

Supplementary Tables

Step	Software	No. Reads	Mean Time (min)	Standard Dev (min)
Alignment	Bismark	1M	19.33	1.74
	BitmapperBS	1M	11.98	3.76
	BSseeker2	1M	65.76	3.5
	bwa-meth	1M	29.92	6.17
Meth Calling	Bismark	1M	2.86	0.9
	BitmapperBS	1M	1.97	0.17
	BSseeker2	1M	39.87	17.4
	bwa-meth	1M	0.24	0.07

Supplementary Table 1. Timing comparison of each alignment and methylation estimation software. Ten permutations of each software were run, and the mean times are reported, as well as the standard deviation per software.

Insert Size	HG001	HG002	HG003	HG004	HG005	HG006	HG007	mean	stDev
EMSeqLAB01	297.1	299.6	349.75	288.95	300.53	298.23	330.3	309.21	22.11
EMSeqLAB02	325.8	327.95	326.35	328.15	320.2	325.47	358.57	330.35	12.72
MethylSeq	249.77	250.93	250.88	247.13	249.67	256.45	247.78	250.37	3.04
SPLAT	214.83	217.81	220.71	223.74	220.34	223.85	224.18	220.78	3.52
TruSeq	218.58	217.68	219.88	216.77	214.67	217.58	215.78	217.28	1.74
TrueMethylOX	207.7	203.83	209.95	205.93	211.8	211.5	204.4	207.87	3.3
TrueMethylBS	228.6	220.67	226.5	224.2	224.73	227.4	219	224.44	3.52
EPIC	225.7	227.45	225.65	227	229.65	229.7	227.7	227.55	1.65
Primary Mapping %	HG001	HG002	HG003	HG004	HG005	HG006	HG007	mean	stDev
EMSeqLAB01	97.48	97.63	97.37	97.87	97.14	97.36	97.32	97.45	0.24
EMSeqLAB02	94.89	93.27	94.09	94.46	91.97	94.27	93.33	93.75	0.98
MethylSeq	98.4	98.36	98.32	98.41	98.16	98.36	98.39	98.34	0.09
SPLAT	96.44	97.18	96.92	97.21	97.07	97.48	97.24	97.08	0.33
TruSeq	95.33	95.45	95.23	95.58	95.51	95.55	95.37	95.43	0.13
TrueMethylOX	85.95	84.53	87.43	87.01	85.64	87.15	85.11	86.12	1.11
TrueMethylBS	85.17	83.5	86.66	86.06	84.73	86.4	84.2	85.25	1.18
EPIC	98.27	98.31	98.37	98.12	98.28	98.27	98.23	98.26	0.08
Duplicate %	HG001	HG002	HG003	HG004	HG005	HG006	HG007	mean	stDev
EMSeqLAB01	9.28	9.13	9.15	8.65	11.28	12.1	10.6	10.03	1.3
EMSeqLAB02	23.61	25.03	23.97	23.37	27.08	23.68	25.11	24.55	1.31
MethylSeq	13.75	13.77	13.84	14.42	13.56	14.44	13.32	13.87	0.42
SPLAT	12.37	12.02	11.63	10.9	11.88	10.86	13.28	11.85	0.84
TruSeq	21.73	22.53	27.26	24.35	25.86	31.57	25.77	25.58	3.28
TrueMethylOX	21.24	21.66	18.19	19.48	20.86	19.56	21.21	20.31	1.26
TrueMethylBS	21.29	21.95	17.89	18.72	20.15	18.66	20.22	19.84	1.48
EPIC	66.42	67.43	62.9	63.92	69.21	69.5	68.24	66.8	2.56
Dinucleotide Bias	HG001	HG002	HG003	HG004	HG005	HG006	HG007	mean	stDev
EMSeqLAB01	3.77	2.97	3.36	2.98	2.7	2.69	2.99	3.07	0.38
EMSeqLAB02	1.12	0.84	1.16	0.98	1.14	1.11	1.1	1.06	0.11
EPIC	26.58	26.74	26.59	26.69	26.43	26.36	26.93	26.62	0.19
MethylSeq	3.4	3.71	3.43	3.41	3.47	3.53	3.52	3.5	0.11
SPLAT	6.95	7.58	5.55	6.36	6.66	6.24	5.8	6.45	0.69
TrueMethylBS	3.43	3.44	3.8	3.3	3.73	3.61	3.99	3.61	0.24
TrueMethylOX	3.3	3.71	3.64	3.71	3.72	3.72	3.71	3.64	0.16
TruSeq	24.66	23.09	24.1	23.69	23.13	23.36	23.83	23.69	0.56
Useable Bases %	HG001	HG002	HG003	HG004	HG005	HG006	HG007	mean	stDev
EMSeqLAB01	90.21	90.35	90.26	90.71	87.88	87.61	88.89	89.42	1.27
EMSeqLAB02	76.01	74.6	75.51	76.16	72.62	76.01	51.56	71.78	9
EPIC	33.2	32.21	36.69	35.67	30.46	30.17	31.4	32.83	2.52
MethylSeq	74.46	74.5	74.55	73.8	74.77	74.01	74.9	74.43	0.39
SPLAT	78.9	80.62	81.19	83	81.43	82.79	81.4	81.33	1.38
TrueMethylBS	70.21	66.6	71.08	70.13	70.03	71.69	71.97	70.24	1.78
TrueMethylOX	67.53	66.49	68.45	68.5	68.07	68.66	66.78	67.78	0.87
TruSeq	62.68	62.98	60.23	61.19	60.29	62.58	51.03	60.14	4.17

Supplementary Table 2 Read and mapping statistics for all cell lines. stDev = standard deviation. Values shown are averages across replicates for each library. Usable bases are calculated as the total mapped bases as a percentage of the total number of bases sequenced.

Number of Common Sites for all Assays	2298846	Assay					
Common Sites with 5X for all Assays (C5X Sites)	1928536						
DM Sites in 3 or more platforms on C5X sites (DM4+)	29802	EM-Seq	Methyl-Seq	Nanopore	SPLAT	TrueMethyl	TruSeq
Percentage of all common sites with 5X Coverage		97%	96%	100%	96%	97%	86%
Number of DM Sites for this assay on C5X Sites (DMA)		74054	67621	26868	76591	59516	87170
Percentage DMA unique to this platform		26%	26%	17%	29%	22%	42%
Percentage of DMA sites in DM4+		36%	38%	56%	35%	42%	27%
Percentage of DM4+ in DMA sites		90%	86%	51%	89%	85%	79%

Supplementary Table 3 Statistics for differentially methylated sites across assays.

	EMSeq	MethylSeq	Nanopore	SPLAT	TrueMethyl	TruSeq
Number of DMAs mapped to array	3296	2964	1027	3228	2750	4267
Number DMAs with PMD > .2	3279	2942	1026	3092	2743	3404
% DMAs with PMD >.2 and array PMD > .2	55.5%	58.8%	67.0%	57.3%	60.0%	49.6%
Number Hypermethylated in HG005-HG007	2505	2358	721	2368	2092	2432
% Hypermethylated DMAs with array PMD > .2	57.0%	60.3%	69.1%	58.6%	62.0%	52.4%
Number Hypomethylated in HG005-HG007	774	584	305	724	651	972
% Hypomethylated DMAs with array PMD < -.2	50.4%	53.1%	62.0%	53.0%	53.8%	42.7%
% of sites with array PMD >.2 identified as DMAs	44.0%	41.9%	16.6%	43.5%	39.8%	42.7%

Supplementary Table 4 Concordance between assays of differentially methylated sites per assay (DMAs) with respect to microarray sites. PMD = Percent Methylation Difference, calculated as an absolute value.

TargetID	African American	Caucasian American	Asian American	Asian-Caucasian	FOR	Chr.	Position (HG19)	Position (HG38)	Gene	Feature	Variance	meQTL	EMSeq	MethylSeq	Nanopore	SPRAT	TrueMethyl	TruSeq	Illumina	
cg1590012	0.84	0.85	0.62	-0.23	2.73E-29	1	1265354	1329974	TAS1R3	Promoter	AS	yes	-0.7629	-0.6364	-0.3709	-0.6693	-0.6275	-0.399	-0.2610	
cg23611477	0.89	0.81	0.75	-0.06	4.11E-11	1	1644835	1713396	CDK11A,CDK11B	Body/Promoter	AF	yes	NA	0.4202	0.0614	NA	NA	NA	0.0538	
cg00649623	0.28	0.15	0.14	-0.01	2.78E-17	1	1653867	1724428	CDK11B,CDK11A	Promoter	AF	no	-0.0133	0.0033	0.0132	0.0111	0.0147	-0.018	0.0057	
cg03196347	0.73	0.69	0.61	0.01	1.08E-05	1	1873803	1944364	NPW4	Promoter	AF	yes	-0.0497	-0.1173	0.0269	0.239	0.0752	-0.04	-0.0131	
cg00095688	0.62	0.66	0.52	-0.14	1.24E-05	1	2003864	2072425	PRKCZ	Promoter	AS	no	-0.0687	-0.0143	-0.0531	-0.2671	-0.1292	-0.0435	-0.0499	
cg10761639	0.74	0.84	0.69	-0.15	1.09E-17	1	2021794	2092355	PRKCZ	Promoter	CA	yes	0.0025	0.0335	-0.1343	-0.0615	-0.0867	-0.0412	-0.0425	
cg24499605	0.45	0.32	0.43	0.11	1.76E-14	1	3142925	3226361	PRDM16	Body	CA	no	0.061	0.0478	0.1165	0.0104	-0.0335	0.129	0.0281	
cg14654471	0.91	0.89	0.75	-0.14	3.57E-14	1	5937169	5977109	NPW4	Body	AS	yes	-0.0249	0.0024	-0.0453	0.0282	-0.0055	0.0189	-0.1263	
cg13549940	0.64	0.81	0.81	0.00	3.29E-12	1	6390053	6329993	ACOT17	Body	AF	yes	-0.2485	-0.1441	-0.2142	-0.1369	-0.0986	-0.1721	-0.1473	
cg23914842	0.32	0.39	0.50	0.11	1.21E-07	1	9327170	9367111	HP6D	3'UTR	AS	yes	-0.0111	-0.1046	-0.0248	-0.2128	-0.1016	-0.0768	0.0269	
cg01017257	0.57	0.48	0.61	0.13	3.62E-05	1	15059738	14733242	KIAA1026/KAZN	Body/Body	CA	yes	0.7907	0.649	0.6319	0.6125	0.5373	0.617	0.6381	
cg04850599	0.31	0.26	0.40	0.14	1.05E-08	1	17019133	16692638	ESPNP	Body	AS	no	-0.1329	NA	-0.0215	-0.0769	NA	NA	-0.0618	-0.0007
cg16588994	0.30	0.21	0.36	0.15	2.37E-05	1	21023322	2099639	KIF17	Body	CA	yes	0	0.0968	0	NA	-0.0175	0.0952	-0.0276	
cg18150584	0.57	0.50	0.64	0.14	6.28E-04	1	23887816	23561326	ID3	Promoter	CA	no	0.083	0.0777	0.025	0.2504	0.0816	0.2045	0.1313	
cg19276111	0.43	0.55	0.49	-0.06	2.33E-03	1	24229232	23002742	CNR2	Promoter	AF	no	-0.0678	0.0343	-0.14	-0.0744	-0.1275	-0.0814	-0.2102	
cg20415053	0.54	0.62	0.74	0.12	1.60E-05	1	26527928	26201437	CATSPER4	Body	AS	yes	0.0882	0.0313	0.1282	0.2414	0.0432	0.1908	0.1243	
cg02131754	0.50	0.29	0.18	-0.11	3.50E-20	1	28572799	28245788	NPW4	AF/3AS	AS	yes	-0.3134	-0.3608	-0.2891	-0.4694	-0.4177	-0.4076	-0.4258	
cg14718142	0.66	0.81	0.84	0.03	9.37E-14	1	32739251	32727500	LCK	Promoter	AF/AS	yes	0.0052	-0.1846	0.0507	0.0287	-0.1409	-0.0417	-0.0160	
cg06917450	0.29	0.27	0.54	0.27	2.31E-16	1	38156652	3769080	C1orf109	Promoter	AS	yes	0.5083	0.5241	0.4751	0.439	0.4331	0.4884	0.5093	
cg26038582	0.69	0.57	0.64	0.07	1.73E-02	1	42384390	41917719	HIVEP3	Promoter	CA	no	-0.2774	-0.185	-0.0661	0.021	-0.234	0.111	-0.2571	
cg02927682	0.37	0.40	0.49	0.09	1.73E-03	1	54844424	54378751	SSBP3	Body	AS	yes	0.2756	0.1371	0.105	0.1135	0.138	0.2669	0.2106	
cg10710651	0.48	0.37	0.50	0.13	1.06E-04	1	80968194	86502001	NA	CA	yes	0.2863	0.3508	0.346	0.323	0.2832	0.2422	0.1701		
cg10631373	0.41	0.29	0.36	0.07	2.25E-04	1	89457642	88991959	RBMLX1,CCBL2	Promoter/Promoter	CA	yes	0.1154	0.0038	0.0936	0.1048	0.1652	-0.0228	0.1258	
cg09480871	0.59	0.66	0.75	0.09	5.84E-07	1	101003634	100538078	GP88B	Promoter	AF	yes	0.1117	0.0329	-0.0697	-0.308	0.2187	0.0961	0.0979	
cg06223162	0.30	0.38	0.53	0.15	5.65E-08	1	101003688	100538132	GP88B	Promoter	AS	yes	0.1265	0.1352	0.1149	0.132	0.0938	0.1667	0.1493	
cg25110835	0.25	0.28	0.46	0.18	1.91E-09	1	110254828	109712206	GSTM5	Promoter	AS	yes	-0.4427	-0.3016	-0.208	0.0643	-0.0629	-0.2639	-0.1339	
cg02193146	0.64	0.79	0.76	-0.03	6.37E-06	1	110752257	11039635	NA	AF	no	0.1198	0.0101	0.0234	0.0045	-0.0575	-0.0532	-0.0285		
cg24833868	0.51	0.49	0.66	0.17	2.26E-05	1	14655624	147084075	NA	AS	yes	0.3135	0.5334	0.1769	0.2033	0.1937	0.2011	0.1400		
cg13502125	0.66	0.63	0.77	0.14	8.15E-05	1	147826191	148354063	NA	AS	yes	-0.0759	-0.1163	-0.0008	0.0058	-0.0483	0.1103	-0.0616		
cg029339103	0.45	0.61	0.22	-0.19	2.67E-15	1	154839999	154867433	KCNK9	Body	AS	yes	-0.5464	-0.3508	-0.4614	-0.541	-0.4958	-0.3729	-0.5728	
cg23915527	0.50	0.34	0.39	0.01	2.45E-05	1	161364797	161398997	NA	AF	yes	0.2098	0.2829	0.1048	0.1548	0.0742	0.2871	0.2315		
cg12092579	0.38	0.23	0.29	0.06	2.07E-06	1	178380975	17811840	RASA2.1	Body	AF	no	-0.2273	-0.1367	-0.0771	-0.3965	-0.1321	-0.3319	-0.3504	
cg21868798	0.36	0.30	0.24	-0.06	3.09E-05	1	199481399	199512771	NA	AF	yes	0.2709	0.1163	-0.0715	0.1599	0.1124	0.1697	0.1315		
cg18222190	0.41	0.35	0.48	0.13	1.21E-10	1	204290972	204321844	PLEKH8A6	Promoter	CA	yes	0.1774	0.1619	0.0542	0.1112	0.1024	0.1	0.1857	
cg20240347	0.46	0.31	0.35	0.04	1.71E-04	1	204465584	204496556	NA	AF	yes	0.0271	0.0594	-0.1258	0.029	-0.0769	0.1178	0.0815		
cg17178900	0.28	0.50	0.24	-0.26	7.76E-10	1	205818956	205849828	PM2DD1	Body	CA	yes	-0.4676	-0.555	-0.4076	-0.4483	-0.5194	-0.6068	-0.5164	
cg26354017	0.31	0.50	0.28	-0.22	1.98E-08	1	205819088	205849960	PM2DD1	Promoter	CA	yes	-0.5308	-0.4969	-0.4043	-0.6205	-0.5653	-0.6419	-0.5504	
cg14159672	0.30	0.48	0.26	-0.22	5.54E-11	1	205819179	205850051	PM2DD1	Promoter	CA	yes	-0.6875	-0.6542	-0.643	-0.5692	-0.6211	-0.6649	-0.6134	
cg14893161	0.26	0.38	0.22	-0.16	2.06E-11	1	205819251	205850123	PM2DD1	Promoter	CA	yes	-0.5273	-0.4899	-0.5048	-0.5089	-0.5619	-0.4667	-0.4443	
cg11869513	0.15	0.30	0.11	-0.19	9.61E-14	1	205819406	205850278	PM2DD1	Promoter	CA	yes	-0.1019	-0.2598	-0.1607	-0.1985	-0.2424	-0.3878	-0.2003	
cg24503407	0.25	0.43	0.21	-0.22	1.11E-13	1	205819492	205850364	PM2DD1	Promoter	CA	yes	-0.6528	-0.5719	-0.5636	-0.5621	-0.6911	NA	-0.5648	
cg07157834	0.33	0.46	0.28	-0.18	2.78E-09	1	205819609	205850481	PM2DD1	Promoter	CA	yes	-0.5907	-0.6364	-0.4552	-0.5556	-0.6012	-0.3885	-0.5729	
cg06939379	0.62	0.48	0.46	-0.02	1.01E-06	1	232941706	232809590	KIAA1383/MAP10	Promoter	AF	yes	0.1941	0.1563	0.2429	0.1696	0.3363	0.2297	0.1942	
cg00911195	0.49	0.32	0.33	0.01	7.12E-09	1	232941775	232809629	KIAA1383/MAP10	Promoter	AF	yes	0.0731	0.0597	0.1212	0.071	0.0593	0.0451	0.0826	
cg02889973	0.46	0.39	0.51	0.12	3.25E-04	1	234977572	234841825	NA	CA	no	0.2911	0.332	0.1873	0.3361	0.3551	0.1044	0.3384		
cg09033006	0.40	0.46	0.22	-0.24	5.74E-19	1	244517177	244358175	C1orf100	Promoter	AS	yes	-0.1061	-0.1756	-0.1901	-0.206	-0.2146	-0.2098	-0.2268	
cg19188911	0.61	0.70	0.75	0.05	1.01E-07	1	245541456	245378154	KIF26B	Body	AF	no	0.2452	0.0935	0.2145	0.2595	0.1635	0.0662	0.1769	
cg04134399	0.28	0.15	0.28	0.13	9.18E-09	1	246331142	246076740	NPW4	Body	CA	no	-0.082	-0.0983	0.0651	0.066	-0.0339	-0.14	-0.0226	
cg04798314	0.51	0.60	0.84	0.18	2.25E-13	1	246668601	246505995	SMYD3	Body	AS	yes	-0.0724	0.1592	-0.0085	-0.0506	-0.0733	0.0421	-0.0321	
cg09226051	0.42	0.40	0.30	-0.10	4.03E-03	1	247611502	247448200	NLRP3	Body	AS	yes	-0.0714	-0.154	-0.017	-0.1633	0.034	-0.0239	-0.2073	
cg15829088	0.33	0.37	0.45	0.08	3.36E-04	1	247802935	247639333	NA	ncRNA promoter	AS	yes	0.0851	-0.0693	0.0111	-0.2654	0	-0.0899	-0.0053	

Supplementary Table 5 Population Variance agreement. A total of 52 CpGs on chromosome 1 that had been identified as differentially methylated between ethnic populations were annotated and compared for concordance of differential signal between microarray and sequencing data.

fmoles of different forms identified

	Name	dC	dmC	dmC	dhmC	dmC/dC	dhmC/dmC	dhmC/dC	
Biological Replicate 1	HG001	CP 01	5680.727	156.3333	2143.964	0.922163	2.75%	0.04%	0.0012%
		CP 01	5721.555	157.2296	2212.077	0.966647	2.75%	0.04%	0.0012%
	HG002	CP 02	6134.437	206.1124	2915.523	1.078334	3.36%	0.04%	0.0012%
		CP 02	6097.877	206.3662	2893.051	0.969016	3.38%	0.03%	0.0011%
	HG003	CP 03	6676.031	212.3023	2979.854	0.756934	3.18%	0.03%	0.0008%
		CP 03	6742.651	211.5162	2948.914	0.739288	3.14%	0.03%	0.0008%
	HG004	CP 04	5223.132	188.2691	2588.429	0.592667	3.60%	0.02%	0.0008%
		CP 04	5224.774	191.126	2560.265	0.56839	3.66%	0.02%	0.0008%
	HG005	CP 05	5487.878	192.3814	2680.448	0.828852	3.51%	0.03%	0.0011%
		CP 05	5523.128	193.3392	2646.98	0.784192	3.50%	0.03%	0.0010%
	HG006	CP 06	5962.204	217.7679	2979.255	0.724408	3.65%	0.02%	0.0009%
		CP 06	6041.553	217.7672	3002.681	0.686946	3.60%	0.02%	0.0008%
	HG007	CP 07	5819.142	205.9319	2860.277	0.884028	3.54%	0.03%	0.0011%
		CP 07	5733.883	204.7114	2814.214	0.937631	3.57%	0.03%	0.0012%
Biological Replicate 2	HG001	CP 08	3620.176	99.23043	1362.303	0.646334	2.74%	0.05%	0.0013%
		CP 08	3674.493	98.73453	1356.403	0.582917	2.69%	0.04%	0.0012%
	HG002	CP 09	2835.62	91.63515	1259.922	0.537728	3.23%	0.04%	0.0014%
		CP 09	2872.229	92.00553	1250.31	0.520239	3.20%	0.04%	0.0013%
	HG003	CP 10	2832.307	85.18032	1167.688	0.370097	3.01%	0.03%	0.0010%
		CP 10	2864.241	86.2764	1175.466	0.351737	3.01%	0.03%	0.0009%
	HG004	CP 11	2987.18	104.5185	1423.736	0.548236	3.50%	0.04%	0.0013%
		CP 11	2989.671	104.0718	1431.635	0.452427	3.48%	0.03%	0.0011%
	HG005	CP 12	2999.949	102.7485	1410.854	0.489238	3.43%	0.03%	0.0012%
		CP 12	3048.796	103.0123	1399.723	0.54343	3.38%	0.04%	0.0013%
	HG006	CP 13	3258.307	113.3027	1535.199	0.48512	3.48%	0.03%	0.0011%
		CP 13	3199.5	111.8209	1519.138	0.476289	3.49%	0.03%	0.0011%
	HG007	CP 14	3324.166	113.9253	1545.66	0.634276	3.43%	0.04%	0.0014%
		CP 14	3266.074	112.9173	1530.64	0.60005	3.46%	0.04%	0.0014%
Blank		CP 15	0		0.681001				
		CP 15	0		0.689081				

Supplementary Table 6 LC-MS/MS quantification of dC, dmC and dhmC in fmoles from digested genomic DNA (HG001-HG007) samples. For the detection of 5hmC a second higher volume injection was performed. The two dmC quantification values correspond to the two injections. Percentage of 5hmC in these samples is very low and below the limit of detection of the method.