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## Supplementary information

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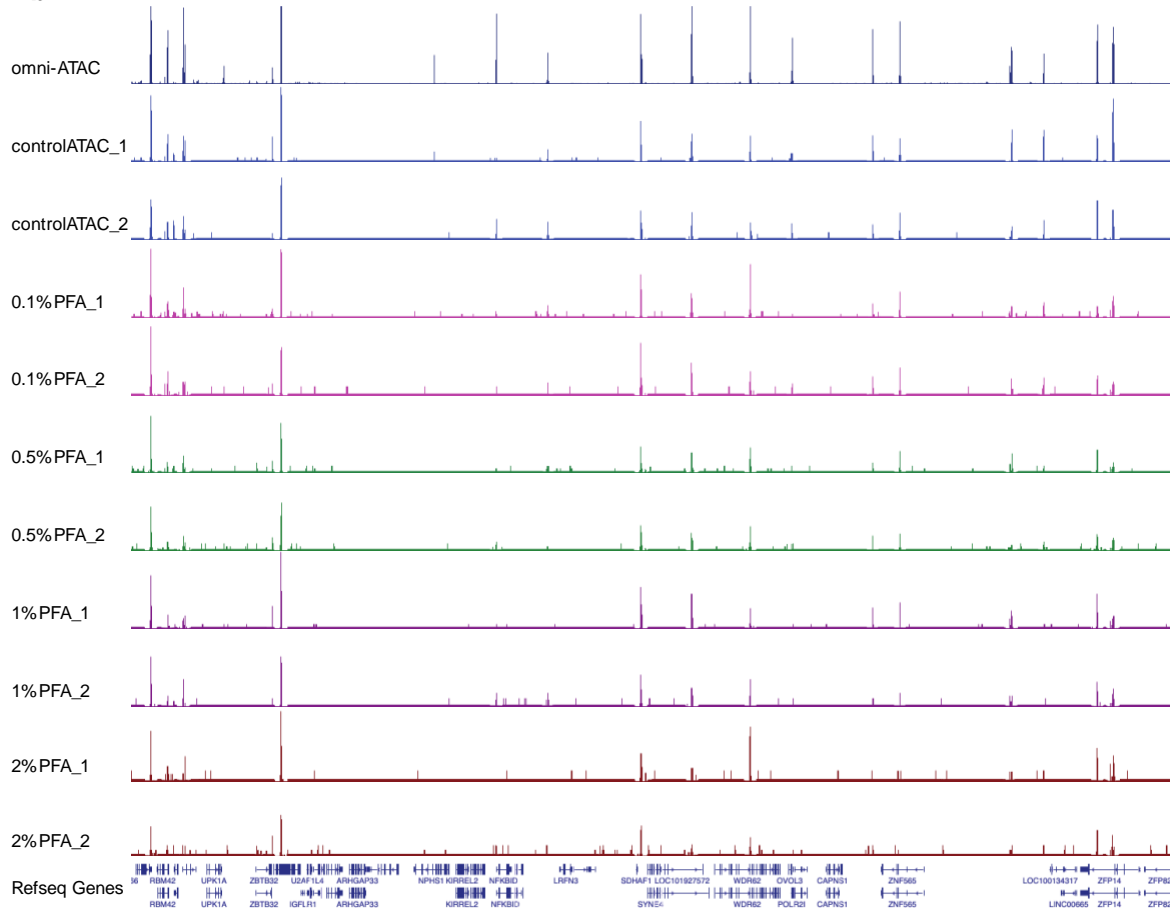
# Scalable dual-omics profiling with single-nucleus chromatin accessibility and mRNA expression sequencing 2 (SNARE-seq2)

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In the format provided by the  
authors and unedited

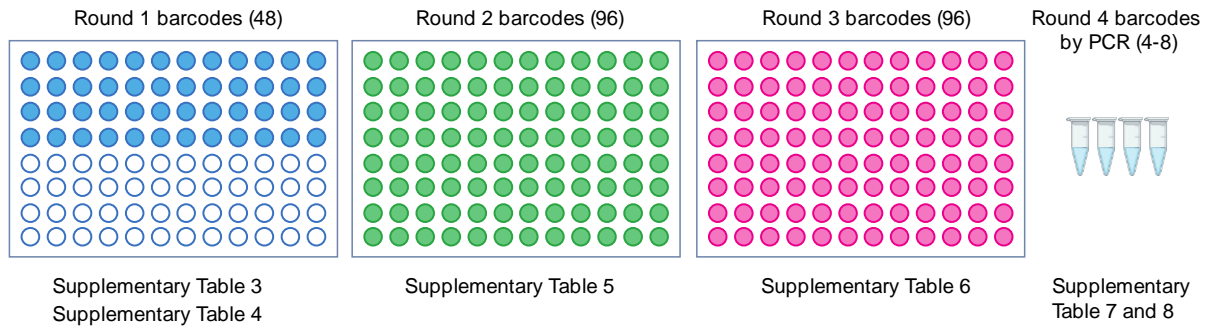
**a**

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<sup>1</sup> (10 millions subsampled reads) on the region of chromosome 19.

**a**

Round 1 barcoded oligo

RT (dT) Round 1 barcoded oligo

5' /5Phos/AGGCCAGAGCATTTCG **NNNNNNNN** TTTTTTTTTTTTTTNN 3'

RT (N6) Round 1 barcoded oligo

5' /5Phos/ AGGCCAGAGCATTTCG **NNNNNNNN** [NNNNNN] [NNNNNN] = random hexamer (N6)

AC Round 1 barcoded oligo

5' /Phos/AGGCCAGAGCATTTCG **NNNNNNNN** ACGTACTGCAGACTATGTCTACAG 3'

3' /InvdT/TGCATGACGTCTGATACAGATGTCCAGAGCACCCGAGCC 5'

AC Round 1 linker

Round 2 barcoded oligo

/5Phos/CATCGGCGTACGACT **NNNNNNNN** ATCCACGTGCTTGAG 3'

3' TAGGTGCACGAACCTCTCCGGTCTCGTAAGC 5'

Round 2 linker

Round 3 barcoded oligo

/5Biosg/CAGACGTGTGCTCTTCCGATCT [ **NNNNNNNNNN** ] **NNNNNNNN** GTGGCCGATGTTTCG 3'

3' CACCGGCTACAAAGCGTAGCCGCATGCTGA 5'

Round 3 linker

[**NNNNNNNNNN**] = unique molecular identifiers (UMI)

**b**

Round 2 barcoded oligo

/5Phos/CATCGGCGTACGACT **NNNNNNNN** ATCCACGTGCTTGAG **AGGCCAGAGCATTTCG** 3' **Steps 40-42**

3' TAGGTGCACGAACCTCTCCGGTCTCGTAAGC 5'

Round 2 linker

5' **ATCCACGTGCTTGAGAGGCCAGAGCATTTCG** 3'

Round 2 blocking oligo

Round 3 barcoded oligo

/5Biosg/CAGACGTGTGCTCTTCCGATCT [ **NNNNNNNNNN** ] **NNNNNNNN** GTGGCCGATGTTTCG **CATCGGCGTACGACT** 3' **Steps 47-48**

3' CACCGGCTACAAAGCGTAGCCGCATGCTGA 5'

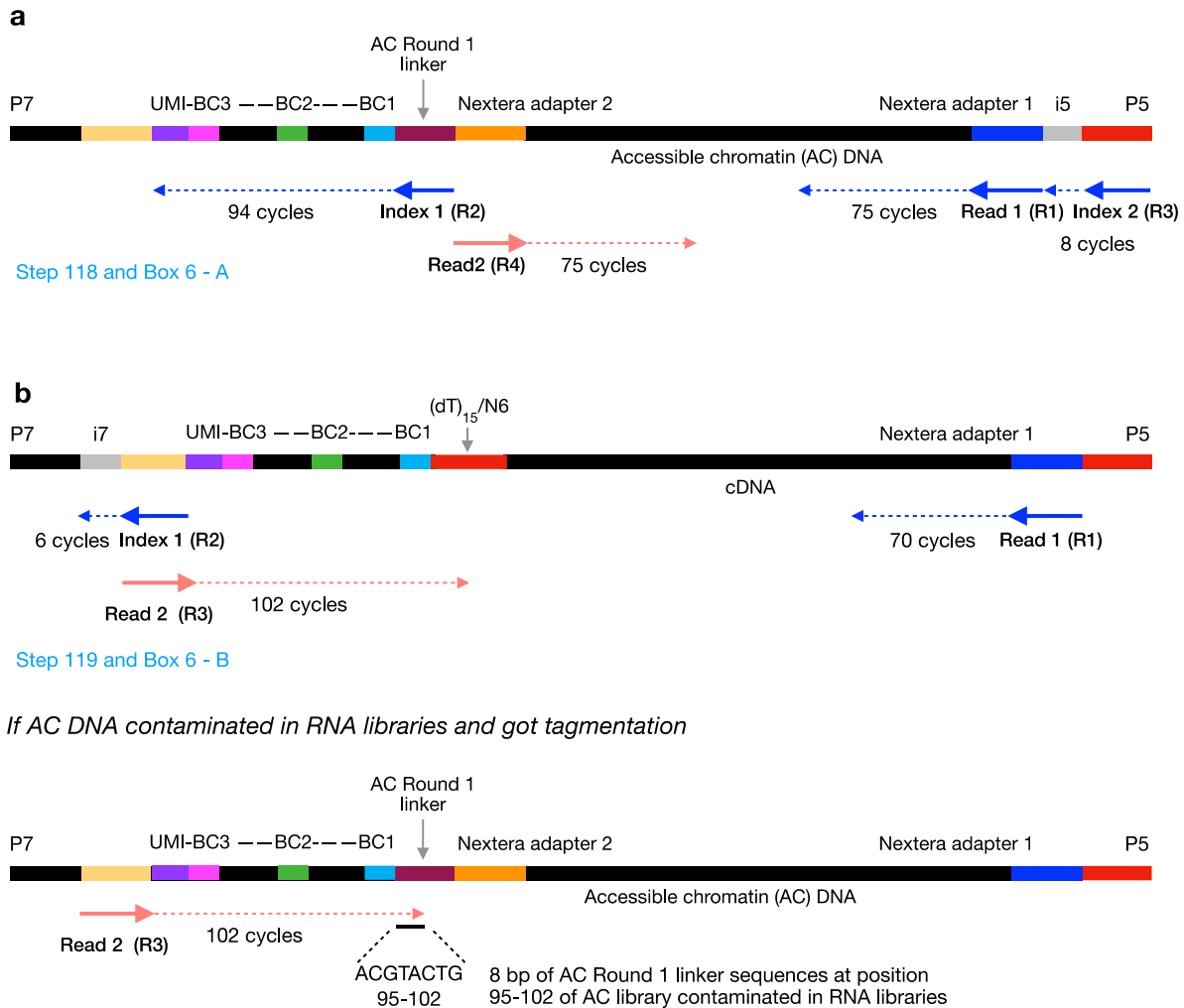
Round 3 linker

5' **GTGGCCGATGTTTCGCATCGGCGTACGACT** 3'

Round 3 blocking oligo

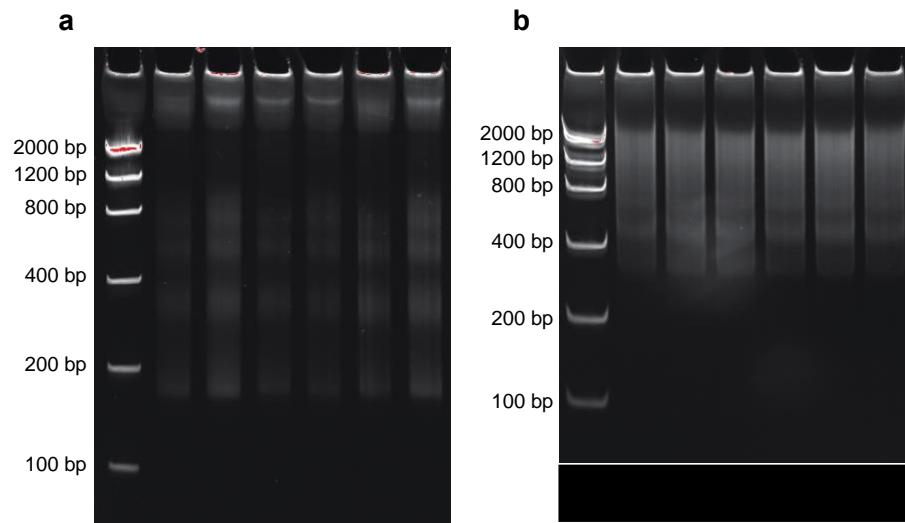
**Supplementary Fig. 2 | Schematics of Round 1-3 barcoded oligos and Round 2-3 blocking oligos.**

**a**, Schemes showing sequences of barcoded oligos in each round and their 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.



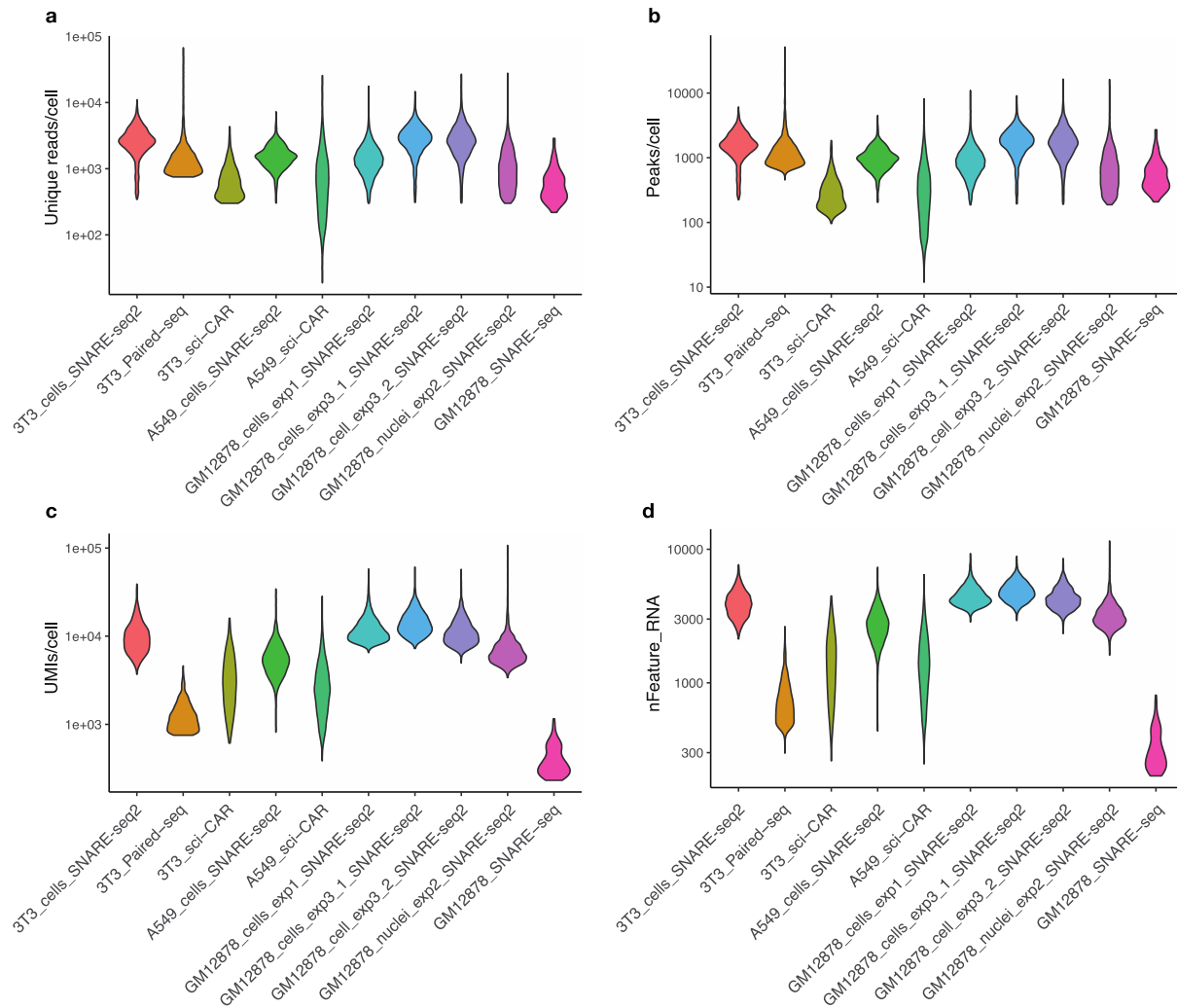
### Supplementary Fig. 3 | Sequencing schemes of SNARE-seq2.

**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
<b>Total cost of day 1 experiment</b>			<b>809.11</b>

**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 $\mu$ m	PALL Corporation	ODM02C35	23.184
<b>Total cost of post-day 1 experiment</b>			<b>623.71</b>

### 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
<b>Total of sequencing cost</b>					<b>14,550</b>

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	CAAGCAGAAGACGGCATAACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATAACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATAACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATAACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATAACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT

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

Well Position	Name	Sequence (5'-3')
A1	AC_v1.2_R1_01	/5Phos/AGGCCAGAGCATTCTGAACGTGATACGTACTGCAGACTATGTCTACAG
A2	AC_v1.2_R1_02	/5Phos/AGGCCAGAGCATTCTGAAACATCGACGTACTGCAGACTATGTCTACAG
A3	AC_v1.2_R1_03	/5Phos/AGGCCAGAGCATTCTGATGCCTAAACGTACTGCAGACTATGTCTACAG
A4	AC_v1.2_R1_04	/5Phos/AGGCCAGAGCATTCTGAGTGGTCAACGTACTGCAGACTATGTCTACAG
A5	AC_v1.2_R1_05	/5Phos/AGGCCAGAGCATTCTGACCACTGTACGTACTGCAGACTATGTCTACAG
A6	AC_v1.2_R1_06	/5Phos/AGGCCAGAGCATTCTGACATTGGCACGTACTGCAGACTATGTCTACAG
A7	AC_v1.2_R1_07	/5Phos/AGGCCAGAGCATTCTGCAGATCTGACGTACTGCAGACTATGTCTACAG
A8	AC_v1.2_R1_08	/5Phos/AGGCCAGAGCATTCTGCATCAAGTACGTACTGCAGACTATGTCTACAG
A9	AC_v1.2_R1_09	/5Phos/AGGCCAGAGCATTCTGCGCTGATCACGTACTGCAGACTATGTCTACAG
A10	AC_v1.2_R1_10	/5Phos/AGGCCAGAGCATTCTGACAAGCTAACGTACTGCAGACTATGTCTACAG
A11	AC_v1.2_R1_11	/5Phos/AGGCCAGAGCATTCTGCTGTAGCCACGTACTGCAGACTATGTCTACAG
A12	AC_v1.2_R1_12	/5Phos/AGGCCAGAGCATTCTGAGTACAAGACGTACTGCAGACTATGTCTACAG
B1	AC_v1.2_R1_13	/5Phos/AGGCCAGAGCATTCTGAACAACCAACGTACTGCAGACTATGTCTACAG
B2	AC_v1.2_R1_14	/5Phos/AGGCCAGAGCATTCTGAACCGAGAACGTACTGCAGACTATGTCTACAG
B3	AC_v1.2_R1_15	/5Phos/AGGCCAGAGCATTCTGAACGCTTAACGTACTGCAGACTATGTCTACAG
B4	AC_v1.2_R1_16	/5Phos/AGGCCAGAGCATTCTGAAGACGGAACGTACTGCAGACTATGTCTACAG
B5	AC_v1.2_R1_17	/5Phos/AGGCCAGAGCATTCTGAAGGTACAACGTACTGCAGACTATGTCTACAG
B6	AC_v1.2_R1_18	/5Phos/AGGCCAGAGCATTCTGACACAGAAACGTACTGCAGACTATGTCTACAG
B7	AC_v1.2_R1_19	/5Phos/AGGCCAGAGCATTCTGACAGCAGAACGTACTGCAGACTATGTCTACAG
B8	AC_v1.2_R1_20	/5Phos/AGGCCAGAGCATTCTGACCTCCAAACGTACTGCAGACTATGTCTACAG
B9	AC_v1.2_R1_21	/5Phos/AGGCCAGAGCATTCTGACGCTCGAACGTACTGCAGACTATGTCTACAG
B10	AC_v1.2_R1_22	/5Phos/AGGCCAGAGCATTCTGACGTATCAACGTACTGCAGACTATGTCTACAG
B11	AC_v1.2_R1_23	/5Phos/AGGCCAGAGCATTCTGACTATGCAACGTACTGCAGACTATGTCTACAG
B12	AC_v1.2_R1_24	/5Phos/AGGCCAGAGCATTCTGAGAGTCAAACGTACTGCAGACTATGTCTACAG

Well Position	Name	Sequence (5'-3')
C1	AC_v1.2_R1_25	/5Phos/AGGCCAGAGCATTTCGAGATCGCAACGTACTGCAGACTATGTCTACAG
C2	AC_v1.2_R1_26	/5Phos/AGGCCAGAGCATTTCGAGCAGGAAACGTACTGCAGACTATGTCTACAG
C3	AC_v1.2_R1_27	/5Phos/AGGCCAGAGCATTTCGAGTCACTAACGTACTGCAGACTATGTCTACAG
C4	AC_v1.2_R1_28	/5Phos/AGGCCAGAGCATTTCGATCCTGTAACGTACTGCAGACTATGTCTACAG
C5	AC_v1.2_R1_29	/5Phos/AGGCCAGAGCATTTCGATTGAGGAACGTACTGCAGACTATGTCTACAG
C6	AC_v1.2_R1_30	/5Phos/AGGCCAGAGCATTTCGCAACCACAACGTACTGCAGACTATGTCTACAG
C7	AC_v1.2_R1_31	/5Phos/AGGCCAGAGCATTTCGGACTAGTAACGTACTGCAGACTATGTCTACAG
C8	AC_v1.2_R1_32	/5Phos/AGGCCAGAGCATTTCGCAATGGAAACGTACTGCAGACTATGTCTACAG
C9	AC_v1.2_R1_33	/5Phos/AGGCCAGAGCATTTCGCACTTCGAACGTACTGCAGACTATGTCTACAG
C10	AC_v1.2_R1_34	/5Phos/AGGCCAGAGCATTTCGCAGCGTTAACGTACTGCAGACTATGTCTACAG
C11	AC_v1.2_R1_35	/5Phos/AGGCCAGAGCATTTCGCATACCAAACGTACTGCAGACTATGTCTACAG
C12	AC_v1.2_R1_36	/5Phos/AGGCCAGAGCATTTCGCCAGTTCAACGTACTGCAGACTATGTCTACAG
D1	AC_v1.2_R1_37	/5Phos/AGGCCAGAGCATTTCGCCGAAGTAACGTACTGCAGACTATGTCTACAG
D2	AC_v1.2_R1_38	/5Phos/AGGCCAGAGCATTTCGCCGTGAGAACGTACTGCAGACTATGTCTACAG
D3	AC_v1.2_R1_39	/5Phos/AGGCCAGAGCATTTCGCCTCCTGAACGTACTGCAGACTATGTCTACAG
D4	AC_v1.2_R1_40	/5Phos/AGGCCAGAGCATTTCGCGAACTTAACGTACTGCAGACTATGTCTACAG
D5	AC_v1.2_R1_41	/5Phos/AGGCCAGAGCATTTCGCGACTGGAACGTACTGCAGACTATGTCTACAG
D6	AC_v1.2_R1_42	/5Phos/AGGCCAGAGCATTTCGCGCATACAACGTACTGCAGACTATGTCTACAG
D7	AC_v1.2_R1_43	/5Phos/AGGCCAGAGCATTTCGCTCAATGAACGTACTGCAGACTATGTCTACAG
D8	AC_v1.2_R1_44	/5Phos/AGGCCAGAGCATTTCGCTGAGCCAACGTACTGCAGACTATGTCTACAG
D9	AC_v1.2_R1_45	/5Phos/AGGCCAGAGCATTTCGCTGGCATAACGTACTGCAGACTATGTCTACAG
D10	AC_v1.2_R1_46	/5Phos/AGGCCAGAGCATTTCGGAATCTGAACGTACTGCAGACTATGTCTACAG
D11	AC_v1.2_R1_47	/5Phos/AGGCCAGAGCATTTCGCAAGACTAACGTACTGCAGACTATGTCTACAG
D12	AC_v1.2_R1_48	/5Phos/AGGCCAGAGCATTTCGGAGCTGAAACGTACTGCAGACTATGTCTACAG

Supplementary Table 4 | Reverse transcription (RT) Round 1 barcoded oligos<sup>2</sup>

Well Position	Oligo Type	Name	Sequences (5' - 3')
A1	dt(15)VN	Round1_01	/5Phos/AGGCCAGAGCATTCTGAACGTGATTTTTTTTTTTTTTTTVN
A2	dt(15)VN	Round1_02	/5Phos/AGGCCAGAGCATTCTGAAACATCGTTTTTTTTTTTTTTTVN
A3	dt(15)VN	Round1_03	/5Phos/AGGCCAGAGCATTCTGATGCCTAATTTTTTTTTTTTTTTTVN
A4	dt(15)VN	Round1_04	/5Phos/AGGCCAGAGCATTCTGAGTGGTCATTTTTTTTTTTTTTTTVN
A5	dt(15)VN	Round1_05	/5Phos/AGGCCAGAGCATTCTGACCACTGTTTTTTTTTTTTTTTVN
A6	dt(15)VN	Round1_06	/5Phos/AGGCCAGAGCATTCTGACATTGGCTTTTTTTTTTTTTTTTVN
A7	dt(15)VN	Round1_07	/5Phos/AGGCCAGAGCATTCTGCAGATCTGTTTTTTTTTTTTTTTVN
A8	dt(15)VN	Round1_08	/5Phos/AGGCCAGAGCATTCTGCATCAAGTTTTTTTTTTTTTTTVN
A9	dt(15)VN	Round1_09	/5Phos/AGGCCAGAGCATTCTGCGCTGATCTTTTTTTTTTTTTTTTVN
A10	dt(15)VN	Round1_10	/5Phos/AGGCCAGAGCATTCTGACAAGCTATTTTTTTTTTTTTTTTVN
A11	dt(15)VN	Round1_11	/5Phos/AGGCCAGAGCATTCTGCTGTAGCCTTTTTTTTTTTTTTTTVN
A12	dt(15)VN	Round1_12	/5Phos/AGGCCAGAGCATTCTGAGTACAAGTTTTTTTTTTTTTTTVN
B1	dt(15)VN	Round1_13	/5Phos/AGGCCAGAGCATTCTGAACAACCATTTTTTTTTTTTTTTTVN
B2	dt(15)VN	Round1_14	/5Phos/AGGCCAGAGCATTCTGAACCGAGATTTTTTTTTTTTTTTTVN
B3	dt(15)VN	Round1_15	/5Phos/AGGCCAGAGCATTCTGAACGCTTATTTTTTTTTTTTTTTTVN
B4	dt(15)VN	Round1_16	/5Phos/AGGCCAGAGCATTCTGAAGACGGATTTTTTTTTTTTTTTTVN
B5	dt(15)VN	Round1_17	/5Phos/AGGCCAGAGCATTCTGAAGGTACATTTTTTTTTTTTTTTTVN
B6	dt(15)VN	Round1_18	/5Phos/AGGCCAGAGCATTCTGACACAGAATTTTTTTTTTTTTTTTVN
B7	dt(15)VN	Round1_19	/5Phos/AGGCCAGAGCATTCTGACAGCAGATTTTTTTTTTTTTTTTVN
B8	dt(15)VN	Round1_20	/5Phos/AGGCCAGAGCATTCTGACCTCCAATTTTTTTTTTTTTTTTVN
B9	dt(15)VN	Round1_21	/5Phos/AGGCCAGAGCATTCTGACGCTCGATTTTTTTTTTTTTTTTVN
B10	dt(15)VN	Round1_22	/5Phos/AGGCCAGAGCATTCTGACGTATCATTTTTTTTTTTTTTTTVN
B11	dt(15)VN	Round1_23	/5Phos/AGGCCAGAGCATTCTGACTATGCATTTTTTTTTTTTTTTTVN
B12	dt(15)VN	Round1_24	/5Phos/AGGCCAGAGCATTCTGAGAGTCAATTTTTTTTTTTTTTTTVN

Well Position	Oligo Type	Name	Sequences (5' - 3')
C1	dt(15)VN	Round1_25	/5Phos/AGGCCAGAGCATTGAGATCGCATTTTTTTTTTTTTTTVN
C2	dt(15)VN	Round1_26	/5Phos/AGGCCAGAGCATTGAGCAGGAATTTTTTTTTTTTTTTVN
C3	dt(15)VN	Round1_27	/5Phos/AGGCCAGAGCATTGAGTCACTATTTTTTTTTTTTTTTVN
C4	dt(15)VN	Round1_28	/5Phos/AGGCCAGAGCATTGATCCTGTATTTTTTTTTTTTTTTVN
C5	dt(15)VN	Round1_29	/5Phos/AGGCCAGAGCATTGATTGAGGATTTTTTTTTTTTTTTVN
C6	dt(15)VN	Round1_30	/5Phos/AGGCCAGAGCATTGCAACCACATTTTTTTTTTTTTTTVN
C7	dt(15)VN	Round1_31	/5Phos/AGGCCAGAGCATTGGACTAGTATTTTTTTTTTTTTTTVN
C8	dt(15)VN	Round1_32	/5Phos/AGGCCAGAGCATTGCAATGGAATTTTTTTTTTTTTTTVN
C9	dt(15)VN	Round1_33	/5Phos/AGGCCAGAGCATTGCACTTCGATTTTTTTTTTTTTTTVN
C10	dt(15)VN	Round1_34	/5Phos/AGGCCAGAGCATTGCGAGCGTTATTTTTTTTTTTTTTTVN
C11	dt(15)VN	Round1_35	/5Phos/AGGCCAGAGCATTGCATACCAATTTTTTTTTTTTTTTVN
C12	dt(15)VN	Round1_36	/5Phos/AGGCCAGAGCATTGCCAGTTCATTTTTTTTTTTTTTTVN
D1	dt(15)VN	Round1_37	/5Phos/AGGCCAGAGCATTGCCGAAGTATTTTTTTTTTTTTTTVN
D2	dt(15)VN	Round1_38	/5Phos/AGGCCAGAGCATTGCCCGTGAGATTTTTTTTTTTTTTTVN
D3	dt(15)VN	Round1_39	/5Phos/AGGCCAGAGCATTGCCTCCTGATTTTTTTTTTTTTTTVN
D4	dt(15)VN	Round1_40	/5Phos/AGGCCAGAGCATTGCGAAGTTATTTTTTTTTTTTTTTVN
D5	dt(15)VN	Round1_41	/5Phos/AGGCCAGAGCATTGCGGACTGGATTTTTTTTTTTTTTTVN
D6	dt(15)VN	Round1_42	/5Phos/AGGCCAGAGCATTGCGGCATACATTTTTTTTTTTTTTTVN
D7	dt(15)VN	Round1_43	/5Phos/AGGCCAGAGCATTGCTCAATGATTTTTTTTTTTTTTTVN
D8	dt(15)VN	Round1_44	/5Phos/AGGCCAGAGCATTGCTGAGCCATTTTTTTTTTTTTTTVN
D9	dt(15)VN	Round1_45	/5Phos/AGGCCAGAGCATTGCTGGCATATTTTTTTTTTTTTTTVN
D10	dt(15)VN	Round1_46	/5Phos/AGGCCAGAGCATTGGAATCTGATTTTTTTTTTTTTTTVN
D11	dt(15)VN	Round1_47	/5Phos/AGGCCAGAGCATTGCAAGACTATTTTTTTTTTTTTTTVN
D12	dt(15)VN	Round1_48	/5Phos/AGGCCAGAGCATTGGAGCTGAATTTTTTTTTTTTTTTVN
E1	random hexamer	Round1_49	/5Phos/AGGCCAGAGCATTGGATAGACANNNNNN

Well Position	Oligo Type	Name	Sequences (5' - 3')
E2	random hexamer	Round1_50	/5Phos/AGGCCAGAGCATTCTGGCCACATANNNNNN
E3	random hexamer	Round1_51	/5Phos/AGGCCAGAGCATTCTGGCGAGTAANNNNNN
E4	random hexamer	Round1_52	/5Phos/AGGCCAGAGCATTCTGGCTAACGANNNNNN
E5	random hexamer	Round1_53	/5Phos/AGGCCAGAGCATTCTGGCTCGGTANNNNNN
E6	random hexamer	Round1_54	/5Phos/AGGCCAGAGCATTCTGGGAGAACANNNNNN
E7	random hexamer	Round1_55	/5Phos/AGGCCAGAGCATTCTGGGTGCGAANNNNNN
E8	random hexamer	Round1_56	/5Phos/AGGCCAGAGCATTCTGGTACGCAANNNNNN
E9	random hexamer	Round1_57	/5Phos/AGGCCAGAGCATTCTGGTCGTAGANNNNNN
E10	random hexamer	Round1_58	/5Phos/AGGCCAGAGCATTCTGGTCTGTCANNNNNN
E11	random hexamer	Round1_59	/5Phos/AGGCCAGAGCATTCTGGTGTTCANNNNNN
E12	random hexamer	Round1_60	/5Phos/AGGCCAGAGCATTCTGTAGGATGANNNNNN
F1	random hexamer	Round1_61	/5Phos/AGGCCAGAGCATTCTGTATCAGCANNNNNN
F2	random hexamer	Round1_62	/5Phos/AGGCCAGAGCATTCTGTCCGTCTANNNNNN
F3	random hexamer	Round1_63	/5Phos/AGGCCAGAGCATTCTGTCTTCACANNNNNN
F4	random hexamer	Round1_64	/5Phos/AGGCCAGAGCATTCTGTGAAGAGANNNNNN
F5	random hexamer	Round1_65	/5Phos/AGGCCAGAGCATTCTGTGGAACAANNNNNN
F6	random hexamer	Round1_66	/5Phos/AGGCCAGAGCATTCTGTGGCTTCANNNNNN
F7	random hexamer	Round1_67	/5Phos/AGGCCAGAGCATTCTGTGGTGGTANNNNNN
F8	random hexamer	Round1_68	/5Phos/AGGCCAGAGCATTCTGTTCACGCANNNNNN
F9	random hexamer	Round1_69	/5Phos/AGGCCAGAGCATTCTGAACCTCACNNNNNN
F10	random hexamer	Round1_70	/5Phos/AGGCCAGAGCATTCTGAAGAGATCNNNNNN
F11	random hexamer	Round1_71	/5Phos/AGGCCAGAGCATTCTGAAGGACACNNNNNN
F12	random hexamer	Round1_72	/5Phos/AGGCCAGAGCATTCTGAATCCGTCNNNNNN
G1	random hexamer	Round1_73	/5Phos/AGGCCAGAGCATTCTGAATGTTGCNNNNNN
G2	random hexamer	Round1_74	/5Phos/AGGCCAGAGCATTCTGACACGACCNNNNNN

Well Position	Oligo Type	Name	Sequences (5' - 3')
G3	random hexamer	Round1_75	/5Phos/AGGCCAGAGCATTTCGACAGATTCNNNNNN
G4	random hexamer	Round1_76	/5Phos/AGGCCAGAGCATTTCGAGATGTACNNNNNN
G5	random hexamer	Round1_77	/5Phos/AGGCCAGAGCATTTCGAGCACCTCNNNNNN
G6	random hexamer	Round1_78	/5Phos/AGGCCAGAGCATTTCGAGCCATGCNNNNNN
G7	random hexamer	Round1_79	/5Phos/AGGCCAGAGCATTTCGAGGCTAACNNNNNN
G8	random hexamer	Round1_80	/5Phos/AGGCCAGAGCATTTCGATAGCGACNNNNNN
G9	random hexamer	Round1_81	/5Phos/AGGCCAGAGCATTTCGATCATTCCNNNNNN
G10	random hexamer	Round1_82	/5Phos/AGGCCAGAGCATTTCGATTGGCTCNNNNNN
G11	random hexamer	Round1_83	/5Phos/AGGCCAGAGCATTTCGCAAGGAGCNNNNNN
G12	random hexamer	Round1_84	/5Phos/AGGCCAGAGCATTTCGCACCTTACNNNNNN
H1	random hexamer	Round1_85	/5Phos/AGGCCAGAGCATTTCGCCATCCTCNNNNNN
H2	random hexamer	Round1_86	/5Phos/AGGCCAGAGCATTTCGCCGACAACNNNNNN
H3	random hexamer	Round1_87	/5Phos/AGGCCAGAGCATTTCGCCTAATCCNNNNNN
H4	random hexamer	Round1_88	/5Phos/AGGCCAGAGCATTTCGCCTCTATCNNNNNN
H5	random hexamer	Round1_89	/5Phos/AGGCCAGAGCATTTCGCGACACACNNNNNN
H6	random hexamer	Round1_90	/5Phos/AGGCCAGAGCATTTCGCGGATTGCNNNNNN
H7	random hexamer	Round1_91	/5Phos/AGGCCAGAGCATTTCGCTAAGGTCNNNNNN
H8	random hexamer	Round1_92	/5Phos/AGGCCAGAGCATTTCGGAACAGGCNNNNNN
H9	random hexamer	Round1_93	/5Phos/AGGCCAGAGCATTTCGGACAGTGCNNNNNN
H10	random hexamer	Round1_94	/5Phos/AGGCCAGAGCATTTCGGAGTTAGCNNNNNN
H11	random hexamer	Round1_95	/5Phos/AGGCCAGAGCATTTCGGATGAATCNNNNNN
H12	random hexamer	Round1_96	/5Phos/AGGCCAGAGCATTTCGGCCAAGACNNNNNN

V = A,C,G

Supplementary Table 5 | Round 2 barcoded oligos<sup>2</sup>

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/CATCGGCGTACGACTGTCTAGAAATCCACGTGCTTGAG
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/CATCGGCGTACGACTTGAACAAATCCACGTGCTTGAG
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/CATCGGCGTACGACTATCATTCATCCACGTGCTTGAG
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<sup>2</sup>

Well Position	Name	Sequences (5'-3')
A1	Round3_01	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAACGTGATGTGGCCGATGTTTCG
A2	Round3_02	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAAACATCGGTGGCCGATGTTTCG
A3	Round3_03	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATGCCTAAGTGGCCGATGTTTCG
A4	Round3_04	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGTGGTCAGTGGCCGATGTTTCG
A5	Round3_05	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACCACTGTGTGGCCGATGTTTCG
A6	Round3_06	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACATTGGCGTGGCCGATGTTTCG
A7	Round3_07	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAGATCTGGTGGCCGATGTTTCG
A8	Round3_08	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCATCAAGTGTGGCCGATGTTTCG
A9	Round3_09	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGCTGATCGTGGCCGATGTTTCG
A10	Round3_10	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACAAGCTAGTGGCCGATGTTTCG
A11	Round3_11	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCTGTAGCCGTGGCCGATGTTTCG
A12	Round3_12	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGTACAAGGTGGCCGATGTTTCG
B1	Round3_13	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAACAACCAGTGGCCGATGTTTCG
B2	Round3_14	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAACCGAGAGTGGCCGATGTTTCG
B3	Round3_15	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAACGCTTAGTGGCCGATGTTTCG
B4	Round3_16	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAAGACGGAGTGGCCGATGTTTCG
B5	Round3_17	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAAGGTACAGTGGCCGATGTTTCG
B6	Round3_18	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACACAGAAGTGGCCGATGTTTCG
B7	Round3_19	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACAGCAGAGTGGCCGATGTTTCG
B8	Round3_20	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACCTCCAAGTGGCCGATGTTTCG
B9	Round3_21	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACGCTCGAGTGGCCGATGTTTCG
B10	Round3_22	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACGTATCAGTGGCCGATGTTTCG
B11	Round3_23	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACTATGCAGTGGCCGATGTTTCG
B12	Round3_24	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGAGTCAAGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
C1	Round3_25	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGATCGCAGTGGCCGATGTTTCG
C2	Round3_26	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGCAGGAAGTGGCCGATGTTTCG
C3	Round3_27	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGTCACTAGTGGCCGATGTTTCG
C4	Round3_28	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATCCTGTAGTGGCCGATGTTTCG
C5	Round3_29	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATTGAGGAGTGGCCGATGTTTCG
C6	Round3_30	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAACCACAGTGGCCGATGTTTCG
C7	Round3_31	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGACTAGTAGTGGCCGATGTTTCG
C8	Round3_32	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAATGGAAGTGGCCGATGTTTCG
C9	Round3_33	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCACTTCGAGTGGCCGATGTTTCG
C10	Round3_34	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAGCGTTAGTGGCCGATGTTTCG
C11	Round3_35	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCATACCAAGTGGCCGATGTTTCG
C12	Round3_36	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCAGTTCAGTGGCCGATGTTTCG
D1	Round3_37	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCGAAGTAGTGGCCGATGTTTCG
D2	Round3_38	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCGTGAGAGTGGCCGATGTTTCG
D3	Round3_39	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCTCCTGAGTGGCCGATGTTTCG
D4	Round3_40	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGAACTTAGTGGCCGATGTTTCG
D5	Round3_41	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGACTGGAGTGGCCGATGTTTCG
D6	Round3_42	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGCATACAGTGGCCGATGTTTCG
D7	Round3_43	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCTCAATGAGTGGCCGATGTTTCG
D8	Round3_44	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCTGAGCCAGTGGCCGATGTTTCG
D9	Round3_45	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCTGGCATAGTGGCCGATGTTTCG
D10	Round3_46	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGAATCTGAGTGGCCGATGTTTCG
D11	Round3_47	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAAGACTAGTGGCCGATGTTTCG
D12	Round3_48	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGAGCTGAAGTGGCCGATGTTTCG
E1	Round3_49	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGATAGACAGTGGCCGATGTTTCG
E2	Round3_50	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGCCACATAGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
E3	Round3_51	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGCGAGTAAGTGGCCGATGTTTCG
E4	Round3_52	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGCTAACGAGTGGCCGATGTTTCG
E5	Round3_53	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGCTCGGTAGTGGCCGATGTTTCG
E6	Round3_54	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGGAGAACAGTGGCCGATGTTTCG
E7	Round3_55	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGGTGCGAAGTGGCCGATGTTTCG
E8	Round3_56	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGTACGCAAGTGGCCGATGTTTCG
E9	Round3_57	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGTCGTAGAGTGGCCGATGTTTCG
E10	Round3_58	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGTCTGTCAGTGGCCGATGTTTCG
E11	Round3_59	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGTGTCTAGTGGCCGATGTTTCG
E12	Round3_60	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTAGGATGAGTGGCCGATGTTTCG
F1	Round3_61	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTATCAGCAGTGGCCGATGTTTCG
F2	Round3_62	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTCCGTCTAGTGGCCGATGTTTCG
F3	Round3_63	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTCTTCACAGTGGCCGATGTTTCG
F4	Round3_64	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTGAAGAGAGTGGCCGATGTTTCG
F5	Round3_65	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTGGAACAAGTGGCCGATGTTTCG
F6	Round3_66	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTGGCTTCAGTGGCCGATGTTTCG
F7	Round3_67	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTGGTGGTAGTGGCCGATGTTTCG
F8	Round3_68	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNTTCACGCAGTGGCCGATGTTTCG
F9	Round3_69	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAACTCACCGTGGCCGATGTTTCG
F10	Round3_70	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAAGAGATCGTGGCCGATGTTTCG
F11	Round3_71	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAAGGACACGTGGCCGATGTTTCG
F12	Round3_72	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAATCCGTCTGGCCGATGTTTCG
G1	Round3_73	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAATGTTGCGTGGCCGATGTTTCG
G2	Round3_74	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACACGACCGTGGCCGATGTTTCG
G3	Round3_75	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNACAGATTCGTGGCCGATGTTTCG
G4	Round3_76	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGATGTACGTGGCCGATGTTTCG

Well Position	Name	Sequences (5'-3')
G5	Round3_77	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGCACCTCGTGGCCGATGTTTCG
G6	Round3_78	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGCCATGCGTGGCCGATGTTTCG
G7	Round3_79	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNAGGCTAACGTGGCCGATGTTTCG
G8	Round3_80	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATAGCGACGTGGCCGATGTTTCG
G9	Round3_81	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATCATTCCGTGGCCGATGTTTCG
G10	Round3_82	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNATTGGCTCGTGGCCGATGTTTCG
G11	Round3_83	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCAAGGAGCGTGGCCGATGTTTCG
G12	Round3_84	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCACCTTACGTGGCCGATGTTTCG
H1	Round3_85	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCATCCTCGTGGCCGATGTTTCG
H2	Round3_86	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCGACAACGTGGCCGATGTTTCG
H3	Round3_87	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCTAATCCGTGGCCGATGTTTCG
H4	Round3_88	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCCTCTATCGTGGCCGATGTTTCG
H5	Round3_89	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGACACACGTGGCCGATGTTTCG
H6	Round3_90	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCGGATTGCGTGGCCGATGTTTCG
H7	Round3_91	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNCTAAGGTCGTGGCCGATGTTTCG
H8	Round3_92	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGAACAGGCGTGGCCGATGTTTCG
H9	Round3_93	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGACAGTGCGTGGCCGATGTTTCG
H10	Round3_94	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGAGTTAGCGTGGCCGATGTTTCG
H11	Round3_95	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGATGAATCGTGGCCGATGTTTCG
H12	Round3_96	/5Biosg/CAGACGTGTGCTCTTCCGATCTNNNNNNNNNNNGCCAAGACGTGGCCGATGTTTCG

/5Biosg/ = 5' Biotin

**Supplementary Table 7 | AC indexed PCR primers**

Primer name	Sequences (5' - 3')
Ad1_N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTG
Ad1_N502	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTTCGTCGGCAGCGTCAGATGTG
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	AATGATACGGCGACCACCGAGATCTACACAGTATCTTTTCGTCGGCAGCGTCAGATGTG

Supplementary Table 8 | RNA indexed PCR primers

Primer name	Sequences (5' - 3')
SPLiT_N701	CAAGCAGAAGACGGCATAACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N702	CAAGCAGAAGACGGCATAACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N703	CAAGCAGAAGACGGCATAACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N704	CAAGCAGAAGACGGCATAACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N705	CAAGCAGAAGACGGCATAACGAGATGTAGCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N706	CAAGCAGAAGACGGCATAACGAGATTACAAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N707	CAAGCAGAAGACGGCATAACGAGATTTGACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N708	CAAGCAGAAGACGGCATAACGAGATGGAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N709	CAAGCAGAAGACGGCATAACGAGATTGACATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N710	CAAGCAGAAGACGGCATAACGAGATGGACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N711	CAAGCAGAAGACGGCATAACGAGATCTCTACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N712	CAAGCAGAAGACGGCATAACGAGATGCGGACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N713	CAAGCAGAAGACGGCATAACGAGATTTTCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N714	CAAGCAGAAGACGGCATAACGAGATGGCCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N715	CAAGCAGAAGACGGCATAACGAGATCGAAACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N716	CAAGCAGAAGACGGCATAACGAGATCGTACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N717	CAAGCAGAAGACGGCATAACGAGATTCAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N718	CAAGCAGAAGACGGCATAACGAGATAGGAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N719	CAAGCAGAAGACGGCATAACGAGATTGCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N720	CAAGCAGAAGACGGCATAACGAGATATTCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N721	CAAGCAGAAGACGGCATAACGAGATGTCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N722	CAAGCAGAAGACGGCATAACGAGATCGATTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N723	CAAGCAGAAGACGGCATAACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N724	CAAGCAGAAGACGGCATAACGAGATGCTACCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N725	CAAGCAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N726	CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N727	CAAGCAGAAGACGGCATAACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N728	CAAGCAGAAGACGGCATAACGAGATCCACTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N729	CAAGCAGAAGACGGCATAACGAGATACAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N730	CAAGCAGAAGACGGCATAACGAGATGCTCATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N731	CAAGCAGAAGACGGCATAACGAGATCGCCTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N732	CAAGCAGAAGACGGCATAACGAGATGCCATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N733	CAAGCAGAAGACGGCATAACGAGATTCTGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Primer name	Sequences (5' - 3')
SPLiT_N734	CAAGCAGAAGACGGCATAACGAGATGAATGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N735	CAAGCAGAAGACGGCATAACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N736	CAAGCAGAAGACGGCATAACGAGATGTATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N737	CAAGCAGAAGACGGCATAACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N738	CAAGCAGAAGACGGCATAACGAGATCCGGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N739	CAAGCAGAAGACGGCATAACGAGATTGAGTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SPLiT_N740	CAAGCAGAAGACGGCATAACGAGATATTATAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Supplementary Table 9 | SNARE-seq2 sequencing primers

Primer name	Description	Sequence (5'-3')
SNARE2_Read1	<ul style="list-style-type: none"> <li>- Read cDNA of RNA libraries (Read1 or R1 order).</li> <li>- Read 1<sup>st</sup> accessible chromatin of AC libraries (Read1 or R1 order).</li> </ul>	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 2 <sup>nd</sup> accessible chromatin of AC libraries (Read2 or R4 order).	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG
PhiX_Read1	<ul style="list-style-type: none"> <li>- Read 1<sup>st</sup> PhiX sequence in Read1 or R1 order of RNA library sequencing.</li> <li>- Read 1<sup>st</sup> PhiX sequence in Read1 or R1 order of AC library sequencing.</li> <li>- Read 1<sup>st</sup> PhiX sequence in Index1 or R2 order of RNA library sequencing.</li> <li>- Read 1<sup>st</sup> PhiX sequence in Index1 or R2 order of AC library sequencing.</li> </ul>	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
PhiX_Read2	<ul style="list-style-type: none"> <li>- Read 2<sup>nd</sup> PhiX sequence in Read2 or R3 order of RNA library sequencing.</li> <li>- Read 2<sup>nd</sup> PhiX sequence in Read2 or R4 order of AC library sequencing</li> </ul>	CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

## Reference

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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).