Table S1 | Mapping statistics of RRBS-based regular, low-input and single-cell MAB-seq

Method	Experiment	Total reads	PF reads after trimming	Uniquely mapped reads	Mapping efficiency	Number of 1×CG	Number of 5×CG
RRBS-based regular MAB-seq (1 μg DNA)	Sperm rep1	29,822,760	28,738,090	16,606,280	0.578	2,598,268	966,519
	Sperm rep2	29,282,903	26,917,843	13,455,072	0.500	1,380,938	966,095
	shTdg mESC	28,908,921	27,165,723	12,502,602	0.460	1,125,162	758,336
RRBS-based liMAB-seq (~100 diploid cells)	2-cell embryos	35,640,277	31,952,632	13,907,478	0.4353	1,659,538	995,323
	shTdg mESC rep2	32,707,440	29,205,704	11,078,150	0.3793	2,031,985	934,280
	Paternal pronuclei rep1	36,014,418	30,873,848	12,527,439	0.4058	1,417,107	891,785
	Tet TKO mESC rep2	30,005,697	27,913,538	13,345,063	0.4781	1,466,108	878,947
	Paternal pronuclei rep2	51,459,994	33,039,317	10,703,204	0.3240	1,008,455	765,432
	Tet TKO mESC rep1	20,720,152	19,201,923	9,053,976	0.4715	1,934,988	764,281
	shTdg mESC rep1	23,297,580	21,636,674	10,265,147	0.4744	1,889,543	756,502
	Dnmt TKO mESC rep2	24,568,185	22,497,650	9,832,898	0.4371	1,251,116	713,976
	Dnmt TKO mESC rep1	31,818,194	27,535,402	10,020,554	0.3639	1,012,523	596,356
RRBS-based	Tet TKO mESC	21,574,462	19,334,066	7,423,872	0.384	480,210	421,649

scMAB-seq	shTdg mESC	35,237,615	29,328,097	13,013,857	0.444	439,729	384,426
	shTdg mESC	17,576,284	14,544,392	6,177,465	0.425	360,136	284,590
	shTdg mESC	21,114,069	16,574,750	6,577,015	0.397	437,599	272,443
	Tet TKO mESC	35,517,471	27,866,527	9,911,731	0.356	285,858	199,704
	shTdg mESC	17,566,012	14,100,383	5,341,181	0.379	217,691	193,710
	shTdg mESC	18,725,584	13,310,417	4,981,658	0.374	206,623	179,259
	shTdg mESC	19,982,722	15,074,769	6,290,954	0.417	237,457	178,104
	shTdg mESC	18,964,678	13,601,420	4,004,439	0.294	245,814	176,566
	shTdg mESC	19,093,335	14,497,019	4,943,628	0.341	238,184	165,249
	2-cell blastomere	31,649,330	23,668,009	8,553,326	0.361	211,703	157,679
	4-cell blastomere	11,412,663	8,482,794	2,171,010	0.256	210,281	156,341
	shTdg mESC	40,899,978	30,492,794	11,475,900	0.376	222,725	153,755
	shTdg mESC	18,911,106	14,293,291	3,732,004	0.261	163,951	144,859
	2-cell blastomere	19,086,346	15,514,778	5,713,036	0.368	175,121	140,792
	2-cell blastomere	18,270,341	14,835,647	4,744,635	0.320	170,154	140,466
	4-cell blastomere	11,172,491	7,889,004	2,495,648	0.316	188,420	139,933
	2-cell blastomere	17,354,821	14,075,671	5,145,670	0.366	157,082	139,850
	4-cell blastomere	10,721,500	7,770,324	2,183,668	0.281	175,480	136,719

	2-cell blastomere	18,112,141	12,952,144	3,143,347	0.243	151,410	128,455
	2-cell blastomere	31,466,481	15,725,860	6,220,382	0.396	152,497	125,772
	2-cell blastomere	30,879,267	21,307,958	3,545,059	0.166	153,881	125,095
	Tet TKO mESC	20,493,844	14,763,476	5,788,015	0.392	184,302	119,621
	2-cell blastomere	19,389,934	11,368,138	3,037,307	0.267	131,870	111,172
	Tet TKO mESC	15,830,015	12,776,248	3,470,479	0.272	151,070	111,063
	2-cell blastomere	24,781,049	20,542,784	8,059,263	0.392	139,317	110,726
	2-cell blastomere	25,160,848	20,678,581	5,994,340	0.290	139,794	108,510
	2-cell blastomere	19,836,447	15,847,026	4,698,879	0.297	156,016	108,436
	2-cell blastomere	19,679,898	16,081,885	4,761,718	0.296	162,213	108,271
	2-cell blastomere	12,567,568	10,047,141	3,174,735	0.316	149,664	105,990
	2-cell blastomere	15,559,143	10,953,254	3,251,724	0.297	118,926	104,215
	2-cell blastomere	14,480,356	11,058,523	3,893,824	0.352	150,970	102,633
	2-cell blastomere	16,030,786	11,045,878	3,861,295	0.350	148,250	102,491

	2-cell blastomere	24,955,952	19,497,636	6,627,878	0.340	136,881	101,833
	4-cell blastomere	11,175,604	6,272,960	1,398,458	0.223	124,601	101,821
	2-cell blastomere	18,342,887	13,949,330	4,177,662	0.299	161,713	100,019
	2-cell blastomere	23,584,661	19,317,452	5,567,865	0.288	136,795	98,721
	2-cell blastomere	13,503,781	10,301,424	2,390,977	0.232	148,443	95,988
	2-cell blastomere	16,613,789	12,827,855	4,486,767	0.350	129,737	93,895
	2-cell blastomere	15,296,830	12,615,030	3,949,754	0.313	142,998	92,229
	2-cell blastomere	14,654,707	11,862,856	4,587,690	0.387	131,842	91,148
	2-cell blastomere	15,187,820	12,635,338	4,187,961	0.331	141,729	90,497
	2-cell blastomere	17,678,818	15,418,582	5,679,811	0.368	139,748	89,739
	2-cell blastomere	12,099,785	9,670,289	3,349,756	0.346	131,093	86,595
	4-cell blastomere	11,048,205	9,176,965	456,875	0.050	151,555	85,174
	4-cell blastomere	12,244,028	8,454,203	2,692,643	0.318	118,029	82,300
	4-cell blastomere	11,819,631	7,466,731	2,195,684	0.294	106,305	81,643

	4-cell blastomere	13,121,379	7,808,799	2,579,086	0.330	98,145	71,902
	2-cell blastomere	13,559,000	10,528,203	3,259,547	0.310	93,634	56,231

Table S1 | Mapping statistics of RRBS-based regular, low-input and single-cell MAB-seq. For regular MAB-seq, 1 µg genomic DNA was used as the starting material. For low-input MAB-seq (liMAB-seq), around 100 diploid cells were used (in the case of the paternal pronuclei which are haploid, around 150 paternal pronuclei were used). For single-cell MAB-seq (scMAB-seq), a single cell was used. 1×CG and 5×CG refer to CpG sites covered for at least 1 time and 5 times by sequencing, respectively. For each method, the samples were sorted by the number of 5×CG.

Table S2 | Increased CpG coverage using longer sequencing length

scMAB-seq sample	Number of reads	Number of 1×CG		Number of 5×CG		Number of 10×CG	
		SE 100	SE 250	SE 100	SE 250	SE 100	SE 250
1	14183724	411425	590004	386658	502038	325798	438787
2	19982722	237457	349383	178104	249946	170509	236627
3	18112141	151410	215121	128455	178726	121910	167205
4	19389934	131870	191898	111172	157666	104029	145112

Table S2 | Increased CpG coverage using longer sequencing length. Four RRBS-based scMAB-seq libraries were sequenced in single-end 250 bp (SE 250) mode. The SE 250 data were compared with the same data trimmed to 100 bp (SE 100). The analysis suggested that when necessary, longer sequencing length can help recover more CpG sites.

Table S3 | Increased CpG coverage using pair-end sequencing

scMAB-seq sample	Number of reads	Number of 1×CG		Number of 5×CG		Number of 10×CG	
		SE 100	PE 100	SE 100	PE 100	SE 100	PE 100
1	17566012	217691	452392	193710	326490	186341	296856
2	18911106	163951	354656	144859	243562	138520	219834
3	9131030	149555	315158	135070	222066	126754	198910
4	8305919	113003	244364	99968	198794	92499	146575

Table S3 | Increased CpG coverage using pair-end sequencing. Four RRBS-based scMAB-seq libraries were sequenced in pair-end 100 bp (PE 100) mode. The PE 100 data were compared with the same data using only read 1 (SE 100). The analysis suggested that when necessary, performing pair-end sequencing can help recover more CpG sites.

Table S4 | Mapping statistics of PBAT-based low-input and single-cell MAB-seq

Method	Experiment	Total reads	PF reads after trimming	Uniquely mapped reads	Mapping efficiency	PCR duplication rate	Number of 1×CG
PBAT-based liMAB-seq (~100 diploid cells)	shTdg mESC	256,302,036	251,815,145	106,783,752	0.424	0.127	25,253,604
	TKO mESC	27,859,841	27,429,466	11,884,783	0.433	0.034	8,196,246
PBAT-based scMAB-seq	Tet TKO mESC	30,800,116	30,033,906	10,624,469	0.354	0.135	6,086,412
	shTdg mESC	31,195,776	30,274,887	7,211,236	0.238	0.111	4,339,008
	Tet TKO mESC	20,309,317	19,720,899	5,344,165	0.271	0.077	3,636,782
	shTdg mESC	29,817,145	28,765,221	5,018,444	0.174	0.084	3,183,118
	Tet TKO mESC	18,876,465	18,329,827	4,259,691	0.232	0.067	2,987,072
	shTdg mESC	28,786,036	27,449,771	3,943,879	0.144	0.122	2,335,982
	4-cell blastomere	16,032,132	15,586,920	3,812,399	0.245	0.177	2,267,937
	4-cell blastomere	13,226,779	12,816,582	2,909,310	0.227	0.151	1,834,911
	4-cell blastomere	14,986,634	14,572,877	3,184,182	0.219	0.191	1,834,503
	shTdg mESC	26,682,776	25,785,216	2,329,210	0.090	0.077	1,674,503
	4-cell blastomere	9,566,516	9,249,967	1,940,987	0.210	0.122	1,374,069

Table S4 | Mapping statistics of PBAT-based low-input and single-cell MAB-seq. For low-input MAB-seq (liMAB-seq), around 100 diploid cells were used. For single-cell MAB-seq (scMAB-seq), a single cell was used. 1×CG refers to CpG sites covered for at least 1 time by sequencing. For each method, the samples were sorted by the number of 1×CG.