

Supplementary Methods

Generation of calibration curve using 76-mer probes

76-mer 4mC containing model DNA was generated as described above with 4mdCTP. Template sequence: 5'-

CCTCACCATCTCAACCAATATTATATTATGTGTATATTCGATATTTTGTGTTAT

AATATTGAGGGAGAAGTGGTGA; forward primer: 5'-

CCTCACCATCTCAACCAATA; reverse primer: 5'-TCACCACTTCTCCCTCAAT.

76-mer dsDNA with 4mC was mixed with the same 76-mer dsDNA with no cytosine modification at different ratio (0%, 25%, 50%, 75%, 100%) and subject to 4mC-TAB-Seq.

Supplementary Results

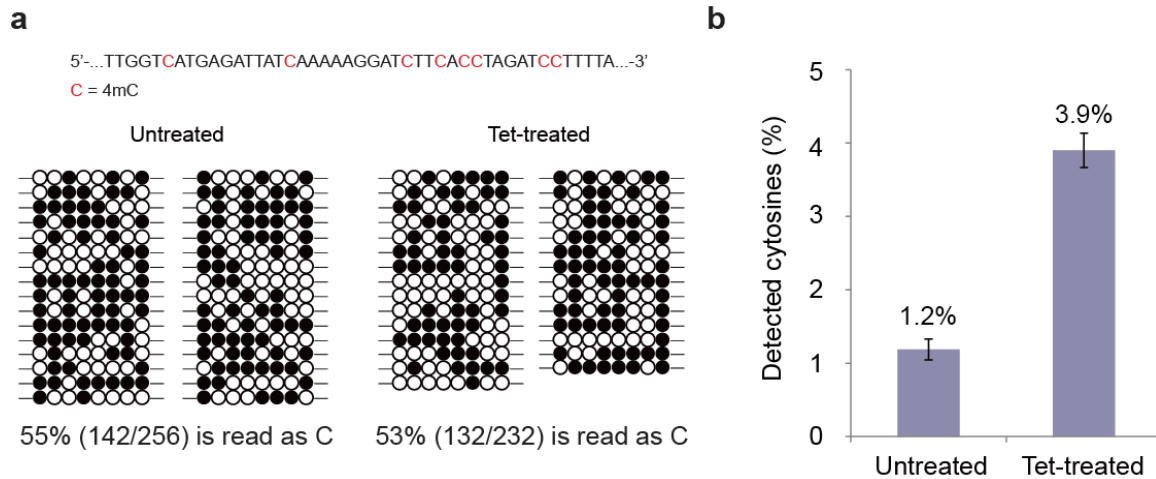


Figure S1. Tet has a weak demethylation activity towards 4mC. (a) Either untreated or Tet-treated 4mC-containing 304 bp model DNA was applied to optimized bisulfite conditions. After purification, the bisulfite-treated templates were amplified and the PCR products were applied to TOPO cloning, colony picking and Sanger sequencing. The 'C' reads were counted at each of eight total modification sites. Open circles represent the 4mCs read as T and black-filled circles represent the 4mCs read as C. (b) Quantification of unmodified cytosine on 4mC-containing model DNA generated with 4mdCTP by LC-MS/MS. The abundance of unmodified cytosines increased by ~2% after Tet treatment, indicating a slight, but detectable 4mC demethylation. Error bars indicate mean \pm s.d., n = 3.

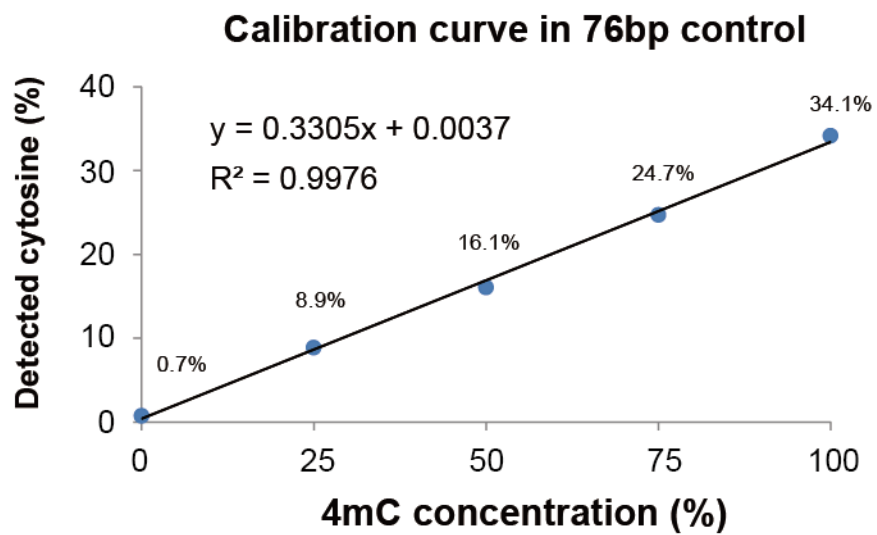


Figure S2. 4mC-TAB-seq results of 76-mer probes with variable 4mC content.

Figure S3. Motif sequence conservation analysis. Sequence conservation was evaluated for flanking 30 bases of each revealed motif in both upstream and downstream by calculating entropy dynamics in each position.

Table S1. Summary of mapping information.

<i>C. kristjanssonii</i>	Non-clonal mapped Reads		Detected Cytosine		Genome Coverage
Untreated	1,491,660	57.91%	971,460	96.6%	78.9
Tet treated	1,032,169	61.21%	970,708	96.5%	54.6

Table S2. Motif distribution in *C. kristjanssonii* using SMRTsequencing

Type	Motif	Total number in the genome	methylated number	Fraction
6mA	G <u>A</u> TC	3108	3101	99.77%
6mA	CGD <u>A</u> G	3591	3578	99.64%
6mA	CC <u>A</u> TTY	4041	3927	97.18%
4mC	GG <u>C</u> C	2990	2961	99.03%
4mC	RCC <u>C</u> C	2908	2827	97.21%
suspected modified base	G <u>C</u> NGCVGC	536	439	81.90%

D: A, G, or T

Y: C or T

R: A or G