

Step 1 and Step 2. Extraction of distal CpGs methylation values. Calculation of average methylation levels

5' end																	3' end																	Average
Average	CpG site																	CpG site																
	1		2		3		4		5		6		7		8			-8		-7		-6		-5		-4		-3		-2		-1		
37.3125	46	33	33	33	36	33	36	0	29	0	40	33	20	100	25	100	B cell female	100	40	100	40	100	25	0	25	0	33	0	60	67	67	60	60	48.5625
65.1875	43	60	57	60	57	50	57	50	63	50	71	100	71	100	71	83	B cell female	50	25	50	25	50	20	25	40	38	50	44	50	38	50	29	50	39.625
48.4375	40	50	40	50	20	50	50	50	0	50	33.333	66.667	66.667	66.667	75	66.667	T cell female	40	37.5	40	37.5	60	33.333	80	50	100	62.5	50	62.5	44.444	66.667	37.5	80	55.1215278
88.13492063	100	100	100	100	100	100	100	100	100	80	100	71.429	57.143	66.667	57.143	77.778	T cell female	40	66.667	36.364	66.667	40	66.667	16.667	33.333	30	0	30	0	36.364	0	36.364	0	31.1931818
38.97916667	50	50	50	33	50	33	25	33	75	33	75	0	66.667	0	50	0	B cell male	25	33	60	50	50	50	33	25	38	25	38	25	38	25	33	25	35.8125
53.4375	50	50	50	71	50	67	50	67	67	83	50	50	50	50	50	0	B cell male	25	67	25	75	33	75	57	75	71	0	57	0	50	40	44	40	45.875
41.09713203	42.857	36.364	33.333	40	33.333	40	42.857	30	50	30	50	57.143	25	66.667	0	80	T cell male	57.143	80	71.429	66.667	62.5	66.667	50	50	33.333	40	33.333	40	25	40	25	40	48.8169643
40.625	50	0	50	0	33.333	0	33.333	0	33.333	50	50	50	100	50	100	50	T cell male	60	0	88.889	100	87.5	0	75	0	83.333	0	75	0	66.667	0	83.333	0	44.9826389
47.3125	86	33	86	20	86	20	57	17	80	20	75	17	80	0	80	0	oxB cell female	33	50	33	0	25	0	50	50	43	57	43	57	43	63	43	63	40.8125
41.66666667	33.333	33.333	33.333	50	33.333	50	33.333	50	0	50	0	100	33.333	66.667	33.333	66.667	oxB cell female	16.667	16.667	33.333	16.667	40	33.333	40	33.333	60	75	41.667	60	54.545	60	50	60	43.2007576
81.69642857	80	83.333	85.714	80	85.714	80	33.333	50	83.333	85.714	80	100	100	100	100	80	oxT cell female	57.143	0	44.444	0	44.444	0	71.429	0	80	0	80	0	75	0	75	0	32.9662698
56.26736111	22.222	100	22.222	100	12.5	100	12.5	100	25	80	37.5	80	37.5	66.667	37.5	66.667	oxT cell female	0	33.333	0	50	0	50	42.857	50	62.5	50	62.5	50	62.5	33.333	62.5	33.333	40.1785714
25	0	0	0	0	0	0	0	0	100	0	50	50	50	50	50	50	ox B cell male	33	38	33	57	50	67	67	50	100	0	100	0	100	0	100	0	49.6875
62.08333333	66.667	66.667	66.667	66.667	50	60	50	50	66.667	0	100	100	100	50	50	50	ox B cell male	0	75	50	66.667	50	66.667	50	50	75	0	66.667	0	66.667	0	66.667	0	42.7083333
53.66071429	85.714	30	85.714	30	85.714	30	71.429	30	66.667	16.667	60	33.333	50	50	33.333	100	ox T cell male	42.857	100	50	75	57.143	75	57.143	66.667	71.429	60	71.429	60	75	25	75	50	63.2291667
60.25748557	77.778	42.857	100	33.333	75	33.333	100	20	100	0	66.667	50	81.818	50	83.333	50	ox T cell male	22.222	20	40	20	50	20	100	12.5	75	33.333	75	33.333	50	33.333	25	33.333	40.1909722

Step 3. Identification of the second highest and second lowest methylation values

	B cell female	B cell female	T cell female	T cell female	B cell male	B cell male	T cell male	T cell male	ox B cell female	ox B cell female	ox T cell female	ox T cell female	ox B cell male	ox B cell male	ox T cell male	ox T cell male
Average methylation at 5' end	37.3125	65.1875	48.4375	88.1349	38.97917	53.4375	41.09713	40.625	47.3125	41.66667	81.69643	56.26736	25	62.08333	53.66071	60.25749
Average methylation at 3' end	48.5625	39.625	55.1215	31.1932	35.8125	45.875	48.81696	44.9826	40.8125	43.20076	32.96627	40.17857	49.6875	42.70833	63.22917	40.19097

Sorting from highest to lowest

	T cell female	2nd highest ox T cell female	B cell female	ox B cell male	ox T cell male	ox T cell female	ox T cell male	B cell male	T cell female	ox B cell female	ox B cell female	T cell male	T cell male	B cell male	2nd lowest B cell female	ox B cell male
Average methylation at 5' end	88.1349	81.6964	65.1875	62.0833	60.25749	56.26736	53.66071	53.4375	48.4375	47.3125	41.66667	41.09713	40.625	38.97917	37.3125	25
	ox T cell male	T cell female	ox B cell male	T cell male	B cell female	B cell male	T cell male	ox B cell female	ox B cell male	ox B cell female	ox T cell male	ox T cell female	B cell female	B cell male	ox T cell female	T cell female
Average methylation at 3' end	63.2292	55.1215	49.6875	48.817	48.5625	45.875	44.98264	43.2008	42.7083	40.8125	40.19097	40.17857	39.625	35.8125	32.96627	31.19318

Step 4. Calculation of computational score of methylation variation

Variation Score = 2nd highest value— 2nd lowest value

Variation at 5’ end = 81.6964 - 37.3125 = 44.3839

Variation at 3’ end = 55.1215 - 32.96627 = 22.15523