nature portfolio

https://doi.org/10.1038/s41596-021-00507-3

Supplementary information

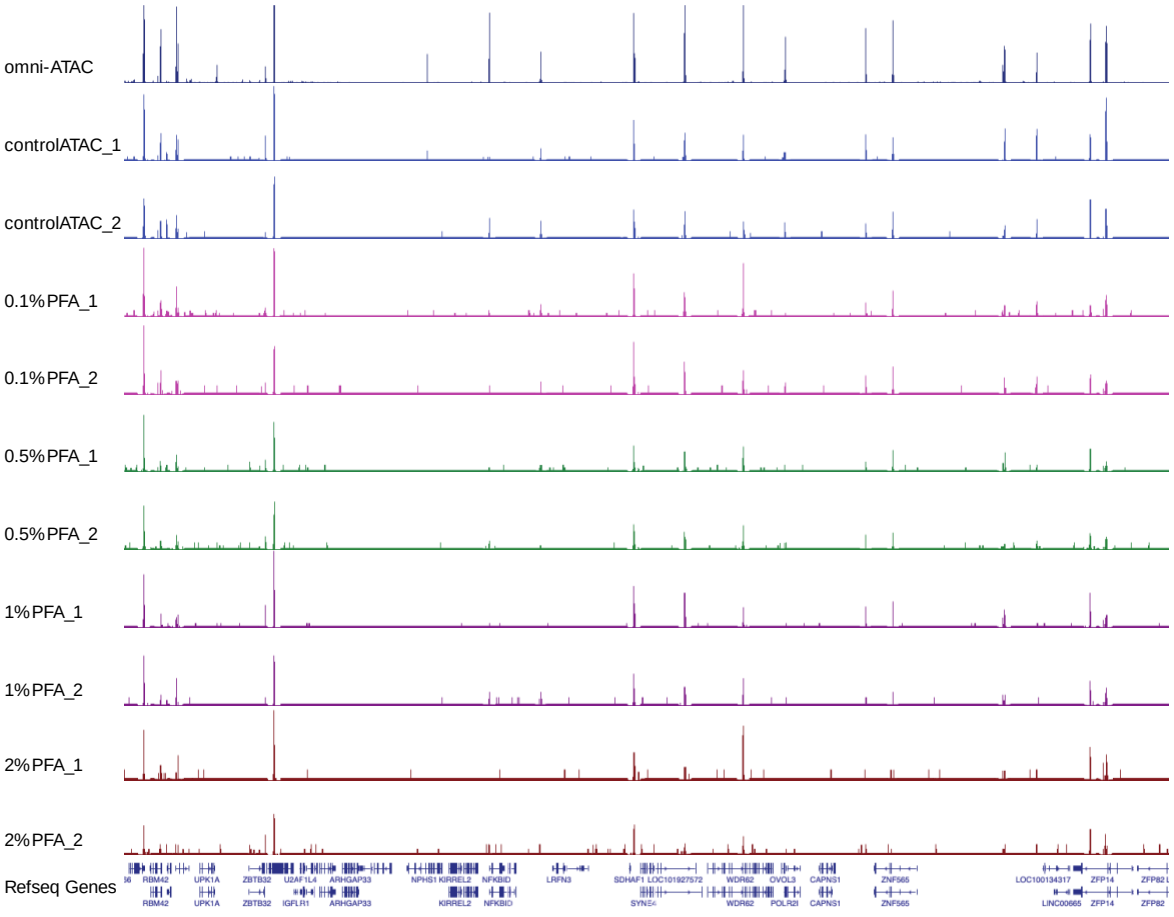
Scalable dual-omics profiling with single-nucleus chromatin accessibility and mRNA expression sequencing 2 (SNARE-seq2)

In the format provided by the authors and unedited

a

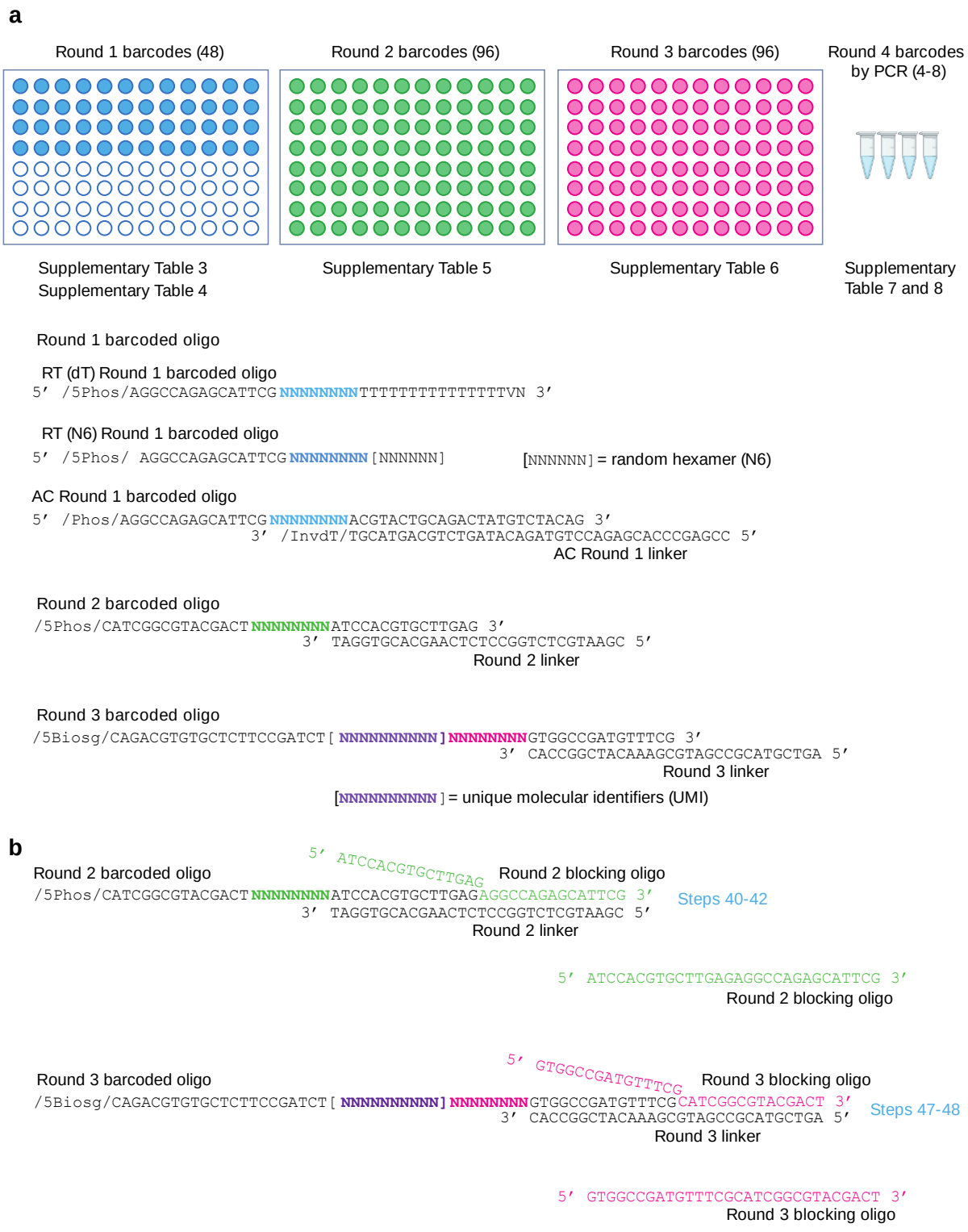
ATAC QC	ATAC control	0.1%PFA	0.5%PFA	1%PFA	2%PFA
Fraction of reads in peaks (FRiP)_1	0.50	0.43	0.42	0.41	0.41
Fraction of reads in peaks (FRiP)_2	0.53	0.43	0.38	0.41	0.38
TSS enrichment score_1	29.59	23.89	21.25	17.60	13.32
TSS enrichment score_2	27.87	22.97	19.70	18.30	13.00

b



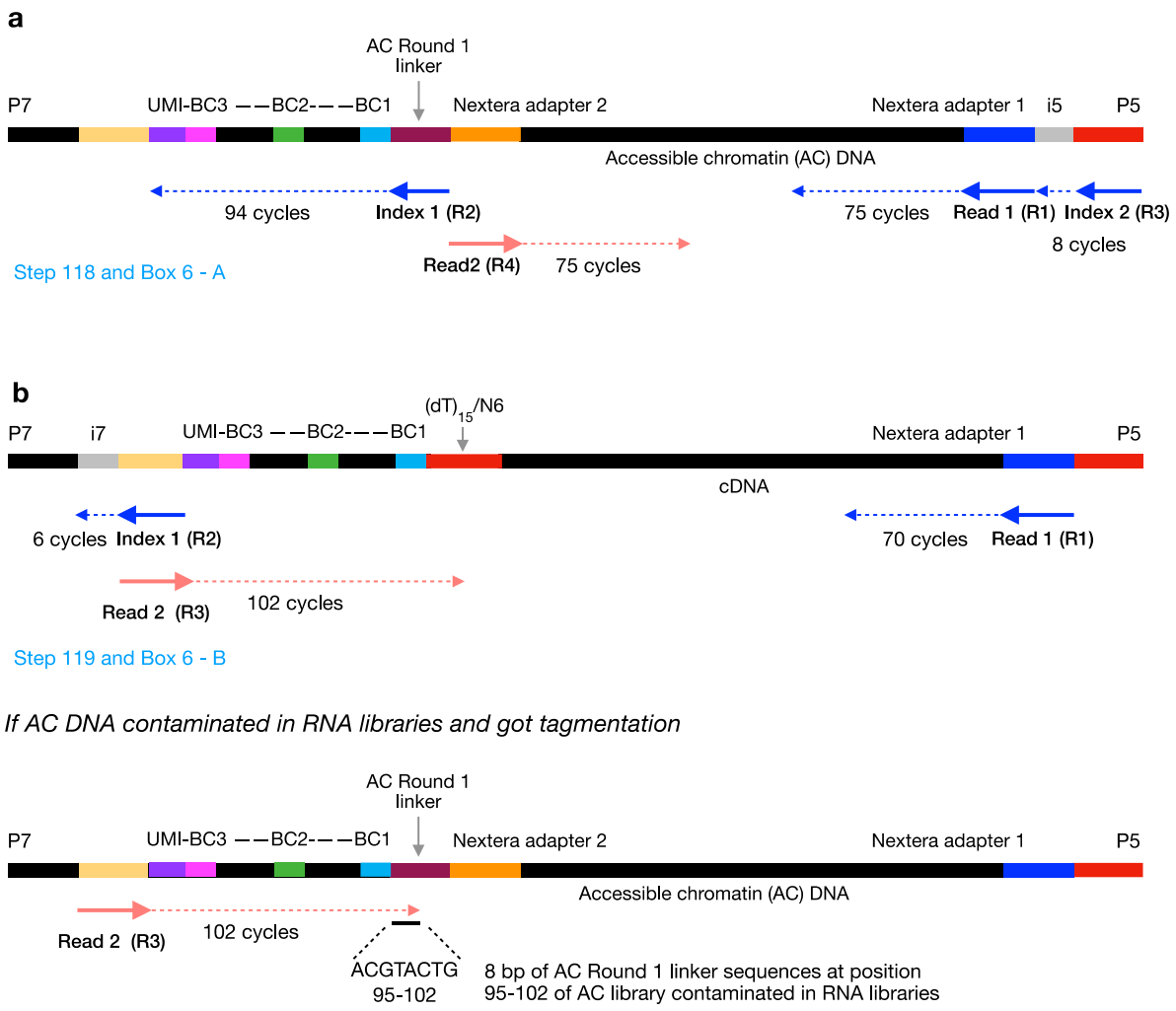
Supplementary Fig. 1 | Optimization of formaldehyde fixation on GM12878 whole cells.

a, The table shows the comparison of fraction of reads in peaks and transcription start site (TSS) enrichment score of bulk ATAC-seq on GM12878 whole cells fixed with 0.1%, 0.5%, 1% and 2% formaldehyde to bulk standard ATAC-seq. There were total 50,000 nuclei subsampled in each replicate, and two million reads were subsampled for ATAC-seq mapping. **b**, Bulk ATAC-seq peak comparison of control GM12878 ATAC-seq (2 million reads), formaldehyde fixed GM12878 whole cell ATAC-seq (2M reads) and the reference omni-ATAC-seq[1] (10 millions subsampled reads) on the region of chromosome 19.



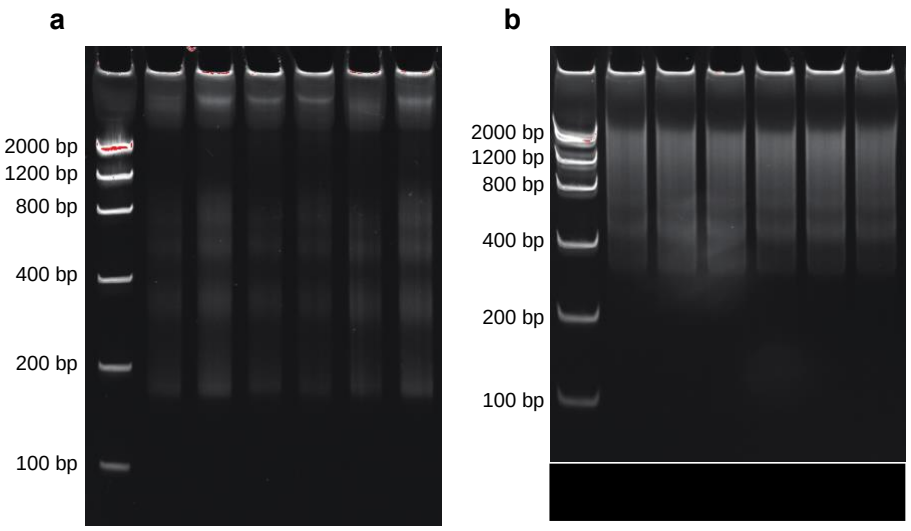
Supplementary Fig. 2 | Schematics of Round 1-3 barcoded oligos and Round 2-3 blocking oligos.

a, Schemes showing sequences of barcoded oligos in each round and theirs corresponding linker oligos. The numbers of unique barcodes in each round were indicated on the top. **b**, Schemes showing blocking oligos anneal to Round 2 or Round 3 linker sequences to prevent cross hybridization of cellular barcodes during nuclei/cell pooling step.



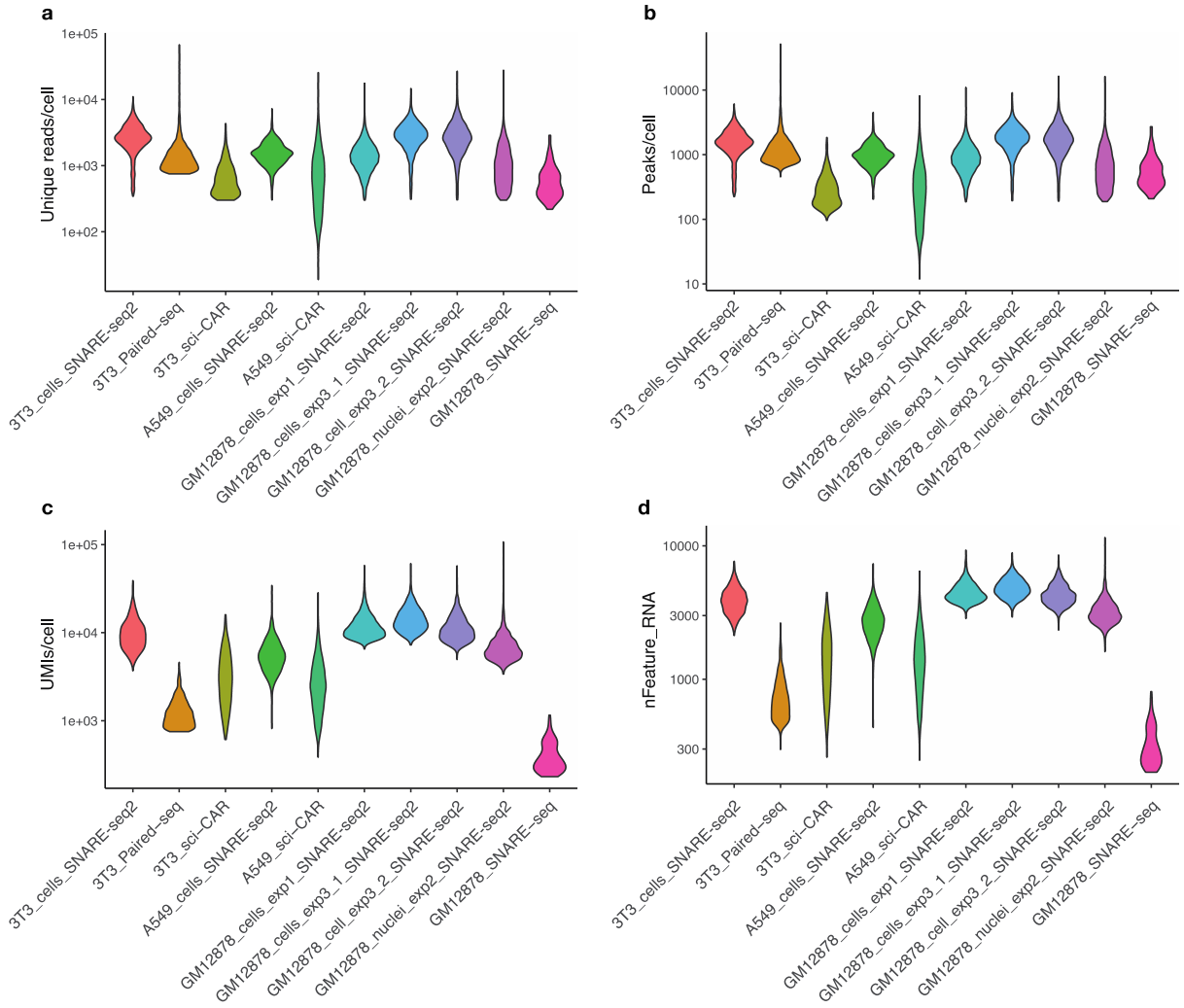
Supplementary Fig. 3 | Sequencing schemes of SNARE-seq2.

a, Sequencing scheme of SNARE-seq2 AC libraries. There are total four reads; 1) R1 or Read 1: AC Read 1 of 75 cycles, 2) R2 or Index 1: UMI and cellular barcode of 94 cycles, 3. R3 or Index 2: i5 index of 8 cycles and 4) R4: AC Read 2 of 75 cycles. Total 252 cycles. **b**, Sequencing scheme of SNARE-seq2 RNA libraries. There are total three reads; 1) R1 or Read 1: cDNA of 70 cycles, 2) R2 or Index 1: i7 index of 6 cycles, and 3) R3 or Read 2: UMI and cellular barcode of 94 cycles and extra 8 cycles (cycle 95-102) to determine the presence of AC libraries in RNA libraries at AC Round 1 linker sequences position 1-8 (ACGTACTG) (bottom scheme). Total 178 cycles.



Supplementary Fig. 4 | Gel image showing nucleosome pattern of standard ATAC-seq libraries and SNARE-seq2 AC libraries.

a, Nucleosome pattern of bulk GM12878 ATAC-seq in 6% TBE PAGE gel (250 volts for 28 min). **b**, Nucleosome pattern of SNARE-seq2 single-nucleus AC libraries in 6% TBE PAGE gel (250 volts for 23 min).



Supplementary Fig. 5 | Comparison of unique reads, accessible sites, UMI, and gene counts per cell/nucleus by SNARE-seq2 with different chromatin accessibility and RNA-seq dual assays.

a, b, Violin plots showing the number of unique reads and accessible sites per cell/nucleus of SNARE-seq2 (GM12878 from different batches and A549 from one batch), sci-CAR, Paired-seq and SNARE-seq methods. Chromatin accessibility data were downloaded from the Gene Expression Omnibus (GEO) database (sci-CAR 3T3: GSM3271041; Paired-seq 3T3: GSM3737488; sci-CAR A549, GSM3271041; SNARE-seq: GSM3590937). **c,d**, Violin plots showing the number of UMI and gene count per cell/nucleus of SNARE-seq2 (GM12878 from different batches and A549 from one batch), sci-CAR, Paired-seq and SNARE-seq methods. Single-cell/nucleus RNA-seq data were downloaded from the GEO database (sci-CAR 3T3: GSM3271040; Paired-seq 3T3: GSM3737489; sci-CAR A549: GSM3271040; SNARE-seq: GSM3590936). SNARE-seq2 GM12878, 3T3, and A549 raw data are available at the GEO database under accession number GSE157660. SNARE-seq processed data are provided in Supplementary file (snare-seq2_code_data.tar.gz). 3T3_cells_SNARE-seq2 and A549_cells_SNARE-seq2 were samples fixed with 1% formaldehyde and cryopreserved in 1x PBS with 10% (vol/vol) DMSO and 0.1% (wt/vol) BSA for three weeks. All GM12878_SNARE-seq2 were fresh cell or nucleus samples.

Supplementary Table 1 | Cost estimation of SNARE-seq2

Library preparation: Day 1

Item	Supplier	Item Code	Cost per plate ($)
Oligonucleotides and primers	IDT		46.850
In-house Tn5 transposase			7.778
Formaldehyde	Thermo Fisher Scientific	28906	5.637
Maxima H Minus Reverse Transcriptase	Thermo Fisher Scientific	EP0753	252.912
SUPERase In RNase Inhibitor	Thermo Fisher Scientific	AM2696	72.352
Enzymatics RNase inhibitor	Enzymatics	Y9240L	67.567
dNTP	Clontech	639132	14.560
ATP	Thermo Fisher Scientific	R0441	3.898
10x NEB Buffer 3.1	NEB	B7203S	1.008
T7 DNA Ligase	NEB	M0318L	86.112
T4 DNA Ligase	NEB	M0202L	232.960
10x T4 DNA Ligase Buffer	NEB	B0202S	3.240
Proteinase K	Thermo Fisher Scientific	25530049	1.992
96-well LoBind PCR plate	Eppendorf	30129504	12.240
Total cost of day 1 experiment			**809.11**

Library preparation: Post-day 1

The cost on post-day 1 was estimated based on 6 pools of nuclei or cells.

Item	Supplier	Item Code	Cost per plate ($)
SUPERase In RNase Inhibitor	Thermo Fisher Scientific	AM2696	23.995
Dynabeads MyOne Streptavidin C1	Thermo Fisher Scientific	65001	72.336
Maxima H Minus Reverse Transcriptase	Thermo Fisher Scientific	EP0753	71.850
ATP	Thermo Fisher Scientific	R0441	4.498
dNTP	Clontech	639132	16.800
Hemo KlenTag	NEB	M0332L	16.200
T7 DNA Ligase	NEB	M0318L	16.560
Ficoll solution	Sigma Aldrich	F5415-50ML	0.252
KAPA HotStart HiFi Ready Mix	KAPA Biosystems	KK2602	57.802
NEBNext High-Fidelity PCR Master Mix	NEB	M0541S	10.800
Nextera XT DNA Library Prep Kit	Illumina	FC-131-1096	173.688
6% Novel TBE Gel	Thermo Fisher Scientific	EC6265BOX	100.800
Glycoblue Coprecipitant	Thermo Fisher Scientific	AM9515	4.920

Item	Supplier	Item Code	Cost per plate ($)
Qubit dsDNA HS assay kit	Thermo Fisher Scientific	Q32854	19.712
DNA Clean & Concentrator	ZymoResearch	D4014	7.770
Low DNA Mass Ladder	Thermo Fisher Scientific	10068013	2.543
PALL Nanosep 0.2 µm	PALL Corporation	ODM02C35	23.184
Total cost of post-day 1 experiment			**623.71**

Sequencing cost

Library type	Sequencing Reagent kit	Cost per lane	# of lane	Estimated reads (M)	Cost ($)
RNA Library (~75,000 passed QC cells, 30,000 reads/cell, 150 cycle kit)	NovaSeq 6000 S4 200 cycles	6,800	1	2,250	6,800
AC Library (~75,000 passed QC cells, 30,000 reads/cell, 300 cycle kit)	NovaSeq 6000 S4 300 cycles	7,750	1	2,250	7,750
Total of sequencing cost					**14,550**

Total cost ($) [Library preparation + Sequencing] = 1,432.82 + 14,550 = 15,982.82
Cost per nucleus or cell ($) by assuming 75,000 passed QC nuclei or cells = 0.21

Supplementary Table 2 | Standard ATAC-seq indexed PCR primers

Primer name	Sequences (5' - 3')
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT

Supplementary Table 3 | Accessible chromatin (AC) Round 1 barcoded oligos

Well Position	Name	Sequence (5'-3')
A1	AC_v1.2_R1_01	/5Phos/AGGCCAGAGCATTCGAACGTGATACGTACTGCAGACTATGTCTACAG
A2	AC_v1.2_R1_02	/5Phos/AGGCCAGAGCATTCGAAACATCGACGTACTGCAGACTATGTCTACAG
A3	AC_v1.2_R1_03	/5Phos/AGGCCAGAGCATTCGATGCCTAAACGTACTGCAGACTATGTCTACAG
A4	AC_v1.2_R1_04	/5Phos/AGGCCAGAGCATTCGAGTGGTCAACGTACTGCAGACTATGTCTACAG
A5	AC_v1.2_R1_05	/5Phos/AGGCCAGAGCATTCGACCACTGTACGTACTGCAGACTATGTCTACAG
A6	AC_v1.2_R1_06	/5Phos/AGGCCAGAGCATTCGACATTGGCACGTACTGCAGACTATGTCTACAG
A7	AC_v1.2_R1_07	/5Phos/AGGCCAGAGCATTCGCAGATCTGACGTACTGCAGACTATGTCTACAG
A8	AC_v1.2_R1_08	/5Phos/AGGCCAGAGCATTCGCATCAAGTACGTACTGCAGACTATGTCTACAG
A9	AC_v1.2_R1_09	/5Phos/AGGCCAGAGCATTCGCGCTGATCACGTACTGCAGACTATGTCTACAG
A10	AC_v1.2_R1_10	/5Phos/AGGCCAGAGCATTCGACAAGCTAACGTACTGCAGACTATGTCTACAG
A11	AC_v1.2_R1_11	/5Phos/AGGCCAGAGCATTCGCTGTAGCCACGTACTGCAGACTATGTCTACAG
A12	AC_v1.2_R1_12	/5Phos/AGGCCAGAGCATTCGAGTACAAGACGTACTGCAGACTATGTCTACAG
B1	AC_v1.2_R1_13	/5Phos/AGGCCAGAGCATTCGAACAACCAACGTACTGCAGACTATGTCTACAG
B2	AC_v1.2_R1_14	/5Phos/AGGCCAGAGCATTCGAACCGAGAACGTACTGCAGACTATGTCTACAG
B3	AC_v1.2_R1_15	/5Phos/AGGCCAGAGCATTCGAACGCTTAACGTACTGCAGACTATGTCTACAG
B4	AC_v1.2_R1_16	/5Phos/AGGCCAGAGCATTCGAAGACGGAACGTACTGCAGACTATGTCTACAG
B5	AC_v1.2_R1_17	/5Phos/AGGCCAGAGCATTCGAAGGTACAACGTACTGCAGACTATGTCTACAG
B6	AC_v1.2_R1_18	/5Phos/AGGCCAGAGCATTCGACACAGAAACGTACTGCAGACTATGTCTACAG
B7	AC_v1.2_R1_19	/5Phos/AGGCCAGAGCATTCGACAGCAGAACGTACTGCAGACTATGTCTACAG
B8	AC_v1.2_R1_20	/5Phos/AGGCCAGAGCATTCGACCTCCAAACGTACTGCAGACTATGTCTACAG
B9	AC_v1.2_R1_21	/5Phos/AGGCCAGAGCATTCGACGCTCGAACGTACTGCAGACTATGTCTACAG
B10	AC_v1.2_R1_22	/5Phos/AGGCCAGAGCATTCGACGTATCAACGTACTGCAGACTATGTCTACAG
B11	AC_v1.2_R1_23	/5Phos/AGGCCAGAGCATTCGACTATGCAACGTACTGCAGACTATGTCTACAG
B12	AC_v1.2_R1_24	/5Phos/AGGCCAGAGCATTCGAGAGTCAAACGTACTGCAGACTATGTCTACAG

Well Position	Name	Sequence (5'-3')
C1	AC_v1.2_R1_25	/5Phos/AGGCCAGAGCATTCGAGATCGCAACGTACTGCAGACTATGTCTACAG
C2	AC_v1.2_R1_26	/5Phos/AGGCCAGAGCATTCGAGCAGGAAACGTACTGCAGACTATGTCTACAG
C3	AC_v1.2_R1_27	/5Phos/AGGCCAGAGCATTCGAGTCACTAACGTACTGCAGACTATGTCTACAG
C4	AC_v1.2_R1_28	/5Phos/AGGCCAGAGCATTCGATCCTGTAACGTACTGCAGACTATGTCTACAG
C5	AC_v1.2_R1_29	/5Phos/AGGCCAGAGCATTCGATTGAGGAACGTACTGCAGACTATGTCTACAG
C6	AC_v1.2_R1_30	/5Phos/AGGCCAGAGCATTCGCAACCACAACGTACTGCAGACTATGTCTACAG
C7	AC_v1.2_R1_31	/5Phos/AGGCCAGAGCATTCGGACTAGTAACGTACTGCAGACTATGTCTACAG
C8	AC_v1.2_R1_32	/5Phos/AGGCCAGAGCATTCGCAATGGAAACGTACTGCAGACTATGTCTACAG
C9	AC_v1.2_R1_33	/5Phos/AGGCCAGAGCATTCGCACTTCGAACGTACTGCAGACTATGTCTACAG
C10	AC_v1.2_R1_34	/5Phos/AGGCCAGAGCATTCGCAGCGTTAACGTACTGCAGACTATGTCTACAG
C11	AC_v1.2_R1_35	/5Phos/AGGCCAGAGCATTCGCATACCAAACGTACTGCAGACTATGTCTACAG
C12	AC_v1.2_R1_36	/5Phos/AGGCCAGAGCATTCGCCAGTTCAACGTACTGCAGACTATGTCTACAG
D1	AC_v1.2_R1_37	/5Phos/AGGCCAGAGCATTCGCCGAAGTAACGTACTGCAGACTATGTCTACAG
D2	AC_v1.2_R1_38	/5Phos/AGGCCAGAGCATTCGCCGTGAGAACGTACTGCAGACTATGTCTACAG
D3	AC_v1.2_R1_39	/5Phos/AGGCCAGAGCATTCGCCTCCTGAACGTACTGCAGACTATGTCTACAG
D4	AC_v1.2_R1_40	/5Phos/AGGCCAGAGCATTCGCGAACTTAACGTACTGCAGACTATGTCTACAG
D5	AC_v1.2_R1_41	/5Phos/AGGCCAGAGCATTCGCGACTGGAACGTACTGCAGACTATGTCTACAG
D6	AC_v1.2_R1_42	/5Phos/AGGCCAGAGCATTCGCGCATACAACGTACTGCAGACTATGTCTACAG
D7	AC_v1.2_R1_43	/5Phos/AGGCCAGAGCATTCGCTCAATGAACGTACTGCAGACTATGTCTACAG
D8	AC_v1.2_R1_44	/5Phos/AGGCCAGAGCATTCGCTGAGCCAACGTACTGCAGACTATGTCTACAG
D9	AC_v1.2_R1_45	/5Phos/AGGCCAGAGCATTCGCTGGCATAACGTACTGCAGACTATGTCTACAG
D10	AC_v1.2_R1_46	/5Phos/AGGCCAGAGCATTCGGAATCTGAACGTACTGCAGACTATGTCTACAG
D11	AC_v1.2_R1_47	/5Phos/AGGCCAGAGCATTCGCAAGACTAACGTACTGCAGACTATGTCTACAG
D12	AC_v1.2_R1_48	/5Phos/AGGCCAGAGCATTCGGAGCTGAAACGTACTGCAGACTATGTCTACAG

Supplementary Table 4 | Reverse transcription (RT) Round 1 barcoded oligos[2]

Well Position	Oligo Type	Name	Sequences (5' - 3')
A1	dt(15)VN	Round1_01	/5Phos/AGGCCAGAGCATTCGAACGTGATTTTTTTTTTTTTTTTVN
A2	dt(15)VN	Round1_02	/5Phos/AGGCCAGAGCATTCGAAACATCGTTTTTTTTTTTTTTTTVN
A3	dt(15)VN	Round1_03	/5Phos/AGGCCAGAGCATTCGATGCCTAATTTTTTTTTTTTTTTTVN
A4	dt(15)VN	Round1_04	/5Phos/AGGCCAGAGCATTCGAGTGGTCATTTTTTTTTTTTTTTTVN
A5	dt(15)VN	Round1_05	/5Phos/AGGCCAGAGCATTCGACCACTGTTTTTTTTTTTTTTTTVN
A6	dt(15)VN	Round1_06	/5Phos/AGGCCAGAGCATTCGACATTGGCTTTTTTTTTTTTTTTTVN
A7	dt(15)VN	Round1_07	/5Phos/AGGCCAGAGCATTCGCAGATCTGTTTTTTTTTTTTTTTTVN
A8	dt(15)VN	Round1_08	/5Phos/AGGCCAGAGCATTCGCATCAAGTTTTTTTTTTTTTTTTVN
A9	dt(15)VN	Round1_09	/5Phos/AGGCCAGAGCATTCGCGCTGATCTTTTTTTTTTTTTTTTVN
A10	dt(15)VN	Round1_10	/5Phos/AGGCCAGAGCATTCGACAAGCTATTTTTTTTTTTTTTTTVN
A11	dt(15)VN	Round1_11	/5Phos/AGGCCAGAGCATTCGCTGTAGCCTTTTTTTTTTTTTTTTVN
A12	dt(15)VN	Round1_12	/5Phos/AGGCCAGAGCATTCGAGTACAAGTTTTTTTTTTTTTTTTVN
B1	dt(15)VN	Round1_13	/5Phos/AGGCCAGAGCATTCGAACAACCATTTTTTTTTTTTTTTTVN
B2	dt(15)VN	Round1_14	/5Phos/AGGCCAGAGCATTCGAACCGAGATTTTTTTTTTTTTTTTVN
B3	dt(15)VN	Round1_15	/5Phos/AGGCCAGAGCATTCGAACGCTTATTTTTTTTTTTTTTTVN
B4	dt(15)VN	Round1_16	/5Phos/AGGCCAGAGCATTCGAAGACGGATTTTTTTTTTTTTTTTVN
B5	dt(15)VN	Round1_17	/5Phos/AGGCCAGAGCATTCGAAGGTACATTTTTTTTTTTTTTTTVN
B6	dt(15)VN	Round1_18	/5Phos/AGGCCAGAGCATTCGACACAGAATTTTTTTTTTTTTTTTVN
B7	dt(15)VN	Round1_19	/5Phos/AGGCCAGAGCATTCGACAGCAGATTTTTTTTTTTTTTTTVN
B8	dt(15)VN	Round1_20	/5Phos/AGGCCAGAGCATTCGACCTCCAATTTTTTTTTTTTTTTTVN
B9	dt(15)VN	Round1_21	/5Phos/AGGCCAGAGCATTCGACGCTCGATTTTTTTTTTTTTTTTVN
B10	dt(15)VN	Round1_22	/5Phos/AGGCCAGAGCATTCGACGTATCATTTTTTTTTTTTTTTTVN
B11	dt(15)VN	Round1_23	/5Phos/AGGCCAGAGCATTCGACTATGCATTTTTTTTTTTTTTTTVN
B12	dt(15)VN	Round1_24	/5Phos/AGGCCAGAGCATTCGAGAGTCAATTTTTTTTTTTTTTTTVN

Well Position	Oligo Type	Name	Sequences (5' - 3')
C1	dt(15)VN	Round1_25	/5Phos/AGGCCAGAGCATTCGAGATCGCATTTTTTTTTTTTTTTVN
C2	dt(15)VN	Round1_26	/5Phos/AGGCCAGAGCATTCGAGCAGGAATTTTTTTTTTTTTTTVN
C3	dt(15)VN	Round1_27	/5Phos/AGGCCAGAGCATTCGAGTCACTATTTTTTTTTTTTTTTVN
C4	dt(15)VN	Round1_28	/5Phos/AGGCCAGAGCATTCGATCCTGTATTTTTTTTTTTTTTTVN
C5	dt(15)VN	Round1_29	/5Phos/AGGCCAGAGCATTCGATTGAGGATTTTTTTTTTTTTTTVN
C6	dt(15)VN	Round1_30	/5Phos/AGGCCAGAGCATTCGCAACCACATTTTTTTTTTTTTTTVN
C7	dt(15)VN	Round1_31	/5Phos/AGGCCAGAGCATTCGGACTAGTATTTTTTTTTTTTTTTVN
C8	dt(15)VN	Round1_32	/5Phos/AGGCCAGAGCATTCGCAATGGAATTTTTTTTTTTTTTTVN
C9	dt(15)VN	Round1_33	/5Phos/AGGCCAGAGCATTCGCACTTCGATTTTTTTTTTTTTTTVN
C10	dt(15)VN	Round1_34	/5Phos/AGGCCAGAGCATTCGCAGCGTTATTTTTTTTTTTTTTTVN
C11	dt(15)VN	Round1_35	/5Phos/AGGCCAGAGCATTCGCATACCAATTTTTTTTTTTTTTTVN
C12	dt(15)VN	Round1_36	/5Phos/AGGCCAGAGCATTCGCCAGTTCATTTTTTTTTTTTTTTVN
D1	dt(15)VN	Round1_37	/5Phos/AGGCCAGAGCATTCGCCGAAGTATTTTTTTTTTTTTTTVN
D2	dt(15)VN	Round1_38	/5Phos/AGGCCAGAGCATTCGCCGTGAGATTTTTTTTTTTTTTTVN
D3	dt(15)VN	Round1_39	/5Phos/AGGCCAGAGCATTCGCCTCCTGATTTTTTTTTTTTTTTVN
D4	dt(15)VN	Round1_40	/5Phos/AGGCCAGAGCATTCGCGAACTTATTTTTTTTTTTTTTTVN
D5	dt(15)VN	Round1_41	/5Phos/AGGCCAGAGCATTCGCGACTGGATTTTTTTTTTTTTTTVN
D6	dt(15)VN	Round1_42	/5Phos/AGGCCAGAGCATTCGCGCATACATTTTTTTTTTTTTTTVN
D7	dt(15)VN	Round1_43	/5Phos/AGGCCAGAGCATTCGCTCAATGATTTTTTTTTTTTTTTVN
D8	dt(15)VN	Round1_44	/5Phos/AGGCCAGAGCATTCGCTGAGCCATTTTTTTTTTTTTTTVN
D9	dt(15)VN	Round1_45	/5Phos/AGGCCAGAGCATTCGCTGGCATATTTTTTTTTTTTTTTVN
D10	dt(15)VN	Round1_46	/5Phos/AGGCCAGAGCATTCGGAATCTGATTTTTTTTTTTTTTTVN
D11	dt(15)VN	Round1_47	/5Phos/AGGCCAGAGCATTCGCAAGACTATTTTTTTTTTTTTTTVN
D12	dt(15)VN	Round1_48	/5Phos/AGGCCAGAGCATTCGGAGCTGAATTTTTTTTTTTTTTTVN
E1	random hexamer	Round1_49	/5Phos/AGGCCAGAGCATTCGGATAGACANNNNNN

Well Position	Oligo Type	Name	Sequences (5' - 3')
E2	random hexamer	Round1_50	/5Phos/AGGCCAGAGCATTCGGCCACATANNNNNN
E3	random hexamer	Round1_51	/5Phos/AGGCCAGAGCATTCGGCGAGTAANNNNNN
E4	random hexamer	Round1_52	/5Phos/AGGCCAGAGCATTCGGCTAACGANNNNNN
E5	random hexamer	Round1_53	/5Phos/AGGCCAGAGCATTCGGCTCGGTANNNNNN
E6	random hexamer	Round1_54	/5Phos/AGGCCAGAGCATTCGGGAGAACANNNNNN
E7	random hexamer	Round1_55	/5Phos/AGGCCAGAGCATTCGGGTGCGAANNNNNN
E8	random hexamer	Round1_56	/5Phos/AGGCCAGAGCATTCGGTACGCAANNNNNN
E9	random hexamer	Round1_57	/5Phos/AGGCCAGAGCATTCGGTCGTAGANNNNNN
E10	random hexamer	Round1_58	/5Phos/AGGCCAGAGCATTCGGTCTGTCANNNNNN
E11	random hexamer	Round1_59	/5Phos/AGGCCAGAGCATTCGGTGTTCTANNNNNN
E12	random hexamer	Round1_60	/5Phos/AGGCCAGAGCATTCGTAGGATGANNNNNN
F1	random hexamer	Round1_61	/5Phos/AGGCCAGAGCATTCGTATCAGCANNNNNN
F2	random hexamer	Round1_62	/5Phos/AGGCCAGAGCATTCGTCCGTCTANNNNNN
F3	random hexamer	Round1_63	/5Phos/AGGCCAGAGCATTCGTCTTCACANNNNNN
F4	random hexamer	Round1_64	/5Phos/AGGCCAGAGCATTCGTGAAGAGANNNNNN
F5	random hexamer	Round1_65	/5Phos/AGGCCAGAGCATTCGTGGAACAANNNNNN
F6	random hexamer	Round1_66	/5Phos/AGGCCAGAGCATTCGTGGCTTCANNNNNN
F7	random hexamer	Round1_67	/5Phos/AGGCCAGAGCATTCGTGGTGGTANNNNNN
F8	random hexamer	Round1_68	/5Phos/AGGCCAGAGCATTCGTTCACGCANNNNNN
F9	random hexamer	Round1_69	/5Phos/AGGCCAGAGCATTCGAACTCACCNNNNNN
F10	random hexamer	Round1_70	/5Phos/AGGCCAGAGCATTCGAAGAGATCNNNNNN
F11	random hexamer	Round1_71	/5Phos/AGGCCAGAGCATTCGAAGGACACNNNNNN
F12	random hexamer	Round1_72	/5Phos/AGGCCAGAGCATTCGAATCCGTCNNNNNN
G1	random hexamer	Round1_73	/5Phos/AGGCCAGAGCATTCGAATGTTGCNNNNNN
G2	random hexamer	Round1_74	/5Phos/AGGCCAGAGCATTCGACACGACCNNNNNN

Well Position	Oligo Type	Name	Sequences (5' - 3')
G3	random hexamer	Round1_75	/5Phos/AGGCCAGAGCATTCGACAGATTCNNNNNN
G4	random hexamer	Round1_76	/5Phos/AGGCCAGAGCATTCGAGATGTACNNNNNN
G5	random hexamer	Round1_77	/5Phos/AGGCCAGAGCATTCGAGCACCTCNNNNNN
G6	random hexamer	Round1_78	/5Phos/AGGCCAGAGCATTCGAGCCATGCNNNNNN
G7	random hexamer	Round1_79	/5Phos/AGGCCAGAGCATTCGAGGCTAACNNNNNN
G8	random hexamer	Round1_80	/5Phos/AGGCCAGAGCATTCGATAGCGACNNNNNN
G9	random hexamer	Round1_81	/5Phos/AGGCCAGAGCATTCGATCATTCCNNNNNN
G10	random hexamer	Round1_82	/5Phos/AGGCCAGAGCATTCGATTGGCTCNNNNNN
G11	random hexamer	Round1_83	/5Phos/AGGCCAGAGCATTCGCAAGGAGCNNNNNN
G12	random hexamer	Round1_84	/5Phos/AGGCCAGAGCATTCGCACCTTACNNNNNN
H1	random hexamer	Round1_85	/5Phos/AGGCCAGAGCATTCGCCATCCTCNNNNNN
H2	random hexamer	Round1_86	/5Phos/AGGCCAGAGCATTCGCCGACAACNNNNNN
H3	random hexamer	Round1_87	/5Phos/AGGCCAGAGCATTCGCCTAATCCNNNNNN
H4	random hexamer	Round1_88	/5Phos/AGGCCAGAGCATTCGCCTCTATCNNNNNN
H5	random hexamer	Round1_89	/5Phos/AGGCCAGAGCATTCGCGACACACNNNNNN
H6	random hexamer	Round1_90	/5Phos/AGGCCAGAGCATTCGCGGATTGCNNNNNN
H7	random hexamer	Round1_91	/5Phos/AGGCCAGAGCATTCGCTAAGGTCNNNNNN
H8	random hexamer	Round1_92	/5Phos/AGGCCAGAGCATTCGGAACAGGCNNNNNN
H9	random hexamer	Round1_93	/5Phos/AGGCCAGAGCATTCGGACAGTGCNNNNNN
H10	random hexamer	Round1_94	/5Phos/AGGCCAGAGCATTCGGAGTTAGCNNNNNN
H11	random hexamer	Round1_95	/5Phos/AGGCCAGAGCATTCGGATGAATCNNNNNN
H12	random hexamer	Round1_96	/5Phos/AGGCCAGAGCATTCGGCCAAGACNNNNNN

V = A,C,G

Supplementary Table 5 | Round 2 barcoded oligos[2]

Well Position	Name	Sequences (5'-3')
A1	Round2_01	/5Phos/CATCGGCGTACGACTAACGTGATATCCACGTGCTTGAG
A2	Round2_02	/5Phos/CATCGGCGTACGACTAAACATCGATCCACGTGCTTGAG
A3	Round2_03	/5Phos/CATCGGCGTACGACTATGCCTAAATCCACGTGCTTGAG
A4	Round2_04	/5Phos/CATCGGCGTACGACTAGTGGTCAATCCACGTGCTTGAG
A5	Round2_05	/5Phos/CATCGGCGTACGACTACCACTGTATCCACGTGCTTGAG
A6	Round2_06	/5Phos/CATCGGCGTACGACTACATTGGCATCCACGTGCTTGAG
A7	Round2_07	/5Phos/CATCGGCGTACGACTCAGATCTGATCCACGTGCTTGAG
A8	Round2_08	/5Phos/CATCGGCGTACGACTCATCAAGTATCCACGTGCTTGAG
A9	Round2_09	/5Phos/CATCGGCGTACGACTCGCTGATCATCCACGTGCTTGAG
A10	Round2_10	/5Phos/CATCGGCGTACGACTACAAGCTAATCCACGTGCTTGAG
A11	Round2_11	/5Phos/CATCGGCGTACGACTCTGTAGCCATCCACGTGCTTGAG
A12	Round2_12	/5Phos/CATCGGCGTACGACTAGTACAAGATCCACGTGCTTGAG
B1	Round2_13	/5Phos/CATCGGCGTACGACTAACAACCAATCCACGTGCTTGAG
B2	Round2_14	/5Phos/CATCGGCGTACGACTAACCGAGAATCCACGTGCTTGAG
B3	Round2_15	/5Phos/CATCGGCGTACGACTAACGCTTAATCCACGTGCTTGAG
B4	Round2_16	/5Phos/CATCGGCGTACGACTAAGACGGAATCCACGTGCTTGAG
B5	Round2_17	/5Phos/CATCGGCGTACGACTAAGGTACAATCCACGTGCTTGAG
B6	Round2_18	/5Phos/CATCGGCGTACGACTACACAGAAATCCACGTGCTTGAG
B7	Round2_19	/5Phos/CATCGGCGTACGACTACAGCAGAATCCACGTGCTTGAG
B8	Round2_20	/5Phos/CATCGGCGTACGACTACCTCCAAATCCACGTGCTTGAG
B9	Round2_21	/5Phos/CATCGGCGTACGACTACGCTCGAATCCACGTGCTTGAG
B10	Round2_22	/5Phos/CATCGGCGTACGACTACGTATCAATCCACGTGCTTGAG
B11	Round2_23	/5Phos/CATCGGCGTACGACTACTATGCAATCCACGTGCTTGAG
B12	Round2_24	/5Phos/CATCGGCGTACGACTAGAGTCAAATCCACGTGCTTGAG

Well Position	Name	Sequences (5'-3')
C1	Round2_25	/5Phos/CATCGGCGTACGACTAGATCGCAATCCACGTGCTTGAG
C2	Round2_26	/5Phos/CATCGGCGTACGACTAGCAGGAAATCCACGTGCTTGAG
C3	Round2_27	/5Phos/CATCGGCGTACGACTAGTCACTAATCCACGTGCTTGAG
C4	Round2_28	/5Phos/CATCGGCGTACGACTATCCTGTAATCCACGTGCTTGAG
C5	Round2_29	/5Phos/CATCGGCGTACGACTATTGAGGAATCCACGTGCTTGAG
C6	Round2_30	/5Phos/CATCGGCGTACGACTCAACCACAATCCACGTGCTTGAG
C7	Round2_31	/5Phos/CATCGGCGTACGACTGACTAGTAATCCACGTGCTTGAG
C8	Round2_32	/5Phos/CATCGGCGTACGACTCAATGGAAATCCACGTGCTTGAG
C9	Round2_33	/5Phos/CATCGGCGTACGACTCACTTCGAATCCACGTGCTTGAG
C10	Round2_34	/5Phos/CATCGGCGTACGACTCAGCGTTAATCCACGTGCTTGAG
C11	Round2_35	/5Phos/CATCGGCGTACGACTCATACCAAATCCACGTGCTTGAG
C12	Round2_36	/5Phos/CATCGGCGTACGACTCCAGTTCAATCCACGTGCTTGAG
D1	Round2_37	/5Phos/CATCGGCGTACGACTCCGAAGTAATCCACGTGCTTGAG
D2	Round2_38	/5Phos/CATCGGCGTACGACTCCGTGAGAATCCACGTGCTTGAG
D3	Round2_39	/5Phos/CATCGGCGTACGACTCCTCCTGAATCCACGTGCTTGAG
D4	Round2_40	/5Phos/CATCGGCGTACGACTCGAACTTAATCCACGTGCTTGAG
D5	Round2_41	/5Phos/CATCGGCGTACGACTCGACTGGAATCCACGTGCTTGAG
D6	Round2_42	/5Phos/CATCGGCGTACGACTCGCATACAATCCACGTGCTTGAG
D7	Round2_43	/5Phos/CATCGGCGTACGACTCTCAATGAATCCACGTGCTTGAG
D8	Round2_44	/5Phos/CATCGGCGTACGACTCTGAGCCAATCCACGTGCTTGAG
D9	Round2_45	/5Phos/CATCGGCGTACGACTCTGGCATAATCCACGTGCTTGAG
D10	Round2_46	/5Phos/CATCGGCGTACGACTGAATCTGAATCCACGTGCTTGAG
D11	Round2_47	/5Phos/CATCGGCGTACGACTCAAGACTAATCCACGTGCTTGAG
D12	Round2_48	/5Phos/CATCGGCGTACGACTGAGCTGAAATCCACGTGCTTGAG
E1	Round2_49	/5Phos/CATCGGCGTACGACTGATAGACAATCCACGTGCTTGAG

Well Position	Name	Sequences (5'-3')
E2	Round2_50	/5Phos/CATCGGCGTACGACTGCCACATAATCCACGTGCTTGAG
E3	Round2_51	/5Phos/CATCGGCGTACGACTGCGAGTAAATCCACGTGCTTGAG
E4	Round2_52	/5Phos/CATCGGCGTACGACTGCTAACGAATCCACGTGCTTGAG
E5	Round2_53	/5Phos/CATCGGCGTACGACTGCTCGGTAATCCACGTGCTTGAG
E6	Round2_54	/5Phos/CATCGGCGTACGACTGGAGAACAATCCACGTGCTTGAG
E7	Round2_55	/5Phos/CATCGGCGTACGACTGGTGCGAAATCCACGTGCTTGAG
E8	Round2_56	/5Phos/CATCGGCGTACGACTGTACGCAAATCCACGTGCTTGAG
E9	Round2_57	/5Phos/CATCGGCGTACGACTGTCGTAGAATCCACGTGCTTGAG
E10	Round2_58	/5Phos/CATCGGCGTACGACTGTCTGTCAATCCACGTGCTTGAG
E11	Round2_59	/5Phos/CATCGGCGTACGACTGTGTTCTAATCCACGTGCTTGAG
E12	Round2_60	/5Phos/CATCGGCGTACGACTTAGGATGAATCCACGTGCTTGAG
F1	Round2_61	/5Phos/CATCGGCGTACGACTTATCAGCAATCCACGTGCTTGAG
F2	Round2_62	/5Phos/CATCGGCGTACGACTTCCGTCTAATCCACGTGCTTGAG
F3	Round2_63	/5Phos/CATCGGCGTACGACTTCTTCACAATCCACGTGCTTGAG
F4	Round2_64	/5Phos/CATCGGCGTACGACTTGAAGAGAATCCACGTGCTTGAG
F5	Round2_65	/5Phos/CATCGGCGTACGACTTGGAACAAATCCACGTGCTTGAG
F6	Round2_66	/5Phos/CATCGGCGTACGACTTGGCTTCAATCCACGTGCTTGAG
F7	Round2_67	/5Phos/CATCGGCGTACGACTTGGTGGTAATCCACGTGCTTGAG
F8	Round2_68	/5Phos/CATCGGCGTACGACTTTCACGCAATCCACGTGCTTGAG
F9	Round2_69	/5Phos/CATCGGCGTACGACTAACTCACCATCCACGTGCTTGAG
F10	Round2_70	/5Phos/CATCGGCGTACGACTAAGAGATCATCCACGTGCTTGAG
F11	Round2_71	/5Phos/CATCGGCGTACGACTAAGGACACATCCACGTGCTTGAG
F12	Round2_72	/5Phos/CATCGGCGTACGACTAATCCGTCATCCACGTGCTTGAG
G1	Round2_73	/5Phos/CATCGGCGTACGACTAATGTTGCATCCACGTGCTTGAG
G2	Round2_74	/5Phos/CATCGGCGTACGACTACACGACCATCCACGTGCTTGAG

Well Position	Name	Sequences (5'-3')
G3	Round2_75	/5Phos/CATCGGCGTACGACTACAGATTCATCCACGTGCTTGAG
G4	Round2_76	/5Phos/CATCGGCGTACGACTAGATGTACATCCACGTGCTTGAG
G5	Round2_77	/5Phos/CATCGGCGTACGACTAGCACCTCATCCACGTGCTTGAG
G6	Round2_78	/5Phos/CATCGGCGTACGACTAGCCATGCATCCACGTGCTTGAG
G7	Round2_79	/5Phos/CATCGGCGTACGACTAGGCTAACATCCACGTGCTTGAG
G8	Round2_80	/5Phos/CATCGGCGTACGACTATAGCGACATCCACGTGCTTGAG
G9	Round2_81	/5Phos/CATCGGCGTACGACTATCATTCCATCCACGTGCTTGAG
G10	Round2_82	/5Phos/CATCGGCGTACGACTATTGGCTCATCCACGTGCTTGAG
G11	Round2_83	/5Phos/CATCGGCGTACGACTCAAGGAGCATCCACGTGCTTGAG
G12	Round2_84	/5Phos/CATCGGCGTACGACTCACCTTACATCCACGTGCTTGAG
H1	Round2_85	/5Phos/CATCGGCGTACGACTCCATCCTCATCCACGTGCTTGAG
H2	Round2_86	/5Phos/CATCGGCGTACGACTCCGACAACATCCACGTGCTTGAG
H3	Round2_87	/5Phos/CATCGGCGTACGACTCCTAATCCATCCACGTGCTTGAG
H4	Round2_88	/5Phos/CATCGGCGTACGACTCCTCTATCATCCACGTGCTTGAG
H5	Round2_89	/5Phos/CATCGGCGTACGACTCGACACACATCCACGTGCTTGAG
H6	Round2_90	/5Phos/CATCGGCGTACGACTCGGATTGCATCCACGTGCTTGAG
H7	Round2_91	/5Phos/CATCGGCGTACGACTCTAAGGTCATCCACGTGCTTGAG
H8	Round2_92	/5Phos/CATCGGCGTACGACTGAACAGGCATCCACGTGCTTGAG
H9	Round2_93	/5Phos/CATCGGCGTACGACTGACAGTGCATCCACGTGCTTGAG
H10	Round2_94	/5Phos/CATCGGCGTACGACTGAGTTAGCATCCACGTGCTTGAG
H11	Round2_95	/5Phos/CATCGGCGTACGACTGATGAATCATCCACGTGCTTGAG
H12	Round2_96	/5Phos/CATCGGCGTACGACTGCCAAGACATCCACGTGCTTGAG

Supplementary Table 6 | Round 3 barcoded oligos[2]

Well Position	Name	Sequences (5'-3')
A1	Round3_01	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAACGTGATGTGGCCGATGTTTCG
A2	Round3_02	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAAACATCGGTGGCCGATGTTTCG
A3	Round3_03	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATGCCTAAGTGGCCGATGTTTCG
A4	Round3_04	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGTGGTCAGTGGCCGATGTTTCG
A5	Round3_05	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACCACTGTGTGGCCGATGTTTCG
A6	Round3_06	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACATTGGCGTGGCCGATGTTTCG
A7	Round3_07	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAGATCTGGTGGCCGATGTTTCG
A8	Round3_08	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCATCAAGTGTGGCCGATGTTTCG
A9	Round3_09	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGCTGATCGTGGCCGATGTTTCG
A10	Round3_10	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACAAGCTAGTGGCCGATGTTTCG
A11	Round3_11	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCTGTAGCCGTGGCCGATGTTTCG
A12	Round3_12	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGTACAAGGTGGCCGATGTTTCG
B1	Round3_13	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAACAACCAGTGGCCGATGTTTCG
B2	Round3_14	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAACCGAGAGTGGCCGATGTTTCG
B3	Round3_15	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAACGCTTAGTGGCCGATGTTTCG
B4	Round3_16	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAAGACGGAGTGGCCGATGTTTCG
B5	Round3_17	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAAGGTACAGTGGCCGATGTTTCG
B6	Round3_18	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACACAGAAGTGGCCGATGTTTCG
B7	Round3_19	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACAGCAGAGTGGCCGATGTTTCG
B8	Round3_20	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACCTCCAAGTGGCCGATGTTTCG
B9	Round3_21	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACGCTCGAGTGGCCGATGTTTCG
B10	Round3_22	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACGTATCAGTGGCCGATGTTTCG
B11	Round3_23	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACTATGCAGTGGCCGATGTTTCG
B12	Round3_24	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGAGTCAAGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
C1	Round3_25	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGATCGCAGTGGCCGATGTTTCG
C2	Round3_26	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGCAGGAAGTGGCCGATGTTTCG
C3	Round3_27	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGTCACTAGTGGCCGATGTTTCG
C4	Round3_28	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATCCTGTAGTGGCCGATGTTTCG
C5	Round3_29	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATTGAGGAGTGGCCGATGTTTCG
C6	Round3_30	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAACCACAGTGGCCGATGTTTCG
C7	Round3_31	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGACTAGTAGTGGCCGATGTTTCG
C8	Round3_32	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAATGGAAGTGGCCGATGTTTCG
C9	Round3_33	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCACTTCGAGTGGCCGATGTTTCG
C10	Round3_34	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAGCGTTAGTGGCCGATGTTTCG
C11	Round3_35	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCATACCAAGTGGCCGATGTTTCG
C12	Round3_36	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCAGTTCAGTGGCCGATGTTTCG
D1	Round3_37	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCGAAGTAGTGGCCGATGTTTCG
D2	Round3_38	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCGTGAGAGTGGCCGATGTTTCG
D3	Round3_39	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCTCCTGAGTGGCCGATGTTTCG
D4	Round3_40	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGAACTTAGTGGCCGATGTTTCG
D5	Round3_41	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGACTGGAGTGGCCGATGTTTCG
D6	Round3_42	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGCATACAGTGGCCGATGTTTCG
D7	Round3_43	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCTCAATGAGTGGCCGATGTTTCG
D8	Round3_44	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCTGAGCCAGTGGCCGATGTTTCG
D9	Round3_45	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCTGGCATAGTGGCCGATGTTTCG
D10	Round3_46	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGAATCTGAGTGGCCGATGTTTCG
D11	Round3_47	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAAGACTAGTGGCCGATGTTTCG
D12	Round3_48	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGAGCTGAAGTGGCCGATGTTTCG
E1	Round3_49	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGATAGACAGTGGCCGATGTTTCG
E2	Round3_50	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCCACATAGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
E3	Round3_51	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCGAGTAAGTGGCCGATGTTTCG
E4	Round3_52	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCTAACGAGTGGCCGATGTTTCG
E5	Round3_53	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCTCGGTAGTGGCCGATGTTTCG
E6	Round3_54	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGGAGAACAGTGGCCGATGTTTCG
E7	Round3_55	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGGTGCGAAGTGGCCGATGTTTCG
E8	Round3_56	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGTACGCAAGTGGCCGATGTTTCG
E9	Round3_57	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGTCGTAGAGTGGCCGATGTTTCG
E10	Round3_58	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGTCTGTCAGTGGCCGATGTTTCG
E11	Round3_59	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGTGTTCTAGTGGCCGATGTTTCG
E12	Round3_60	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTAGGATGAGTGGCCGATGTTTCG
F1	Round3_61	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTATCAGCAGTGGCCGATGTTTCG
F2	Round3_62	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTCCGTCTAGTGGCCGATGTTTCG
F3	Round3_63	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTCTTCACAGTGGCCGATGTTTCG
F4	Round3_64	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTGAAGAGAGTGGCCGATGTTTCG
F5	Round3_65	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTGGAACAAGTGGCCGATGTTTCG
F6	Round3_66	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTGGCTTCAGTGGCCGATGTTTCG
F7	Round3_67	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTGGTGGTAGTGGCCGATGTTTCG
F8	Round3_68	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNTTCACGCAGTGGCCGATGTTTCG
F9	Round3_69	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAACTCACCGTGGCCGATGTTTCG
F10	Round3_70	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAAGAGATCGTGGCCGATGTTTCG
F11	Round3_71	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAAGGACACGTGGCCGATGTTTCG
F12	Round3_72	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAATCCGTCGTGGCCGATGTTTCG
G1	Round3_73	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAATGTTGCGTGGCCGATGTTTCG
G2	Round3_74	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACACGACCGTGGCCGATGTTTCG
G3	Round3_75	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNACAGATTCGTGGCCGATGTTTCG
G4	Round3_76	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGATGTACGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
G5	Round3_77	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGCACCTCGTGGCCGATGTTTCG
G6	Round3_78	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGCCATGCGTGGCCGATGTTTCG
G7	Round3_79	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNAGGCTAACGTGGCCGATGTTTCG
G8	Round3_80	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATAGCGACGTGGCCGATGTTTCG
G9	Round3_81	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATCATTCCGTGGCCGATGTTTCG
G10	Round3_82	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNATTGGCTCGTGGCCGATGTTTCG
G11	Round3_83	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCAAGGAGCGTGGCCGATGTTTCG
G12	Round3_84	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCACCTTACGTGGCCGATGTTTCG
H1	Round3_85	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCATCCTCGTGGCCGATGTTTCG
H2	Round3_86	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCGACAACGTGGCCGATGTTTCG
H3	Round3_87	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCTAATCCGTGGCCGATGTTTCG
H4	Round3_88	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCCTCTATCGTGGCCGATGTTTCG
H5	Round3_89	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGACACACGTGGCCGATGTTTCG
H6	Round3_90	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGGATTGCGTGGCCGATGTTTCG
H7	Round3_91	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCTAAGGTCGTGGCCGATGTTTCG
H8	Round3_92	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGAACAGGCGTGGCCGATGTTTCG
H9	Round3_93	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGACAGTGCGTGGCCGATGTTTCG
H10	Round3_94	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGAGTTAGCGTGGCCGATGTTTCG
H11	Round3_95	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGATGAATCGTGGCCGATGTTTCG
H12	Round3_96	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCCAAGACGTGGCCGATGTTTCG

/5Biosg/ = 5' Biotin

Supplementary Table 7 | AC indexed PCR primers

Primer name	Sequences (5' - 3')
Ad1_N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTG
Ad1_N502	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTCAGATGTG
Ad1_N503	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTCAGATGTG
Ad1_N504	AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTCAGATGTG
Ad1_N505	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTCAGATGTG
Ad1_N506	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTCAGATGTG
Ad1_N507	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTCAGATGTG
Ad1_N508	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTCAGATGTG
Ad1_N510	AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTCGGCAGCGTCAGATGTG
Ad1_N511	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTCAGATGTG
Ad1_N513	AATGATACGGCGACCACCGAGATCTACACTCGACTAGTCGTCGGCAGCGTCAGATGTG
Ad1_N515	AATGATACGGCGACCACCGAGATCTACACTTCTAGCTTCGTCGGCAGCGTCAGATGTG
Ad1_N516	AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTCGTCGGCAGCGTCAGATGTG
Ad1_N517	AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTCAGATGTG
Ad1_N518	AATGATACGGCGACCACCGAGATCTACACCTATTAAGTCGTCGGCAGCGTCAGATGTG
Ad1_N520	AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTCGGCAGCGTCAGATGTG
Ad1_N521	AATGATACGGCGACCACCGAGATCTACACGAGCCTTATCGTCGGCAGCGTCAGATGTG
Ad1_N522	AATGATACGGCGACCACCGAGATCTACACTTATGCGATCGTCGGCAGCGTCAGATGTG
Ad1_N523	AATGATACGGCGACCACCGAGATCTACACTGACAAGCTCGTCGGCAGCGTCAGATGTG
Ad1_N524	AATGATACGGCGACCACCGAGATCTACACCTAGCTTGTCGTCGGCAGCGTCAGATGTG
Ad1_N525	AATGATACGGCGACCACCGAGATCTACACTCGATCCATCGTCGGCAGCGTCAGATGTG
Ad1_N526	AATGATACGGCGACCACCGAGATCTACACCCTGAACTTCGTCGGCAGCGTCAGATGTG
Ad1_N527	AATGATACGGCGACCACCGAGATCTACACTTCAGGTCTCGTCGGCAGCGTCAGATGTG
Ad1_N528	AATGATACGGCGACCACCGAGATCTACACAGTAGAGATCGTCGGCAGCGTCAGATGTG

Primer name	Sequences (5' - 3')
Ad1_N529	AATGATACGGCGACCACCGAGATCTACACGCAGAATCTCGTCGGCAGCGTCAGATGTG
Ad1_N530	AATGATACGGCGACCACCGAGATCTACACCACTACGATCGTCGGCAGCGTCAGATGTG
Ad1_N531	AATGATACGGCGACCACCGAGATCTACACTGTCGTAGTCGTCGGCAGCGTCAGATGTG
Ad1_N532	AATGATACGGCGACCACCGAGATCTACACACCACTTATCGTCGGCAGCGTCAGATGTG
Ad1_N533	AATGATACGGCGACCACCGAGATCTACACGTTGTCCGTCGTCGGCAGCGTCAGATGTG
Ad1_N534	AATGATACGGCGACCACCGAGATCTACACATCCATATTCGTCGGCAGCGTCAGATGTG
Ad1_N535	AATGATACGGCGACCACCGAGATCTACACGCTTGCGCTCGTCGGCAGCGTCAGATGTG
Ad1_N536	AATGATACGGCGACCACCGAGATCTACACAGTATCTTTCGTCGGCAGCGTCAGATGTG

Supplementary Table 8 | RNA indexed PCR primers

Primer name	Sequences (5' - 3')
SPLiT_N701	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N702	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N703	CAAGCAGAAGACGGCATACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N704	CAAGCAGAAGACGGCATACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N705	CAAGCAGAAGACGGCATACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N706	CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N707	CAAGCAGAAGACGGCATACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N708	CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N709	CAAGCAGAAGACGGCATACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N710	CAAGCAGAAGACGGCATACGAGATGGACGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N711	CAAGCAGAAGACGGCATACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N712	CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N713	CAAGCAGAAGACGGCATACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N714	CAAGCAGAAGACGGCATACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N715	CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N716	CAAGCAGAAGACGGCATACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N717	CAAGCAGAAGACGGCATACGAGATTCAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N718	CAAGCAGAAGACGGCATACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N719	CAAGCAGAAGACGGCATACGAGATTGCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N720	CAAGCAGAAGACGGCATACGAGATATTCCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N721	CAAGCAGAAGACGGCATACGAGATGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N722	CAAGCAGAAGACGGCATACGAGATCGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N723	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N724	CAAGCAGAAGACGGCATACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N725	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N726	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N727	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N728	CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N729	CAAGCAGAAGACGGCATACGAGATATCAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N730	CAAGCAGAAGACGGCATACGAGATGCTCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N731	CAAGCAGAAGACGGCATACGAGATCGCCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N732	CAAGCAGAAGACGGCATACGAGATGCCATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N733	CAAGCAGAAGACGGCATACGAGATTCTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Primer name	Sequences (5' - 3')
SPLiT_N734	CAAGCAGAAGACGGCATACGAGATGAATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N735	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N736	CAAGCAGAAGACGGCATACGAGATGTATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N737	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N738	CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N739	CAAGCAGAAGACGGCATACGAGATTGAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N740	CAAGCAGAAGACGGCATACGAGATATTATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Supplementary Table 9 | SNARE-seq2 sequencing primers

Primer name	Description	Sequence (5'-3')
SNARE2_Read1	- Read cDNA of RNA libraries (Read1 or R1 order). - Read 1st accessible chromatin of AC libraries (Read1 or R1 order).	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
SNARE2-R _Index1	Read Index1 (i7) of RNA libraries (Index1 or R2 order).	AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC
SNARE2-R _Read2	Read cell barcodes and UMI of RNA libraries (Read2 or R3 order).	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SNARE2-AC _BCread	Read cell barcodes and UMI region of AC libraries (Index1 or R2 order).	CCACGAGACCTGTAGACATAGTCTGCAGTACGT
SNARE2-AC _Read2	Read 2nd accessible chromatin of AC libraries (Read2 or R4 order).	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
PhiX_Read1	- Read 1st PhiX sequence in Read1 or R1 order of RNA library sequencing. - Read 1st PhiX sequence in Read1 or R1 order of AC library sequencing. - Read 1st PhiX sequence in Index1 or R2 order of RNA library sequencing. - Read 1st PhiX sequence in Index1 or R2 order of AC library sequencing.	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
PhiX_Read2	- Read 2nd PhiX sequence in Read2 or R3 order of RNA library sequencing. - Read 2nd PhiX sequence in Read2 or R4 order of AC library sequencing	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

Reference

1. Corces, M.R. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. *Nat Methods* **14**, 959-962 (2017).
2. Rosenberg, A.B. et al. Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science* **360**, 176-182 (2018).