82.2%	0.00406	0.7041	0.0065	0.70289	0.00225	8.16
Pool_1795_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	6774890	4674545	69.0%	78.2%	78.2%	61.1%	2819134	52.0%	2360604	82.1%	0.0074	0.76565	0.03343	0.7339	0.02602	7.68
Pool_1818_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	6539312	2034995	31.1%	74.2%	74.2%	27.6%	1407758	69.2%	1147622	81.5%	0.00636	0.8002	0.01841	0.75869	0.01213	4.1
Pool_1819_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	2406064	1796216	68.0%	78.2%	78.2%	60.3%	1309192	72.9%	1061298	81.1%	0.0077	0.76619	0.03404	0.80449	0.02719	8.87
Pool_1822_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	4300150	2744384	68.1%	75.0%	75.0%	58.1%	1837180	66.9%	1562084	81.8%	0.0079	0.80339	0.03372	0.81816	0.02616	5.33
Pool_1842_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	8629838	5800883	67.2%	78.1%	78.1%	58.4%	3318085	57.2%	2654476	80.0%	0.01225	0.8168	0.06121	0.84151	0.04947	8.36
Pool_1856_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	13687300	9374645	67.9%	74.8%	74.8%	60.3%	4463713	47.7%	3653306	90.8%	0.00468	0.759	0.0074	0.75787	0.00273	11.59
Pool_1859_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	8148338	5627615	69.1%	78.6%	78.6%	61.5%	3002908	53.4%	2464681	82.1%	0.00417	0.72484	0.00657	0.72533	0.00241	7.99
Pool_1872_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	14746568	10344147	70.1%	79.2%	79.2%	61.1%	5218386	59.4%	4251183	81.5%	0.00619	0.78445	0.01665	0.76301	0.01062	12.73
Pool_1873_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	10000000	6900000	69.0%	79.2%	79.2%	61.1%	2866622	69.2%	2302704	81.8%	0.00571	0.79133	0.00825	0.71966	0.00265	9.32
Pool_1985_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	6184242	4073801	65.9%	74.4%	74.4%	58.3%	2868449	70.4%	2372522	82.7%	0.01112	0.821	0.04481	0.81899	0.03163	8.26
Pool_1992_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	7349248	4827472	65.7%	74.4%	74.4%	58.3%	2868449	70.4%	2372522	82.7%	0.01112	0.821	0.04481	0.81899	0.03163	8.26
Pool_2015_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	2043714	1592465	65.1%	73.2%	73.2%	54.0%	9817919	82.0%	7719668	79.7%	0.00653	0.77969	0.04276	0.7862	0.01266	11.06
Pool_2020_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	16338262	1119953	67.9%	77.8%	77.8%	58.0%	6265181	56.3%	5208446	83.1%	0.01503	0.77023	0.01827	0.73238	0.00443	15.93
Pool_2035_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	35850918	2459485	6.9%	78.2%	78.2%	61.5%	3499883	7.4%	2819809	80.9%	0.00493	0.80623	0.01603	0.76678	0.01101	13.61
Pool_2035_AD002_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	9784064	6235000	63.7%	72.0%	72.0%	55.4%	3847132	56.9%	3045050	81.0%	0.01022	0.79716	0.01601	0.79057	0.04182	8.68
Pool_2043_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	3693776	612822	63.0%	77.0%	77.0%	50.1%	3317954	54.1%	2714460	81.8%	0.00801	0.80106	0.03702	0.79495	0.02924	8.58
Pool_2051_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	1038348	674742	64.6%	78.2%	78.2%	57.4%	4833749	67.8%	4038247	81.8%	0.00948	0.78781	0.02187	0.7124	0.01239	9.49
Pool_2051_AD002_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	11514236	7882377	68.5%	77.1%	77.1%	59.8%	4286806	53.8%	3481560	81.9%	0.0085	0.78699	0.04276	0.78475	0.03451	10.93
Pool_2065_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	2043714	1592465	65.1%	73.2%	73.2%	54.0%	9817919	82.0%	7719668	79.7%	0.00653	0.77969	0.04276	0.7862	0.01266	11.06
Pool_2069_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	598329	292591	48.9%	73.2%	73.2%	53.9%	2425751	51.9%	1932629	81.5%	0.00629	0.77865	0.02336	0.71784	0.0064	8.46
Pool_2068_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	8327790	4920449	59.1%	70.0%	70.0%	48.3%	2919054	59.3%	2382841	78.4%	0.0195	0.8279	0.06654	0.82448	0.04798	6.99
Pool_2082_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	9023870	6541422	69.2%	78.2%	78.2%	61.5%	3499883	57.2%	2819809	80.9%	0.01022	0.81605	0.01603	0.80623	0.01603	10.93
Pool_2084_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5068272	3241253	64.0%	70.2%	70.2%	55.3%	2614435	63.0%	2165169	81.0%	0.00909	0.75584	0.03652	0.7358	0.02837	5.47
Pool_2089_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	6516974	412269	63.3%	73.7%	73.7%	53.4%	2443313	59.0%	2038919	78.9%	0.00977	0.7762	0.04691	0.79799	0.03751	6.1
Pool_2108_AD001_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snmC-seq + SAP	Illumina HSS4000	5178160	359968	6.9%	77.0%	77.0%	51.7%	3478011	60.9%	2839692	81.6%	0.00781	0.80968	0.01601	0.81072	0.00246	9.54
Pool_2108_AD002_indeed	Homo sapiens	BA10	NeuN-Zymo EZ26 direct	snm																	





















## Supplementary References

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