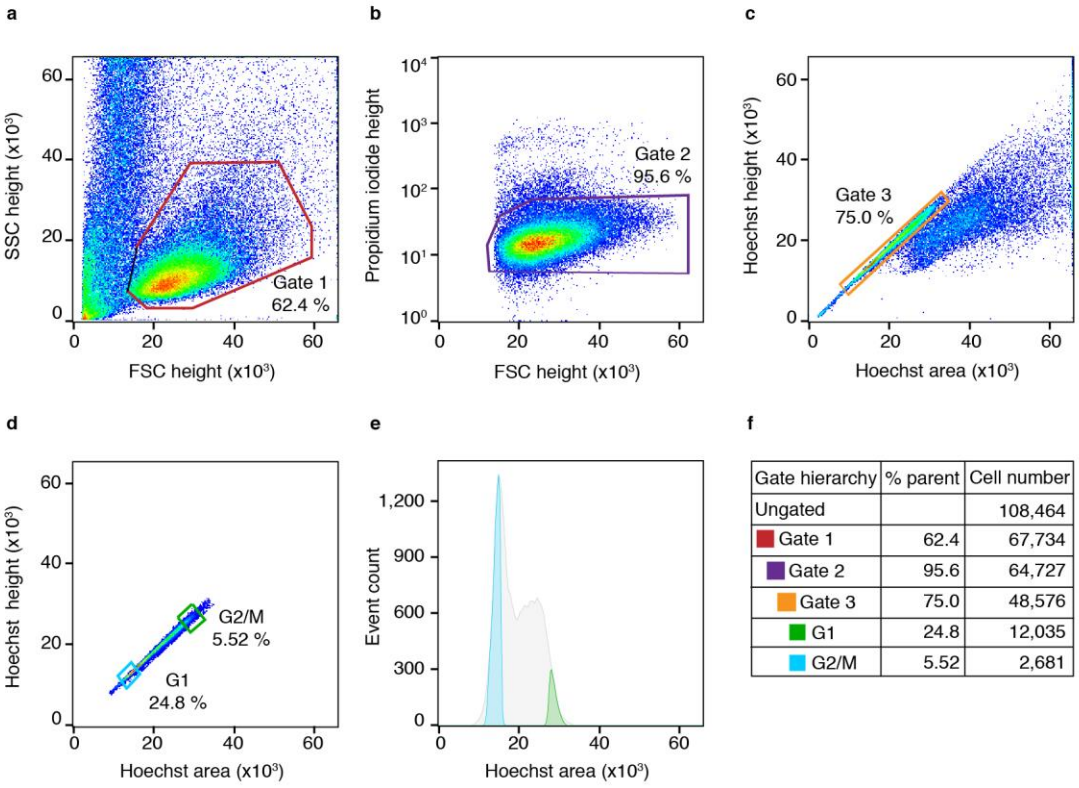

f

Gate hierarchy	% parent	Cell number
Ungated		108,464
Gate 1	62.4	67,734
Gate 2	95.6	64,727
Gate 3	75.0	48,576
G1	24.8	12,035
G2/M	5.52	2,681

g

Population	Mean (Hoechst area)
G1	14,463
G2/M	28,670

Supplementary Figure 1

Gating strategy for FACS

a. Dot plot of ungated mESCs showing gating strategy to exclude debris in FSC (Forward Scatter) versus SSC (Side Scatter). Percentage of events in Gate 1 is indicated.

b. Dot plot of mESCs passing Gate 1 showing gating strategy to exclude dead cells in Propidium iodide versus FSC. Percentage of events in Gate 2 is indicated.

c. Dot plot of mESCs passing Gate 2 showing gating strategy to exclude duplet cells in Hoechst versus Hoechst area. Percentage of events in Gate 3 is indicated.

d. Dot plot of mESCs passing Gate 3 were gated for DNA content in G1 and G2/M phase of the cell cycle. The gate for the G2/M population was defined by doubling the intensity value of the G1 peak maximum.

e. DNA content histogram events in Gate 3 showing counted events versus Hoechst area. Only cells passing gate G2/M were sorted.

f. Table indicating gate hierarchy, percentage of events in each gate relative to parent population and total numbers of events within each gate.

g. Table indicating the mean value for the G1 and G2/M populations.

All measurements were done on the BD FACSJazz and analyzed with the FlowJo software, version 10.1r5.

Supplementary Methods

scDamID2

Lysis plate preparation – Timing 45 min for one plate

CRITICAL It is crucial that the working area is sufficiently clean when working with single cell material. DNAZap and RNAseZAP treatment is required in steps 1-5 and 8-25. RNAseZAP treatment is sufficient for steps 26-33. Ethanol 80 % (vol/vol) treatment is sufficient for steps 34-35.

CRITICAL We recommend preparing lysis plates, adapter plates and master mixes before single-cell material amplification by IVT in a PCR workstation.

1. Pipet 5 µl of mineral oil in each well of a 384-well plate and seal the plate with an aluminium seal.
2. Prepare the lysis mix according to the table below. Keep the mix on ice at all times.

Reagent	Amount for 1 well (nl)	Amount for 1 plate (µl)	Final concentration in mix
Lysis buffer 1.03 x	290	162.5	1 x
Proteinase K (20 mg/ml)	10	5.6	0.67 mg/ml
Total volume	300		

3. In an 8-well PCR strip, aliquot 20.7 µl of mix per well of the strip. Spin for 3-5 sec on tabletop spinner. Keep on ice at all times.
4. Dispense 300 nl per well with the Nanodrop II robot (Box 2). The cumulative reaction volume is 300 nl.
5. Seal the plate and centrifuge at 2,000 g for 1 min at 4 °C.

   PAUSE POINT The lysis plates can be kept at 4 °C until sort on the same day or maximum overnight.

Dam-POI induction – Timing 45 min performed in a cell culture hood

6. Induce cells as stated in **scDam&T Procedure** steps 4-6.

Prepare cells for FACS sorting by Hoechst staining – Timing 1 hr 30 min

7. Prepare cells for FACS sorting as stated in **scDam&T Procedure** steps 7-31.

   PAUSE POINT Proceed with lysis after FACS sorting or store the sorted plates at -20 °C for several months.

Lysis – Timing 8 hr 20 min

8. Thaw the plates on ice if stored at -20 °C.
9. Put the plate in a thermocycler at 50 °C for 8 hr, then 80 °C for 20 min with the thermocycler lid at 100 °C and let the machine go to 4 °C at the end of the program.

   PAUSE POINT The lysed plates can be kept at -20 °C for several months.

DpnI digestion – Timing 8 hr 35 min

10. Thaw the plates on ice if stored at -20 °C.
11. Thaw the 10x CutSmart buffer at room temperature and keep on ice. Keep the DpnI enzyme on an ice block at all times.
12. Prepare the DpnI mix according to the table below. Keep the mix on ice at all times.

Reagent	**Amount for 1 well (nl)**	**Amount for 1 plate (µl)**	**Final concentration in mix**
Nuclease-free water	590	272.8	
10x CutSmart buffer	100	46.2	1.6 x
DpnI (20 U/µl)	10	4.6	0.33 U/µl
Total volume	700		

13. In an 8-well PCR strip, aliquot 39.9 µl of mix per well of the strip. Spin for 3-5 sec on tabletop spinner. Keep on ice at all times.
14. Dispense 700 nl per well with the Nanodrop II robot (Box 2). The cumulative reaction volume is 1000 nl.
15. Seal the plate and centrifuge at 2,000 g for 1 min at 4 °C
16. Put the plate in a thermocycler at 37 °C for 8 hr, then at 80 °C for 20 min with the lid at 100 °C and then place on ice for 1-2 min to cool down.
17. Centrifuge at 2,000 g for 1 min at 4 °C

Adapter dispension – Timing 45 min for 1 plate

18. Dispense adapters as stated in **scDam&T Procedure** steps 67-71 but dispense 400 nl of adapter instead of 50 nl. The cumulative reaction volume is 1400 nl.

Adapter ligation – Timing 12 h 45 min overnight reaction

19. Thaw the 10x Ligase buffer on ice. Keep the T4 ligase on an ice block at all times.
20. Prepare the ligation mix according to the table below. Keep the mix on ice at all times.

Reagent	**Amount for 1 well (nl)**	**Amount for 1 plate (µl)**	**Final concentration in mix**
Nuclease-free water	375	178.0	
10x Ligase buffer	200	94.9	3.33 x
T4 Ligase (5 U/µl)	25	11.8	0.20 U/µl
Total volume	600		

21. In an 8-well PCR strip, aliquot 35.1 µl of mix per well of the strip. Spin for 3-5 sec on tabletop spinner. Keep on ice at all times.
22. Dispense 600 nl per well with the Nanodrop II robot (Box 2). The cumulative reaction volume is 2000 nl.
23. Seal the plate and centrifuge at 2,000 g for 1 min at 4 °C
24. Put the plate in a thermocycler at 16 °C for 12 hr, then 65 °C for 10 min with the lid at 100 °C. Let the thermocycler go to 4 °C at the end of the program.
25. Centrifuge at 2,000 g for 1 min at 4 °C

    PAUSE POINT The processed plate can be kept at -20 °C up to a month.

Pool cells – Timing 1 hr for one plate

26. Pool cells as stated in **Procedure** steps 79-82.

Purification of barcoded material – Timing 1 hr

27. Purify the barcoded material as stated in **scDam&T Procedure** steps 83-91 but use a lower dilution of AMPure XP beads (Reagent setup) and 1.0 volume diluted AMPure XP beads to purify material instead of 0.8 volume.

Amplification by *in vitro* transcription – Timing 14 hr 15 min

28. Amplify the barcoded material as stated in **scDam&T Procedure** steps 92-96.

Purification of aRNA – Timing 1 hr

29. Purify the aRNA as stated in **scDam&T Procedure** steps 97-108.

aRNA fragmentation – Timing 5 min

30. Fragment the aRNA as stated in **scDam&T Procedure** steps 109-113.

Purification and quantification of fragmented aRNA – Timing 1 hr 45 min

31. Purify the aRNA as stated in **scDam&T Procedure** steps 114-125.

Reverse transcription – Timing 1 hr 30 min

32. Reverse transcribe the aRNA for library preparation as stated in **scDam&T Procedure** steps 126-127.

PCR indexing – Timing 30 min

33. Index PCR the reverse transcribed material as stated in **scDam&T Procedure** step 128.

Library purification – Timing 1 hr 30 min

34. Purify the libraries as stated in **scDam&T Procedure** steps 129-141.

Library quantification and sequencing – Timing 19 hr

35. Prepare the libraries for sequencing submission as stated in **scDam&T Procedure** steps 142-145.

    PAUSE POINT The finished libraries can be kept at -20 °C indefinitely.

DamID2 in bulk

Dam-POI induction – Timing 45 min performed in a cell culture hood

1. Induce cells as stated in **scDam&T Procedure** steps 4-6.

Genomic DNA isolation – Timing 3 hr

2. Harvest cells χ hr after induction and extract gDNA following a standard gDNA extraction protocol. We use the Wizard Genomic DNA Purification Kit by Promega.

DpnI digestion – Timing 12 hr 30 min

3. Prepare the following mix.

Reagent	Amount per reaction (µl)	Final concentration in reaction
gDNA (50 ng/µl)	5	25 ng/µl
10x CutSmart buffer	1	1 x
DpnI (20 U/µl)	0.25	0.5 (U/µl)
Nuclease-free water	3.75	
Total volume	10	

4. Digest the gDNA at 37 °C for 12 hr, then 80 °C for 20 min with the lid at 100 °C. Let the thermocycler go to 4 °C at the end of the program.

Adapter ligation – Timing 16 hr 30 min overnight reaction

5. Prepare the following mix.

   CRITICAL In case of more than one sample, use a uniquely barcoded DamID adapter per sample.

Reagent	Amount per reaction (µl)	Final concentration
Digested gDNA (step 4)	10	
DamID adapter (50 µM)	0.5	2 µM
10x Ligase buffer	1.25	1 x
Ligase (5 U/µl)	0.25	0.1 U/µl
Nuclease-free water	0.5	

Total volume	12.5	

6. Ligate the adapters by incubating reaction at 16 °C for 16 hr, then 65 °C for 10 min with the lid at 100 °C. Let the thermocycler go to 4 °C at the end of the program.

Pool samples – Timing 10 min

CRITICAL The pooling weight of each sample depends on the yield estimated from a methyl-PCR, and can vary between 0.5 and 10 µl per sample.

CRITICAL Work on a clean bench free from RNAses. RNAseZAP treatment is sufficient for steps 7-25. Ethanol 80 % (vol/vol) treatment is sufficient for steps 26-28.

7. Pool samples to a maximum total of 2 µg with non-overlapping barcodes in a clean tube.

Purification of barcoded material – Timing 1 hr

8. Purify the barcoded material as stated in **scDam&T Procedure** steps 83-91 but use undiluted instead of diluted AMPure XP beads, 1.0 volume AMPure XP beads to purify material instead of 0.8 volume and elute in 50 µl nuclease-free water instead of 7 µl.
9. Repeat step 8, but use 0.8 volume AMPure XP beads to purify material instead of 1.0 volume and elute in 25 µl nuclease-free water instead of 50 µl.

Amplification by *in vitro* transcription – Timing 2 hr 15 min

10. Thaw the Megascript T7 10x buffer at room temperature. Vortex thoroughly to dissolve precipitates and keep at room temperature.
11. Thaw the Megascript T7 NTPs on ice and keep on ice. Keep the enzyme mix on an ice block at all times.
12. Prepare the Megascript T7 mix as indicated in the table below

Reagent	**Amount (µl)**	**Final concentration in reaction**
Cleaned material (step 9)	4	
Nuclease-free water	4	
10x T7 buffer	2	1 x
ATP (75 mM)	2	7.5 mM
UTP (75 mM)	2	7.5 mM
GTP (75 mM)	2	7.5 mM

CTP (75 mM)	2	7.5 mM
T7 enzyme mix	2	
Total volume	20	

13. Incubate the mix in a thermocycler at 37 °C for 2 hr with the lid heated to 70 °C. Let the thermocycler go to 4 °C at the end of the program.

Purification of aRNA – Timing 1 hr

14. Add 40 µl nuclease-free water to the aRNA (step 13).
15. Purify the aRNA as stated in **scDam&T Procedure** steps 97-108 but elute in 50 µl instead of 23 µl.
16. Quantify the aRNA by measurement on a Nanodrop spectrophotometer machine and dilute aRNA to 50 ng/µl.

aRNA fragmentation – Timing 5 min

17. Take 20 µl of diluted aRNA (step 16) and fragment as stated in **scDam&T Procedure** steps 109-113.

Purification and quantification of fragmented aRNA – Timing 1 hr 45 min

18. Purify the aRNA as stated in **scDam&T Procedure** steps 114-125 but elute in 15 µl instead of 13 µl.
19. Quantify the aRNA by measurement on a Nanodrop spectrophotometer machine and dilute to a concentration between 2 and 15 ng/µl for subsequent quantification on a Bioanalyzer RNA pico chip. Run chip according to the instructions of the kit.

Reverse transcription – Timing 1 hr 30 min

20. Prepare the randomhexRT mix as indicated in the table below.

Reagent	**Amount (µl)**	**Final concentration in reaction**
aRNA (step 19)	4	
randomhexRT primer (20 µM)	1	3.33 µM
dNTP mix (10 mM)	1	1.66 mM
Total volume	6	

21. Heat mix in thermocycler at 65 °C for 5 min with lid at 100 °C. Immediately put on ice.
22. Prepare the RT mix as indicated in the table below. Keep mix on ice and enzymes on ice block at all times.

Reagent	Amount (µl)	Final concentration in reaction
hexRT mix with aRNA (step 21)	6	
5x First strand buffer	2	1 x
DTT (0.1 M)	1	10 mM
RNase OUT (40 U/µl)	0.5	2 U/µl
Superscript II (200 U/µl)	0.5	10 U/µl
Total volume	10	

23. Heat mix in thermocycler at 25 °C for 10 min, then 42 °C for 1 hr with the cycler lid at 50 °C. Let the thermocycler go to 4 °C at the end of the program.

PCR indexing – Timing 30 min

CRITICAL PCR amplification must be kept to a minimum to avoid over-amplification. We recommend 6-8 PCR cycles.

24. Prepare the indexing mix as indicated in the table below. Keep mix on ice. Index each sample with a unique RPi primer for multiplexing.

Reagent	Amount (µl)	Final concentration in reaction
Reverse transcribed material (step 23)	10	
Nuclease-free water	11	
2x NEBNext High-Fidelity PCR Master Mix	25	1x
RNA PCR primer RP1 (10 µM)	2	0.4 µM
RNA PCR index primer RPi (10 µM)	2	0.4 µM
Total volume	50	

25. Put mix in thermocycler and run PCR program with the lid heated at 105 °C as indicated in the table below.

Cycle number	Denature	Anneal	Extend

1	98°C, 30 s		
2-11	98°C, 10 s	60°C, 30 s	72°C, 30 s
12			72°C, 10 min

Library purification – Timing 1 hr 30 min

26. Purify the libraries as stated in **scDam&T Procedure** steps 129-141.
27. Quantify the aRNA by measurement on a Nanodrop spectrophotometer machine and dilute aRNA to 2 ng/µl for quantification on a Bioanalyzer HS DNA chip. Run chip according to the kit manual.

Library quantification and sequencing – Timing 19 hr

28. Prepare the libraries for sequencing submission as stated in **scDam&T Procedure** steps 142-145.

PAUSE POINT The finished libraries can be kept at -20 °C indefinitely.

Supplementary Manual

Molecule after each protocol step – DamID

After ligation of ds DamID adapters (example adapter with barcode 1) 5′→ 3′

Fork T7 promoter TSS Illumina P5 sequence UMI barcode UMI barcode
GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTGCANNNTATGGA-**DNA sequence**
TTCGAGGGCCATTATGCTGAGTGATATCCCCAAGTCTCAAGATGTCAGGCTGCTAGNNNACGTNNNATACCT-**DNA sequence**

After IVT 5′→ 3′

TSS Illumina P5 sequence UMI barcode UMI barcode
GGGGUUCAGAGUUCUACAGUCCGACGAUCNNNUGCANNNUAUGGA-**DNA sequence**

After hexRT-mediated RT (library prep) 5′ → 3′

By using the random hexRT primer, containing a sequence used as in the RA3 sequence (Illumina Truseq Small RNA) and serving as the P7

TSS Illumina P5 sequence UMI barcode UMI barcode hexRT containing P7
GGGGUUCAGAGUUCUACAGUCCGACGAUCNNNUGCANNNUAUGGA-**DNA sequence-**
CCCCAAGTCTCAAGATGTCAGGCTGCTAGNNNACGTNNNATACCT-**DNA sequence-**
NNNNNNTGGAATTCTCGGGTGCCAAGGC

After PCR (library prep; example RPi index primer 1) 5′ → 3′

By using the universal RP1 primer, containing an overlapping sequence with the Illumina P5 sequence in the adapter (Illumina Truseq Small RNA)
By using the indexed RPi primer, containing an overlapping sequence with the P7 sequence of the hexRT, an index, and a sequence to anneal to the flow cell

universal RP1 primer Illumina P5 sequence UMI barcode UMI barcode hexRT containing P7
universal RP1 overlapping with P7 sequence (index underlined)
ATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGATCNNNUGCANNNUAUGGA-**DNA sequence-**
NNNNNNACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTGTAGTGCTAGAGCATACGGCAGAAGACGAAC
TACTATGCCGCTGGTGGCTCTAGATGTGCAAGTCTCAAGATGTCAGGCTAGNNNACGTNNNATACCT-**DNA sequence-**
NNNNNNTGGAATTCTCGGGTGCCAAGGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG

All Illumina Truseq Small RNA sequences can be found at:
www.nature.com/protocolexchange/system/uploads/6661/original/SupplementaryDocument2-illumina-adapter-sequences-Feb2018.pdf?1530635414

Molecule after each protocol step – CEL-Seq (as in CEL-Seq2 protocol)

After annealing of CEL-Seq primer to the mRNA molecule (example primer with barcode 1) 5′→ 3′

polyT barcode UMI barcode UMI Illumina P5 sequence TSS T7 promoter
mRNA sequence-AAAAAAAAAAAAAAAAAAAAAAAA
VTTTTTTTTTTTTTTTTTTTTTTTTACTNNNGTAGNNNCTAGCAGCCTGACATCTTGAGGGATATCACTCAGCATAATGGCCG

After RT with the CEL-Seq primer 5′→ 3′

polyT barcode UMI barcode UMI Illumina P5 sequence TSS T7 promoter
mRNA sequence-AAAAAAAAAAAAAAAAAAAAAAAA
mRNA sequence-TTTTTTTTTTTTTTTTTTTTTTTTACTNNNGTAGNNNCTAGCAGCCTGACATCTTGAGGGATATCACTCAGCATAATGGCCG

After second strand synthesis 5′ → 3′

polyT barcode UMI barcode UMI Illumina P5 sequence TSS T7 promoter
mRNA sequence-AAAAAAAAAAAAAAAAAAAAAAAAATGANNNCATCNNNGATCGTCGGACTGTAGAACTCCCTATAGTGAGTCGTATTACCGGC
mRNA sequence-TTTTTTTTTTTTTTTTTTTTTTTTACTNNNGTAGNNNCTAGCAGCCTGACATCTTGAGGGATATCACTCAGCATAATGGCCG

After IVT 5′→ 3′

TSS Illumina P5 sequence UMI barcode UMI barcode
GGGAGUUCUACAGUCCGACGAUCNNNGAUGNNNUCAU-UUUUUUUUUUUUUUUUUUUUUUUU-**mRNA sequence**

After hexRT-mediated RT (library prep) 5′ → 3′

By using the random hexRT primer, containing a sequence used as in the RA3 sequence (Illumina Truseq Small RNA) and serving as the P7

TSS Illumina P5 sequence UMI barcode UMI barcode
hexRT containing P7
GGGAGUUCUACAGUCCGACGAUCNNNGAUGNNNUCAU-UUUUUUUUUUUUUUUUUUUUUUUU
CCCTCAAGATGTCAGGCTGCTAGNNNCTACNNNAGTA-AAAAAAAAAAAAAAAAAAAAAAAA-**mRNA sequence-**
NNNNNNTGGAATTCTCGGGTGCCAAGGC

After PCR (library prep; example RPi index primer 1) 5′ → 3′

By using the universal RP1 primer, containing an overlapping sequence with the Illumina P5 sequence in the adapter (Illumina Truseq Small RNA)
By using the indexed RPi primer, containing an overlapping sequence with the P7 sequence of the hexRT, an index, and a sequence to anneal to the flow cell

RP1 primer Illumina P5 sequence UMI barcode UMI barcode hexRT containing
P7 universal RP1 overlapping with P7 sequence (index underlined)
ATGATACGGCGACCACCGAGATCTACACGTTCAGAGTTCTACAGTCCGACGATCNNNGATGNNNTCAT-TTTTTTTTTTTTTTTTTTTTTTTT-**mRNA sequence-**
NNNNNNACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTGTAGTGCTAGAGCATACGGCAGAAGACGAAC
TACTATGCCGCTGGTGGCTCTAGATGTGCAAGTCTCAAGATGTCAGGCTGCTAGNNNCTACNNNAGTA-AAAAAAAAAAAAAAAAAAAAAAAA-**mRNA sequence-**
NNNNNNTGGAATTCTCGGGTGCCAAGGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG

Supplementary Table 1

1	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGATGNNNTCATTTTTTTTTTTTTTTTTTTTTTTV
2	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTCTCNNNCGAGTTTTTTTTTTTTTTTTTTTTTTTV
3	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCCANNNGACATTTTTTTTTTTTTTTTTTTTTTTV
4	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTACGNNNCGGATTTTTTTTTTTTTTTTTTTTTTV
5	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCAGNNNTCGTTTTTTTTTTTTTTTTTTTTTTTV
6	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGCANNNAGTTTTTTTTTTTTTTTTTTTTTTTTV
7	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTCTCNNNGATGTTTTTTTTTTTTTTTTTTTTTTV
8	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTCACNNNATGATTTTTTTTTTTTTTTTTTTTTTV
9	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTTCNNNGCGTTTTTTTTTTTTTTTTTTTTTTTV
10	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCACANNNCGTGTTTTTTTTTTTTTTTTTTTTTTV
11	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNATCANNNGAACTTTTTTTTTTTTTTTTTTTTTTV
12	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCGCTNNNCTCTTTTTTTTTTTTTTTTTTTTTTTV
13	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCGTANNNAGTCTTTTTTTTTTTTTTTTTTTTTTV
14	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTGGANNNTACATTTTTTTTTTTTTTTTTTTTTTV
15	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGTTCNNNCTGTTTTTTTTTTTTTTTTTTTTTTTV
16	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGTCANNNCGTTTTTTTTTTTTTTTTTTTTTTTTV
17	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTACNNNATCTTTTTTTTTTTTTTTTTTTTTTTV
18	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGACTNNNTACTTTTTTTTTTTTTTTTTTTTTTTV
19	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTATNNNGCGCTTTTTTTTTTTTTTTTTTTTTTTV
20	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTCGANNNCTAGTTTTTTTTTTTTTTTTTTTTTTV
21	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCACTNNNACCTTTTTTTTTTTTTTTTTTTTTTTV
22	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTCATNNNGTGTTTTTTTTTTTTTTTTTTTTTTTV
23	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTGANNNAGAGTTTTTTTTTTTTTTTTTTTTTTV
24	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTGTNNNTAGTTTTTTTTTTTTTTTTTTTTTTTV
25	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCGAGNNNGTCTTTTTTTTTTTTTTTTTTTTTTTV
26	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTACTNNNCTTCTTTTTTTTTTTTTTTTTTTTTTV
27	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTACANNNCTCTTTTTTTTTTTTTTTTTTTTTTTV
28	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCATNNNGCTGTTTTTTTTTTTTTTTTTTTTTTV
29	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCACGNNNATAGTTTTTTTTTTTTTTTTTTTTTTV
30	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNATCGNNNGCTGTTTTTTTTTTTTTTTTTTTTTTV
31	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGTGANNNTGGATTTTTTTTTTTTTTTTTTTTTTV
32	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNATCTNNNGTAGTTTTTTTTTTTTTTTTTTTTTTV
33	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCCANNNCAACTTTTTTTTTTTTTTTTTTTTTTV
34	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGACNNNCAACTTTTTTTTTTTTTTTTTTTTTTV
35	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGCANNNGAGATTTTTTTTTTTTTTTTTTTTTTV
36	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCAGNNNAGCTTTTTTTTTTTTTTTTTTTTTTTV
37	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCAGTNNNCATGTTTTTTTTTTTTTTTTTTTTTTV
38	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCATCNNNCTGCTTTTTTTTTTTTTTTTTTTTTTV
39	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGTGNNNTAGATTTTTTTTTTTTTTTTTTTTTV
40	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTGATNNNTCTCTTTTTTTTTTTTTTTTTTTTTV
41	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCCTNNNTGCATTTTTTTTTTTTTTTTTTTTTV
42	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGCGNNNCAAGTTTTTTTTTTTTTTTTTTTTTV
43	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGCGNNNACCATTTTTTTTTTTTTTTTTTTTTV
44	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGACNNNCTGATTTTTTTTTTTTTTTTTTTTTV
45	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNAGGANNNGATGTTTTTTTTTTTTTTTTTTTTTV
46	GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCGCGNNNCTTATTTTTTTTTTTTTTTTTTTTTV

47 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCGANNNATGCTTTTTTTTTTTTTTTTTTTTTTTTV
48 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNATAGNNNTAGCTTTTTTTTTTTTTTTTTTTTTTTTV
49 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCTGANNNGATTTTTTTTTTTTTTTTTTTTTTTTTV
50 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGAACNNNCTATTTTTTTTTTTTTTTTTTTTTTTTV
51 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNTAAGNNNGCGTTTTTTTTTTTTTTTTTTTTTTTTV
52 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGAATNNNCTCATTTTTTTTTTTTTTTTTTTTTTTTV
53 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNGCGTNNNGTGATTTTTTTTTTTTTTTTTTTTTTTTTV
54 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCACANNNTAACTTTTTTTTTTTTTTTTTTTTTTTTV
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247 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNACAGNNNAGGCTTTTTTTTTTTTTTTTTTTTTTTTV
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383 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCAGANNNGCGTTTTTTTTTTTTTTTTTTTTTTTTTTV

384 GCCGGTAATACGACTCACTATAGGGAGTTCTACAGTCCGACGATCNNNCATGNNNACAATTTTTTTTTTTTTTTTTTTTTTTTTTV

Supplementary Table 2

	Top Sequence
1	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTGCANNNTATGGA
2	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGTGCNNNTGAAGA
3	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGCTGNNNTCTCGA
4	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGTAGNNNATCGGA
5	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNATCTNNNGACTGA
6	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGTTGNNNAGCCGA
7	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGCACNNNATCCGA
8	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGAATNNNGACTGA
9	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGCTANNNGCCAGA
10	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTGAGNNNCTAAGA
11	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTGCGNNNTCAGGA
12	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCGACNNNGTAAGA
13	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNATACNNNACGTGA
14	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGTTANNNGTCTGA
15	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTAGANNNGATGGA
16	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTCGANNNCAATGA
17	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTAGANNNTGGAGA
18	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCTTCNNNGCACGA
19	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTATGNNNGACGGA
20	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCGGTNNNTATCGA
21	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCATCNNNGAAGGA
22	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNAGTANNNGCGTGA
23	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCTGANNNACTTGA
24	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTCTANNNTAGCGA
25	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNGTCANNNTGATGA
26	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNTGGTNNNCGGTGA
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28	GGTGATCCGGTAATACGACTCACTATAGGGGTTCAGAGTTCTACAGTCCGACGATCNNNCAGTNNNGAGAGA
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Bottom Sequence

1	/5Phos/TCCATANNNTGCANNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
2	/5Phos/TCTTCANNNGCACNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
3	/5Phos/TCGAGANNNCAGCNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
4	/5Phos/TCCGATNNNCTACNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
5	/5Phos/TCAGTCNNNAGATNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
6	/5Phos/TCGGCTNNNCAACNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
7	/5Phos/TCGGATNNNGTGCNNNGATCGTCGGACTGTAGAACTCTGAACCCCTATAGTGAGTCGTATTACCGGGAGCTT
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