Supplementary Table 1. AUC result for BSmooth t-like statistic. Response is a binary variable that classifies CpGs based on whether their original fractional methylation differences between groups are over some criteria or not.

Window size	Honey Bee			Human		
	Diff =0.05	Diff =0.1	Diff =0.2	Diff =0.05	Diff =0.1	Diff =0.2
4000 bp	0.5774	0.6045	0.6739	0.5278	0.5451	0.5579
2000 bp	0.6203	0.6461	0.6706	0.5668	0.5828	0.6009
1000 bp	0.6706	0.7110	0.7443	0.5919	0.6113	0.6359
500 bp	0.7344	0.7686	0.7363	0.6194	0.6425	0.6732

Supplementary Table 2. Differentially methylated CpGs (DMLs) and DMRs between young and old human in the unfiltered and filtered data sets using RadMeth. Top 20 CpGs according to the q-values are shown. The numbers of CpGs that are significantly differentially methylated for q-value thresholds of 0.05, 0.10, and 0.20 are also shown.

Rank	Unfiltered dataset					Filtered dataset				
	Location (scaffold:bp)	Log-OR[1]	Original-p	Combined-p	FDR q	Location (scaffold:bp)	Log-OR	Original-p	Combined-p	FDR q
1	Chr1:3145020	-2.75	0.016	0[2]	0	Chr1:3145020	-2.75	0.016	0	0
2	Chr1:3145021	-2.25	0.042	0	0	Chr1:3145021	-2.25	0.042	0	0
3	Chr1:3145031	-2.91	0.016	0	0	Chr1:3145031	-2.91	0.016	0	0
4	Chr1:3145032	-10.61	0.076	0	0	Chr1:3145032	-10.61	0.076	0	0
5	Chr1:3145036	-13.77	0.0064	0	0	Chr1:3145036	-13.77	0.0064	0	0
6	Chr1:3145037	-2.35	0.074	0	0	Chr1:3145037	-2.35	0.074	0	0
7	Chr1:3145040	-16.22	0.0052	0	0	Chr1:3145040	-16.22	0.0052	0	0
8	Chr1:3145041	-2.30	0.079	0	0	Chr1:3145041	-2.30	0.079	0	0
9	Chr1:3145043	-16.22	0.0052	0	0	Chr1:3145043	-16.22	0.0052	0	0
10	Chr1:3145044	-1.50	0.19	0	0	Chr1:3145044	-1.50	0.19	0	0
11	Chr1:3145051	-611.25	0.0056	0	0	Chr1:3145051	-611.25	0.0056	0	0
12	Chr1:3145052	-2.44	0.066	0	0	Chr1:3145052	-2.44	0.066	0	0
13	Chr1:3145060	-2.59	0.025	0	0	Chr1:3145060	-2.59	0.025	0	0
14	Chr1:3145061	-2.14	0.048	0	0	Chr1:3145061	-2.14	0.048	0	0
15	Chr1:3145069	-3.22	0.013	0	0	Chr1:3145069	-3.22	0.013	0	0
16	Chr1:3145070	-2.54	0.024	0	0	Chr1:3145070	-2.54	0.024	0	0
17	Chr1:3145082	-2.77	0.017	0	0	Chr1:3145082	-2.77	0.017	0	0
18	Chr1:3145083	-1.51	0.13	0	0	Chr1:3145083	-1.51	0.13	0	0
19	Chr1:3145090	-3.30	0.020	0	0	Chr1:3145090	-3.30	0.020	0	0
20	Chr1:3145091	-2.67	0.054	0	0	Chr1:3145091	-2.67	0.054	0	0
q < 0.05	36532 (3641)[3]					56783 (5213)				
q < 0.10	54112 (6621)					86984 (10206)				
q < 0.20	91936 (13722)					153583 (22314)				

[1]Odds Ratio of cytosine reads between forager and nurse groups.
[2]Too low value to approximate accurately

[3]Numbers in parenthesis indicate the number of significant DMRs.

Supplementary Table 3. Differentially methylated CpG clusters (DMRs) between old and young human brains in the unfiltered and filtered data sets using Biseq. Top 20 CpG clusters according to the q-values are shown. We used only chromosome 22 for the analysis.

Rank	Unfiltered dataset (N*=14123)				Filtered dataset (N=14076)			
	Location (start-end)	Methylation Difference	Cluster-p	FDR q	Location (start-end)	Methylation Difference	Cluster-p	FDR q
1	37466162-37466165	0.403	0.34	0.9995	27737516-27737516	0.370	0.37	0.9993
2	34107586-34107586	0.208	0.37	0.9995	36127525-36127526	0.519	0.38	0.9993
3	27737516-27737516	0.370	0.37	0.9995	36678490-36678490	0.250	0.38	0.9993
4	47442538-47442538	0.400	0.38	0.9995	27770812-27770815	0.450	0.38	0.9993
5	20266875-20266875	0.426	0.38	0.9995	45847882-45847883	0.516	0.39	0.9993
6	45703964-45703964	0.281	0.38	0.9995	42841885-42841923	0.300	0.39	0.9993
7	27770812-27770815	0.450	0.38	0.9995	34065152-34065152	0.333	0.39	0.9993
8	47411541-47411542	0.416	0.38	0.9995	36390794-36390794	0.667	0.39	0.9993
9	32232349-32232350	0.349	0.38	0.9995	40239507-40239508	0.536	0.39	0.9993
10	47448253-47448253	0.357	0.39	0.9995	41911670-41911686	0.350	0.39	0.9993
11	36994805-36994812	0.560	0.39	0.9995	18494163-18494164	0.533	0.39	0.9993
12	34065152-34065152	0.333	0.39	0.9995	42463792-42463792	0.833	0.39	0.9993
13	29819309-29819342	0.153	0.39	0.9995	37466224-37466229	0.327	0.39	0.9993
14	43002916-43002974	0.350	0.39	0.9995	29037408-29037409	0.409	0.40	0.9993
15	36390794-36390794	0.667	0.39	0.9995	36594467-36594467	0.000	0.40	0.9993
16	28754998-28754998	0.331	0.39	0.9995	24630479-24630517	0.302	0.40	0.9993
17	16430527-16430531	0.342	0.39	0.9995	18633058-18633059	0.500	0.40	0.9993
18	21909909-21909949	0.387	0.39	0.9995	21909518-21909518	0.204	0.40	0.9993
19	21893127-21893170	0.267	0.39	0.9995	31348589-31348590	0.145	0.40	0.9993
20	42034617-42034643	0.388	0.39	0.9995	27737516-27737516	0.370	0.40	0.9993

* Number of tested clusters in chromosome 22.

Supplementary Table 4. AUC result for HMM-Fisher statistic. Response is a binary variable that classifies CpGs based on whether their original fractional methylation differences between groups are over some criteria or not. We used p-value of each CpG from HMM-Fisher tool as a predictor.

Number of Classes	Honey bee			Human		
	Diff =0.05	Diff =0.1	Diff =0.2	Diff =0.05	Diff =0.1	Diff =0.2
Two	0.5473	0.618	0.6935	0.5562	0.573	0.6115
Three	0.5658	0.6557	0.7401	0.6145	0.6477	0.7106

Supplementary Table 5. Summary of two alignment results to chromosome 22 of the human dataset. We summarized the number of CpGs, mean and standard deviation of read coverage, mean fractional methylation level, proportion of highly, or non-methylated CpGs, and correlation of fractional methylation level between the two alignments. Overall, they have similar values and patterns.

| Sample ID		Number of CpGs	Mean and standard deviation of coverage	Mean fractional methylation	Proportion of Highly methylated CpGs	Proportion of non-methylated CpGs	Correlation of fractional methylation
GSM1167005	Bowtie	1054909	7.97±4.54	0.7768	0.7479	0.100	0.799
	BSMAP	1074201	7.43±5.69	0.7738	0.7367	0.099	
GSM1166274	Bowtie	1052604	7.70±4.48	0.7779	0.7487	0.102	0.731
	BSMAP	997348	4.18±3.85	0.7850	0.7298	0.111	
GSM1173775	Bowtie	767852	2.16±1.45	0.7824	0.7362	0.162	0.605
	BSMAP	756774	2.29±2.44	0.7767	0.7260	0.160	
GSM1173772	Bowtie	1044444	6.45±4.07	0.7639	0.7253	0.110	0.712
	BSMAP	1066613	7.07±6.67	0.7205	0.6519	0.113	

Supplementary Table 6. In line with our second recommendation, we counted globally unmethylated CpG sites used for filtering. In the last column, we calculated the proportion of removed CpGs that are globally unmethylated. This proportion is similar in both alignments.

|	Number of CpGs in filtered dataset.	Number of CpGs in un-filtered dataset.	Proportion of removed CpGs
Bowtie	980120	1069498	0.084
BSMAP	984156	1069267	0.080

Supplementary Table 7. The classification results using HMM-Fisher tool in chromosome 22 using the two aligners. The classified proportions and number of DMLs are similar and do not contradict our conclusion.

		Proportion of Methylation state			Classification results (number of DMLs)	
		Un	Partial	Full	Two class	Three class
Human (Bowtie)	Young	0.1171	0.1287	0.7542	2082	7049
	Old	0.1452	0.1131	0.7417		
Human (BSMAP)	Young	0.1155	0.1411	0.7434	1415	5516
	Old	0.1440	0.1627	0.6933		

Supplementary Figure 1. Comparison of smoothing patterns in our human dataset using two aligners. (A) Bowtie and (B) BSMAP. They show very similar patterns of smoothing and correlation coefficients between estimated and original fractional methylation levels.

(A)

(B)
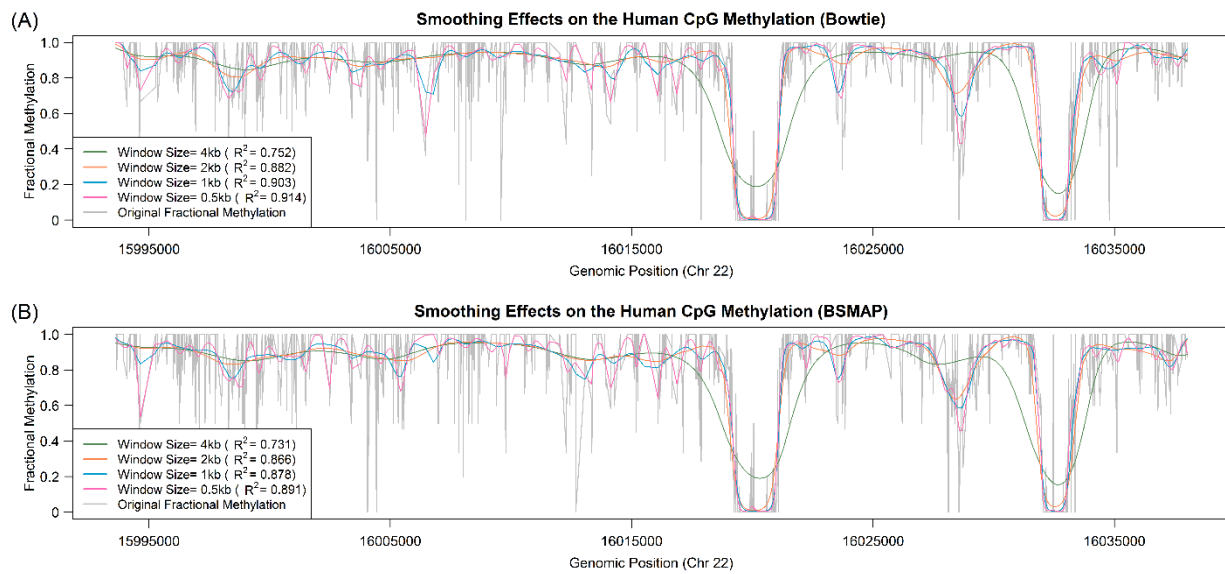
Supplementary Figure 2. Plot of relative ratios between numbers of the DMLs from the 4000bp window size and those from the other window sizes. We used BSmooth to detect DMLs for each window size. The gold line shows the trend from the Bowtie aligned data and the green line is from the BSMAP aligned data. They show similar patterns in variation, that do not increase much over varying window sizes.

Comparison of DML Varying Trends
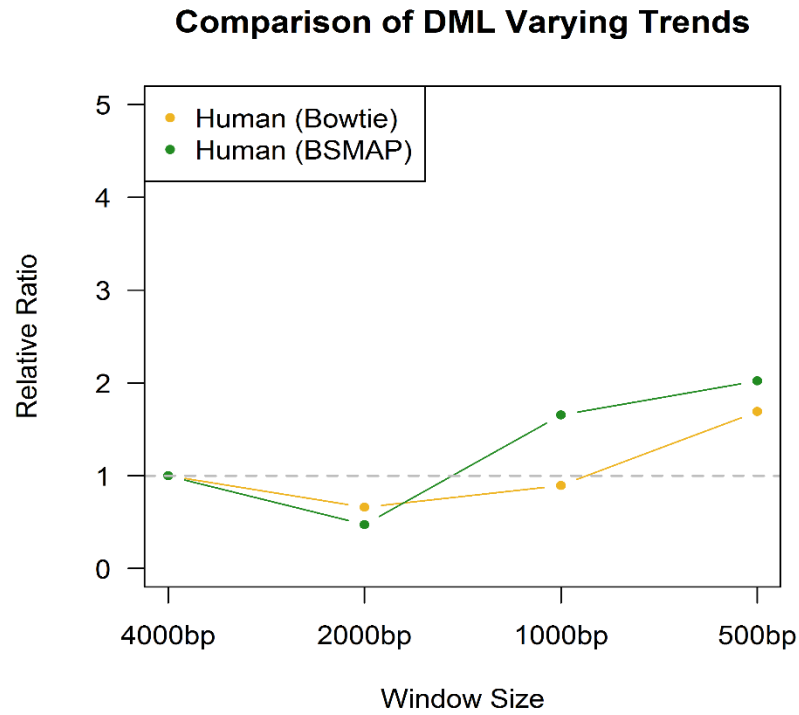
Supplementary Figure 3. Histogram of the distribution of fractional methylation in chromosome 22. Gold bars represent the distribution of methylation levels aligned using Bowtie while green bars represent the distribution of methylation levels aligned with BSMAP. They show very similar patterns and high correlation coefficient.

Histogram of CpGs by Fractional Methylation (Human Chromosome 22)