

Supplementary Tables

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Data	Ryszard						
Analysis	BS-Miner						
Sample	SRR039815						
Methylation type	CpG		CHH		CHG		
	Region	Count CpG	Percent	Count CHH	Percent	Count CHG	Percent
	DOWNSTREAM	97,300	29.05%	92,823	14.05%	3,561	15.81%
	EXON	100,930	30.13%	17,150	2.60%	1,181	5.24%
	INTERGENIC	18,652	5.57%	259,923	39.34%	8,009	35.56%
	INTRAGENIC						
	INTRON	24,967	7.45%	205,544	31.11%	6,390	28.37%
	SPLICE_SITE_ACCEPTOR			217	0.03%	54	0.24%
	SPLICE_SITE_DONOR	359	0.11%	245	0.04%	8	0.04%
	UPSTREAM	92,741	27.69%	84,837	12.84%	3,321	14.74%
	Total	334,949		660,739		22,524	
	Percent	33%		65%		2%	

Table S1. BS-Miner analyses of data from Lyko *et al.* (2010).

Data	Ryszard					
Analysis	Bismark					
Sample	SRR039815					
Methylation type	CpG		CHH		CHG	
Region	Count CpG	Percent	Count CHH	Percent	Count CHG	Percent
DOWNSTREAM	125,846	23.30%	57,536	12.96%	8,737	12.09%
EXON	132,953	24.61%	27,882	6.28%	8,972	12.41%
INTERGENIC	78,899	14.61%	168,454	37.95%	24,219	33.50%
INTRAGENIC	20	0.00%	1	0%		
INTRON	80,428	14.89%	135,288	30.48%	20,735	28.68%
SPLICE_SITE_ACCEPTOR	1	0%	77	0.02%	90	0.12%
SPLICE_SITE_DONOR	395	0.07%	125	0.03%	28	0.04%
UPSTREAM	121,666	22.52%	54,486	12.28%	9,518	13.17%
Total	540,208		443,849		72,299	
Percent	51%		42%		7%	

Table S2. Bismark analysis of data from Lyko *et al.* (2010).

Type (alphabetical order) AHB_CpG	Analyzed	Methylated	Methylated %
Cds	1,273,706	40,288	3.163%
Downstream	2,086,041	37,798	1.812%
Exon	1,273,706	40,288	3.163%
Gene	7,105,672	62,011	0.873%
Intergenic	9,767,537	35,409	0.363%
Intron	5,887,038	22,463	0.382%
SpliceSiteAcceptor	14	1	7.143%
SpliceSiteDonor	2,257	81	3.589%
Transcript	7,806,591	64,990	0.833%
Upstream	2,294,855	38,169	1.663%

Type (alphabetical order) EHB_CpG	Analyzed	Methylated	Methylated %
Cds	1,318,533	82,558	6.261%
Downstream	2,255,737	89,512	3.968%
Exon	1,318,533	82,558	6.261%
Gene	7,555,968	149,371	1.977%
Intergenic	10,471,326	111,682	1.067%
Intron	6,293,493	68,460	1.088%
SpliceSiteAcceptor	18	1	5.556%
SpliceSiteDonor	2,504	215	8.586%
Transcript	8,297,751	158,204	1.907%
Upstream	2,468,142	87,960	3.564%

Table S3a.

Type (alphabetical order) AHB_CHH	Analyzed	Methylated	Methylated %
Cds	3,316,927	26,207	0.79%
Downstream	5,075,583	79,404	1.564%
Exon	3,316,927	26,207	0.79%
Gene	12,684,330	212,020	1.672%
Intergenic	16,317,387	325,139	1.993%
Intron	9,480,307	186,385	1.966%
SpliceSiteAcceptor	14,774	87	0.589%
SpliceSiteDonor	26,957	121	0.449%
Transcript	13,795,281	234,094	1.697%
Upstream	5,213,214	74,112	1.422%

Type (alphabetical order) EHB_CHH	Analyzed	Methylated	Methylated %
Cds	3,648,122	85,111	2.333%
Downstream	5,967,723	216,950	3.635%
Exon	3,648,122	85,111	2.333%
Gene	14,525,454	521,600	3.591%
Intergenic	19,011,050	773,971	4.071%
Intron	10,997,021	438,582	3.988%
SpliceSiteAcceptor	17,187	538	3.13%
SpliceSiteDonor	34,121	620	1.817%
Transcript	15,784,142	570,790	3.616%
Upstream	6,096,005	211,100	3.463%

Table S3b.

Type (alphabetical order) AHB_CHG	Analyzed	Methylated	Methylated %
Cds	1,002,706	3,493	0.348%
Downstream	1,119,219	4,559	0.407%
Exon	1,002,706	3,493	0.348%
Gene	3,188,727	11,619	0.364%
Intergenic	3,777,833	14,009	0.371%
Intron	2,225,532	8,217	0.369%
SpliceSiteAcceptor	16,858	43	0.255%
SpliceSiteDonor	4,407	15	0.34%
Transcript	3,470,589	12,577	0.362%
Upstream	1,189,498	4,526	0.38%

Type (alphabetical order) EHB_CHG	Analyzed	Methylated	Methylated %
Cds	1,059,063	11,898	1.123%
Downstream	1,242,115	16,772	1.35%
Exon	1,059,063	11,898	1.123%
Gene	3,419,998	37,559	1.098%
Intergenic	4,099,352	45,117	1.101%
Intron	2,401,495	25,966	1.081%
SpliceSiteAcceptor	18,474	257	1.391%
SpliceSiteDonor	5,072	57	1.124%
Transcript	3,718,898	40,695	1.094%
Upstream	1,308,187	16,511	1.262%

Table S3c. Distribution of CpG, CHH, and CHG DNA methylation.

EHB_>90%_CpG_514 genes								
GO	Count	%	List Total	Pop Hits	Pop Total	Fold Enrichment	PValue	FDR
mitochondrion	43	11.40584	164	571	4786	2.197663	5.85E-07	7.58E-04
ribosomal protein	17	4.509284	367	157	12980	3.82964	9.13E-06	0.011197
AHB_>90%_CpG_160 genes								
GO								
gtp-binding	15	7.8125	190	152	12980	6.74169	4.47E-08	5.14E-05
establishment of protein localization	17	8.854167	117	285	7937	4.046454	3.41E-06	0.005281

Table S4. Housekeeping genes have the most CpG DNA methylation. As found previously, the genes with high o/e ratios are primarily housekeeping genes (1-3) (Table S2). By ‘housekeeping’ genes, we mean genes that encode proteins with a higher degree of conservation than proteins encoded by non-methylated genes (4). Highly conserved genes are utilized in most tissues, whereas less conserved genes are typically expressed in specialized tissues. Examples are the genes coding for odorant-binding proteins or odorant receptors (5), which are not methylated (not shown, see also (3)).

Term_AHB	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	FDR
phosphoprotein	45	21.32701	3.49E-13	206	815	12980	3.479064	4.27E-10
alternative splicing	25	11.84834	4.02E-05	206	616	12980	2.557212	0.049222

Table S5a. AHB genes with methylation splice junctions.

Term_EHB	Count	%	PValue	List Total	Pop Hits	Pop Total	Fold Enrichment	FDR
phosphoprotein	109	12.80846	9.10E-14	827	815	12980	2.099124	1.20E-10
GO:0001882~nucleoside binding	111	13.04348	1.35E-10	585	824	7918	1.823289	2.05E-07
nucleotide-binding	93	10.92832	3.20E-10	827	743	12980	1.964551	4.23E-07
GO:0001883~purine nucleoside binding	109	12.80846	3.64E-10	585	817	7918	1.805777	5.56E-07
GO:0030554~adenyl nucleotide binding	108	12.69095	5.55E-10	585	812	7918	1.800227	8.46E-07
atp-binding	78	9.165687	6.23E-10	827	585	12980	2.092705	8.25E-07
GO:0017076~purine nucleotide binding	123	14.45358	1.84E-09	585	989	7918	1.683327	2.80E-06
GO:0000166~nucleotide binding	142	16.68625	2.92E-09	585	1206	7918	1.593678	4.45E-06
GO:0005524~ATP binding	98	11.51586	9.75E-09	585	748	7918	1.773308	1.49E-05
GO:0032559~adenyl ribonucleotide binding	98	11.51586	1.13E-08	585	750	7918	1.768579	1.72E-05
GO:0032555~purine ribonucleotide binding	112	13.16099	5.03E-08	585	923	7918	1.642389	7.67E-05
GO:0032553~ribonucleotide binding	112	13.16099	5.03E-08	585	923	7918	1.642389	7.67E-05
cytoplasm	52	6.110458	1.71E-05	827	436	12980	1.871915	0.022614
alternative splicing	66	7.755582	3.52E-05	827	616	12980	1.681638	0.046545

Table S5b. EHB genes with methylated splice junctions.

chh_Intron M-EHB / M-AHB						
	Iteration	p-value	p-value adj	Latest result	Size	Description
	1	6.95E-17	5.19E-13	GO:0001071	395	nucleic acid binding transcription factor activity
	2	4.20E-23	2.34E-15	GO:0044459	369	plasma membrane part
	3	1.87E-27	7.80E-16	GO:0009605	363	response to external stimulus
	4	3.21E-30	9.98E-15	GO:0031012	37	extracellular matrix
	5	6.39E-33	1.48E-13	GO:0022838	197	substrate-specific channel activity
	6	2.54E-35	4.39E-12	GO:0005576	370	extracellular region
	7	2.01E-37	2.59E-10	GO:0005085	60	guanyl-nucleotide exchange factor activity
	8	1.65E-39	1.59E-08	GO:0006887	35	exocytosis
	9	1.31E-41	9.38E-07	GO:0030594	53	neurotransmitter receptor activity
	10	1.84E-43	9.84E-05	GO:0044449	32	contractile fiber part

Table S6. Differential CHH methylation is primarily in the introns of neuronal genes.