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Supplemental information

CUT&Tag-BS for simultaneous profiling of histone modification and DNA methylation with high efficiency and low cost

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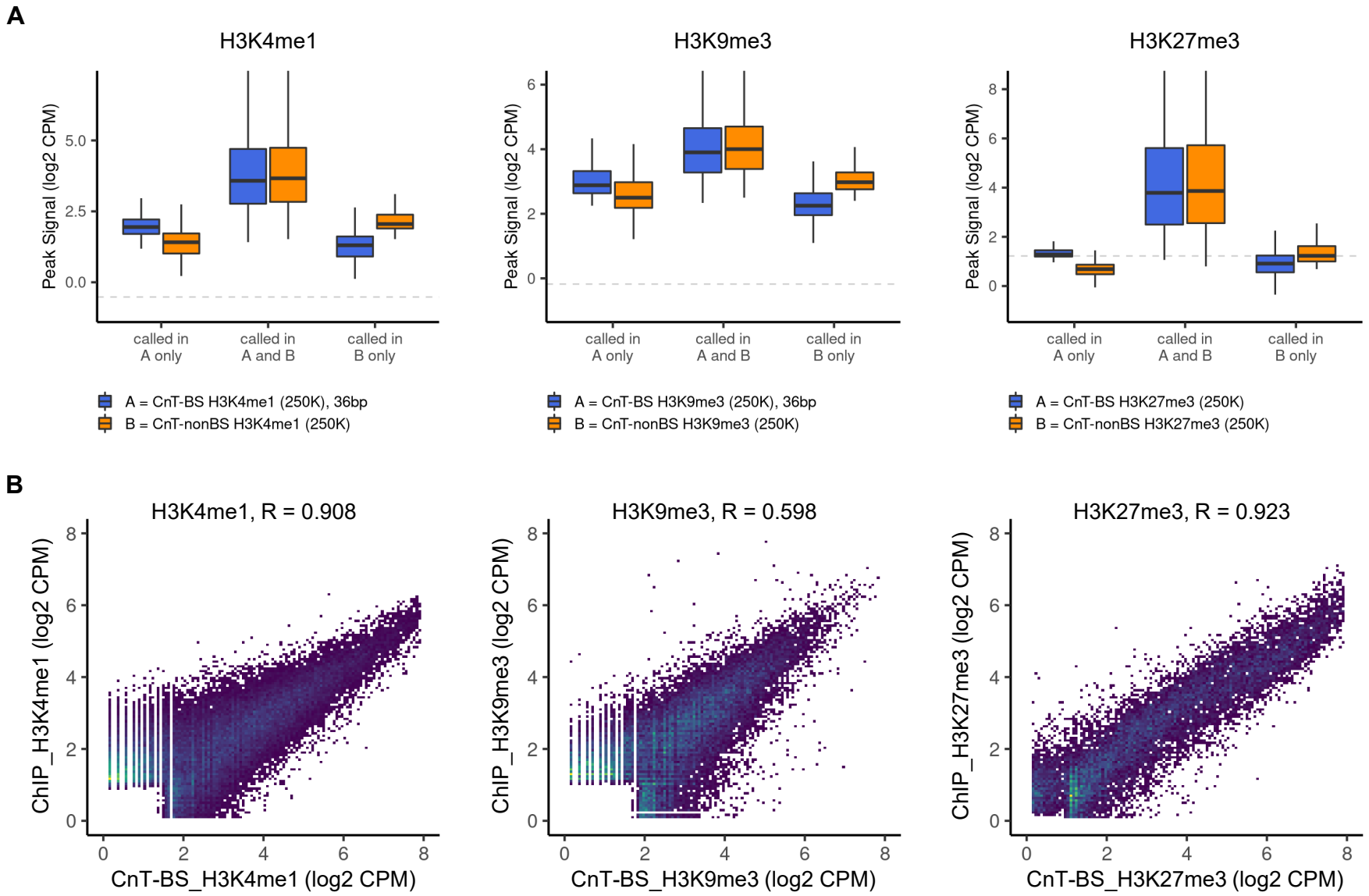


Figure S1. Evaluating the consistency of histone modification enrichments between CUT&Tag-BS and conventional methods, CUT&Tag_nonBS and ChIP-seq, Related to Figure 3. (A) Non-overlapped peaks show much lower enrichment signals than common peaks in the comparison of CUT&Tag-BS with CUT&Tag_nonBS. The gray dashed line represents random background. (B) Density-scatter plots displaying correlation of peak signals between CUT&Tag-BS and ChIP-seq. Each dot represents an individual peak with viridis color scale indicating density. Pearson's *r* value was shown at the top of each plot.

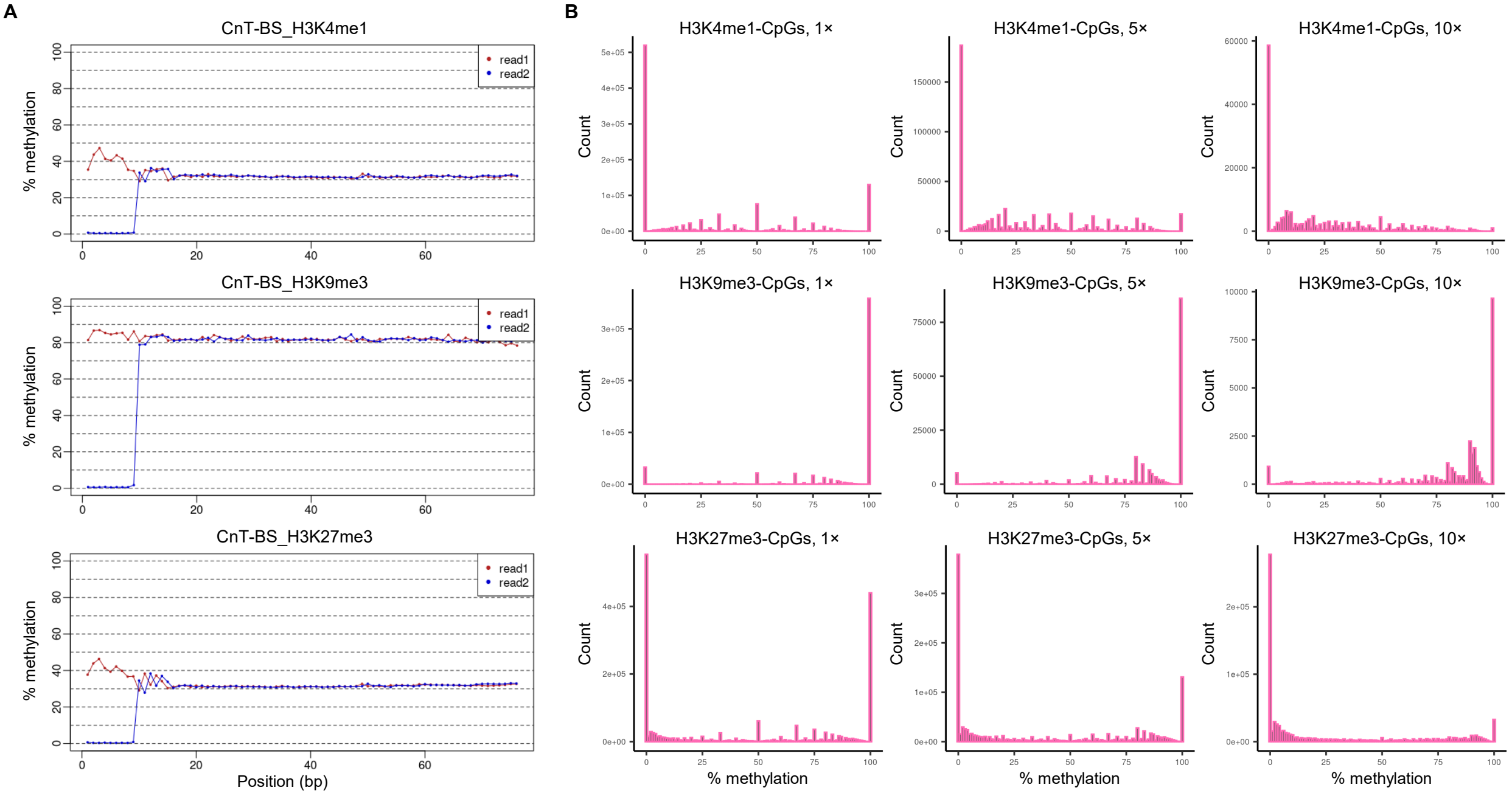


Figure S2. CUT&Tag-BS simultaneously measures DNA methylation, Related to Figure 4. (A) M-bias plots showing averaged CpG methylation rates per position along the reads. (B) Histograms displaying the distribution of methylation levels of individual CpGs at H3K4me1-, H3K9me3-, or H3K27me3-peaks with minimum coverage of 1×, 5×, or 10×.

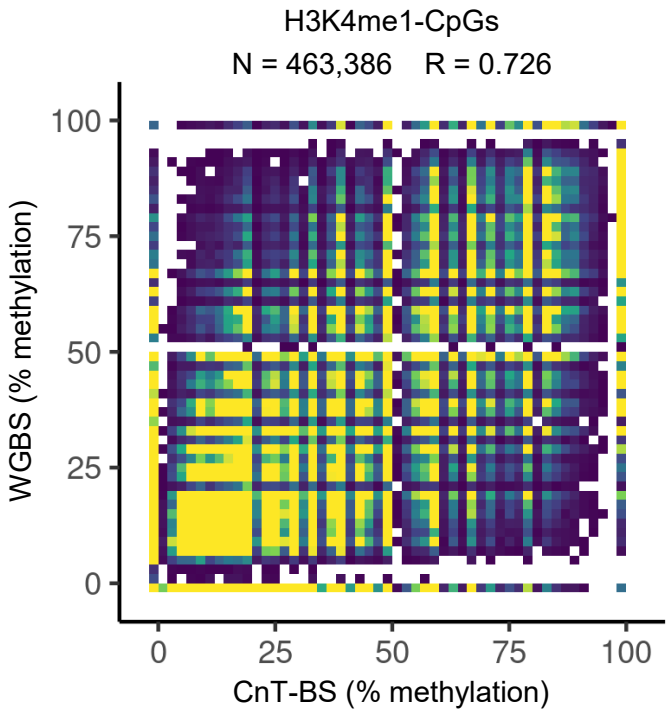


Figure S3. Density scatter plot displaying correlation of methylation at H3K4me1-CpGs between CUT&Tag-BS and WGBS**,** Related to Figure 4. Only CpGs at H3K4me1-peaks with minimum coverage of 5× in both datasets were included. Each dot represents an individual CpG with viridis color scale indicating density. Pearson's *r* value was shown at the top of the plot.

H3K4me1-CpGs (1,225,519)

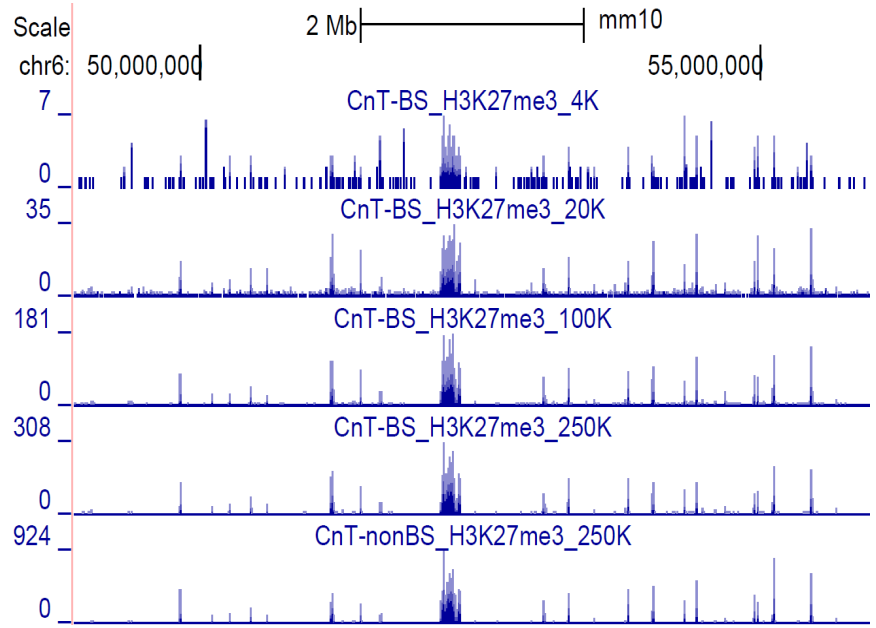
Sample	minimum CpG depth		
	1×	5×	10×
CnT-BS_run1 (PE36)	945,053 77.1%	227,952 18.6%	52,984 4.3%
CnT-BS_run2 (PE76)	1,129,111 92.1%	526,411 43.0%	182,927 14.9%

H3K9me3-CpGs (591,273)

sample	minimum CpG depth		
	1×	5×	10×
CnT-BS_run1 (PE36)	317,347 53.7%	18,541 3.1%	1,487 0.3%
CnT-BS_run2 (PE76)	522,923 88.4%	165,672 28.0%	28,568 4.8%

Figure S4. Coverage of CpGs at H3K4me1- or H3K9me3-peaks when using different sequencing read length (PE36 vs. PE76), Related to Figure 5. The number and percentage of CpGs at H3K4me1- or H3K9me3-peaks with minimum coverage of 1×, 5×, or 10× were shown.

A



B

H3K27me3-CpGs (1,843,093)

Sample	minimum CpG depth		
	1×	5×	10×
CnT-BS_H3K27me3_4K	222,370 12.1%	1,726 0.1%	20 0.0%
CnT-BS_H3K27me3_20K	1,137,938 61.7%	293,835 15.9%	71,560 3.9%
CnT-BS_H3K27me3_100K	1,681,526 91.2%	970,830 52.7%	624,006 33.9%
CnT-BS_H3K27me3_250K	1,747,774 94.8%	1,155,472 62.7%	784,908 42.6%

Figure S5. Robustness and input requirement of CUT&Tag-BS, Related to Figure 7. (A) UCSC genome browser view of H3K27me3 coverage tracks at chr6:48,879,717-55,974,833 generated with different numbers of input cells in CUT&Tag-BS. (B) The number and percentage of CpGs at H3K27me3-peaks with 1×, 5×, or 10× minimum coverage in each sample.

Table S1. List of oligonucleotides used in this study, related to STAR Methods.

Oligo/Primer name	Sequence (5' to 3')	Modifications
Tn5mC-Apt1	TcGTcGGcAGcGTcAGATGTGTATAAGAGAcAG	c: 5C-methylated
Tn5mC1.1-A1block	pCTGTCTCTTATACAddC	p: phosphate, ddC: dideoxycytidylate
Tn5mC-ReplO1	pcTGTcTcTTATAcAcATcTccGAGccCAcGAGAcinvT	p: phosphate, c: 5C-methylated, invT: inverted deoxythymidylate
i5 universal PCR primer	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG	
i7 barcode PCR primer_Ad2.25	CAAGCAGAAGACGGCATACGAGATCACTTTGTGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.26	CAAGCAGAAGACGGCATACGAGATTTCAAGTAGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.27	CAAGCAGAAGACGGCATACGAGATGCTATCACGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.28	CAAGCAGAAGACGGCATACGAGATAATCTACTGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.1	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.2	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.3	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.4	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGTG	
i7 barcode PCR primer_Ad2.6	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGTG	