

Supplementary Figures

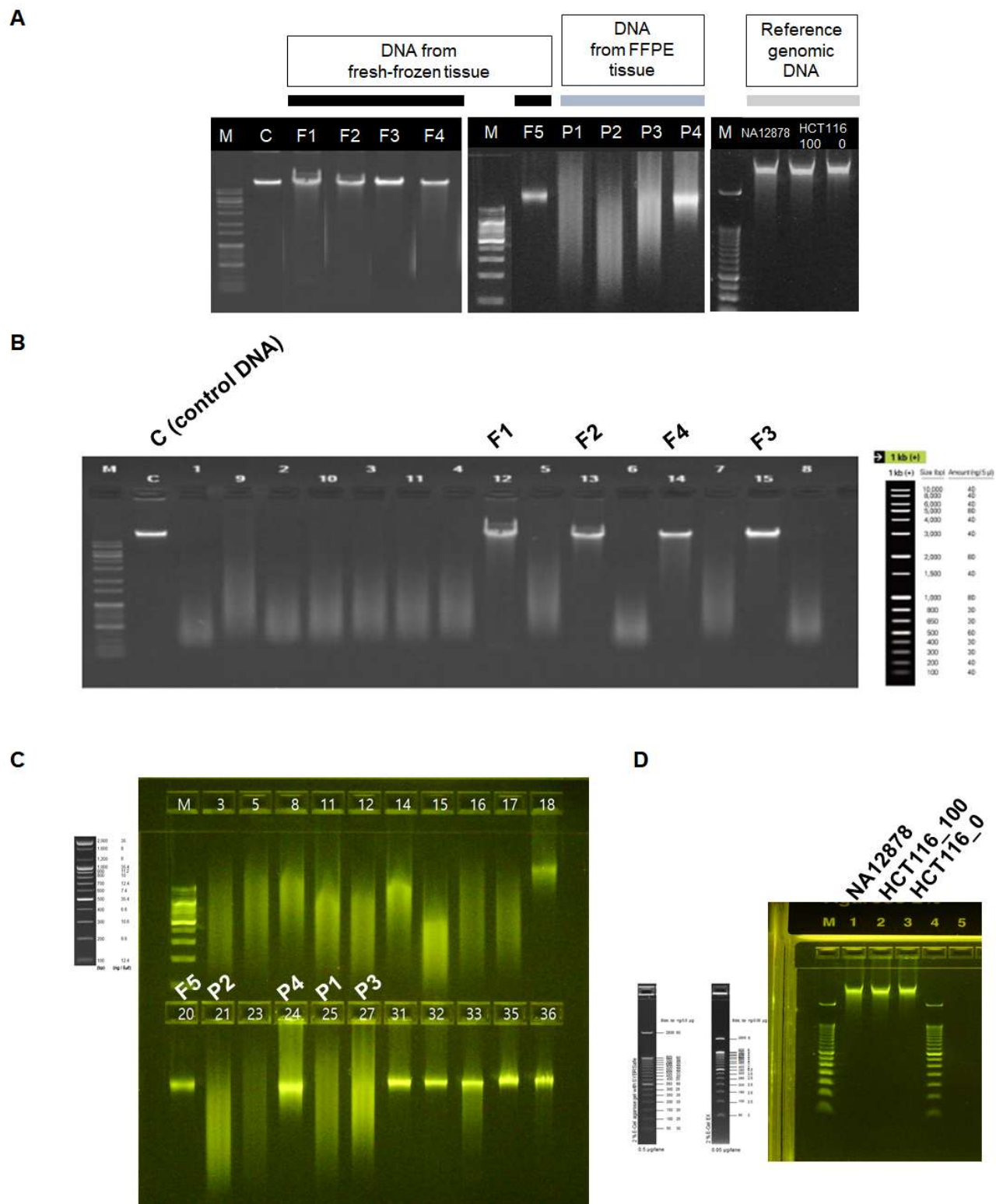


Figure S1. Gel electrophoresis of initial genomic DNA samples.

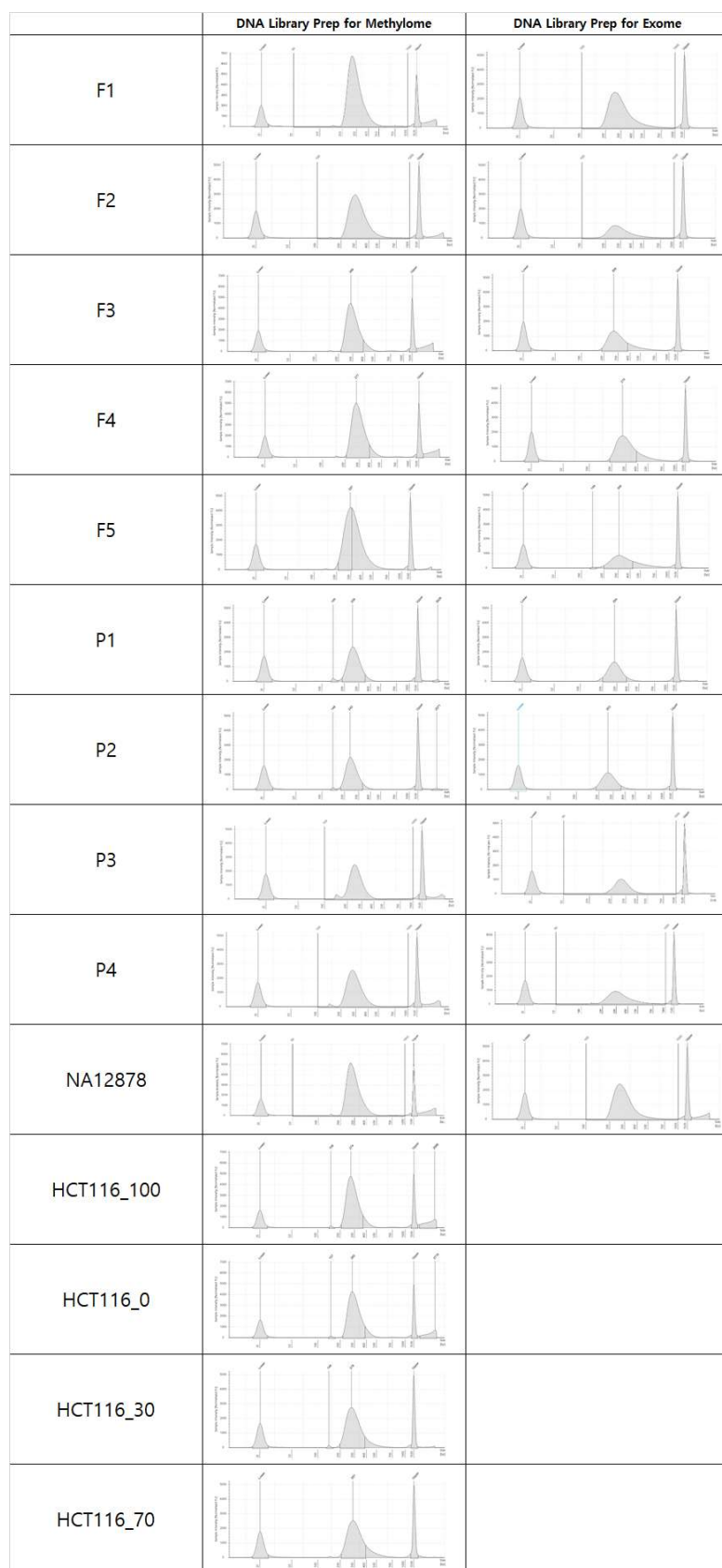


Figure S2. Tapestation electropherogram reports of single-stranded exome and methylome libraries

Supplementary Tables

Table S1. Detailed information of sequencing libraries

Experiment step		Instruments	Unit	Exome and methylome									Methylome				
				DNA from pediatric brain cancer tissue								Reference genomic DNA					
				Medulloblastoma						Secondary glioblastoma							
				DNA from FFPE tissue				DNA from fresh-frozen tissue									
				P1	P2	P3	P4	F1	F2	F3	F4	F5	NA12878	HCT116			
				100% methylated	70% methylated	30% methylated	0% methylated										
Initial genomic DNA		Qubit	Conc. (ng/ul)	183	74.2	479	101	993	420	83.8	86.6	142	270.4	20.4			43.2
		Nano-drop	Conc. (ng/ul)	475.4	190.9	1295.9	177.2	1084	479.2	137.7	128.2	248.5					
			260/280	1.8	1.8	1.84	1.87	1.86	1.87	1.85	1.83	1.84					
			260/230	2.09	1.73	2.2	2	2.29	2.18	1.63	1.64	1.79					
Genomic DNA after shearing		Tape-Station	Conc. (ng/ul)	4.47	1.67	11.7	11.4	5.77	8.36	118	55.3	29.6	23.1	8.17			7.39
			Average size (bp)	262	260	330	277	330	324	201	214	293	199	198			199
Exome library	Input amount of DNA for exome panel DNA library		total amount (ng)	50	26.7	50	50	50	50	50	50	50	50				
	Exome panel DNA Libraries	Tape-Station	Conc. (ng/ul)	11.5	10.4	24.1	11.7	24.7	22.6	14.3	19.6	13.7	24.2				
			Average size (bp)	293	278	235	340	307	306	322	327	378	300				
			Molarity (nM)	63.6	61.1	165	58.8	133	122	74.2	100	63.1	131				
Methylome library	Input amount of DNA for bisulfite conversion		total amount (ng)	100	33.4	100	100	100	100	100	100	100	100	100	100	100	100
	Bisulfite-converted methylome panel DNA libraries	Tape-Station	Conc. (ng/ul)	17.8	15.3	17.4	20.5	48.2	27.3	31.4	36	42.1	41.6	36.7	22.5	26.4	35.2
			Average size (bp)	270	257	268	296	298	321	312	303	330	298	297	323	307	310
			Region Molarity (nmol/l)	105	95.5	106	111	259	136	161	190	206	223	197	113	139	181

Table S2. Statistics of whole exome sequencing data

Study											
Exome capture kit	Sample	No. of raw reads (bases)	No. of uniquely aligned reads (%)	No. of uniquely aligned bases	No. of on-target bases	No. of near-target bases (±100 bp)	No. of off-target bases (%)	No. of duplicated reads (%)	Mean target coverage	Median insert size (bases)	Fold 80 base penalty
read length											
platform											
Current study	NA12878	117,639,490 (17,763,562,990)	117,080,055 (99.5%)	14,226,936,734	8,718,746,284 (61.3%)	4,074,167,355 (28.6%)	1,434,023,095 (10.1%)	19,690,216 (16.8%)	112.0	131	1.35
	P1	53,214,014 (8,035,316,114)	53,017,880 (99.6%)	6,431,457,874	3,968,844,789 (61.7%)	1,687,225,588 (26.2%)	775,387,497 (12.1%)	17,797,789 (33.6%)	36.3	122	1.9
	P2	72,132,994 (10,892,082,094)	71,865,015 (99.6%)	8,415,472,043	5,396,338,868 (64.1%)	2,151,732,602 (25.6%)	867,400,573 (10.3%)	27,159,900 (37.8%)	45.8	114	1.66
	P3	73,473,430 (11,094,487,930)	73,090,904 (99.5%)	6,683,957,721	4,207,604,247 (63.0%)	1,321,752,669 (19.8%)	1,154,600,805 (17.3%)	17,445,876 (23.9%)	42.7	86	2.23
	P4	58,292,892 (8,802,226,692)	58,097,216 (99.7%)	7,783,290,683	4,322,021,311 (55.5%)	2,522,160,518 (32.4%)	939,108,854 (12.1%)	13,998,204 (24.1%)	54.3	164	1.36
	F1	48,953,088 (7,391,916,288)	48,761,986 (99.5%)	5,750,374,311	3,500,476,030 (60.9%)	1,577,855,231 (27.4%)	672,043,050 (11.7%)	11,796,550 (23.2%)	40.2	122	1.47
	F2	95,475,634 (14,416,820,734)	94,976,361 (99.5%)	11,347,318,200	6,938,737,251 (61.1%)	2,981,869,959 (26.3%)	1,426,710,990 (12.6%)	34,017,325 (35.8%)	66.2	125	1.78
	F3	59,806,420 (9,030,769,420)	59,537,155 (99.5%)	7,288,198,678	4,325,190,731 (59.3%)	2,010,672,333 (27.6%)	952,335,614 (13.1%)	22,714,178 (38.2%)	40.5	128	1.53
	F4	84,101,754 (12,699,364,854)	83,688,885 (99.5%)	10,313,776,705	6,086,612,940 (59.0%)	2,876,168,736 (27.9%)	1,350,995,029 (13.1%)	31,945,107 (38.2%)	57.5	131	1.51
	F5	65,565,868 (9,900,446,068)	65,349,664 (99.7%)	8,890,786,773	4,686,051,689 (52.7%)	2,917,176,825 (32.8%)	1,287,558,259 (14.5%)	13,221,882 (20.2%)	64.5	175	1.5
Previous study	P1	150,032,804 (15,153,313,204)	149,518,623 (99.7%)	14,658,148,181	9,761,148,349 (66.6%)	1,732,389,245 (11.8%)	3,164,610,587 (21.6%)	27,722,710 (18.5%)	118.2	151	2.77
	P2	175,088,442 (17,683,932,642)	174,783,906 (99.8%)	16,928,922,642	9,006,639,343 (53.2%)	1,559,118,575 (9.2%)	6,363,164,724 (37.6%)	27,316,671 (15.6%)	109.9	145	3.04
	P3	162,477,064 (16,410,183,464)	162,101,905 (99.8%)	15,966,844,366	10,145,201,691 (63.5%)	2,180,332,157 (13.7%)	3,641,310,518 (22.8%)	38,281,027 (23.6%)	124.0	165	2.9
	P4	151,536,674 (15,305,204,074)	151,157,630 (99.7%)	15,123,321,530	9,261,962,212 (61.2%)	2,813,746,526 (18.6%)	3,047,612,792 (20.2%)	41,602,388 (27.5%)	116.4	193	2.07
	F1	170,673,646 (17,238,038,246)	168,319,945 (98.6%)	16,630,912,289	10,322,307,829 (62.1%)	3,198,818,011 (19.2%)	3,109,786,449 (18.7%)	17,200,657 (10.2%)	160.7	191	1.98
	F2	173,345,918 (17,507,937,718)	170,751,576 (98.5%)	16,863,262,917	10,337,679,825 (61.3%)	3,201,663,447 (19.0%)	3,323,919,645 (19.7%)	11,778,756 (6.9%)	167.4	192	1.99
	F3	129,861,412 (13,116,002,612)	129,694,236 (99.9%)	12,999,001,252	7,675,402,476 (59.0%)	2,462,240,889 (18.9%)	2,861,357,887 (22.0%)	4,039,836 (3.1%)	129.9	196	2.15
	F4	116,929,448 (11,809,874,248)	116803079 (99.9%)	11,708,156,341	6,836,362,788 (58.4%)	2,115,285,137 (18.1%)	2,756,508,416 (23.5%)	3,080,006 (2.6%)	115.4	192	2.18
	F5	147,803,700 (14,928,173,700)	147,466,257 (99.8%)	14,777,551,235	8,542,531,645 (57.8%)	2,693,876,784 (18.2%)	3,541,142,806 (24.0%)	4,186,417 (2.8%)	144.3	194	2.17

Table S3. Unfiltered and SOBDetector-filtered autosomal SNV calls (depth ≥ 10) in the context of SNV type

Exome capture kit	Sample		SNV context	Total number of SNV calls	Number of SNV calls retained after filtering with SOBDetector	Number of SNV calls removed after filtering with SOBDetector	% of retained SNV calls after filtering with SOBDetector	% of removed SNV calls after filtering with SOBDetector	Total number of SNV calls retained after filtering with SOBDetector	Total number of SNV calls removed after filtering with SOBDetector	Average % of retained SNV calls after filtering with SOBDetector	Average % of removed SNV calls after filtering with SOBDetector
Twist (current study)	FFPE	P1	C>T/G>A	4,473	2,289	2,184	51.2	48.8				
			C>G/G>C	429	377	52	87.9	12.1				
			C>A/G>T	1,043	455	588	43.6	56.4				
			A>T/T>A	178	147	31	82.6	17.4				
			A>G/T>C	1,248	1,047	201	83.9	16.1				
		P2	A>C/T>G	251	226	25	90	10	4,541	3,081	59.6	40.4
			C>T/G>A	3,680	2,333	1,347	63.4	36.6				
			C>G/G>C	441	388	53	88	12				
			C>A/G>T	1,873	767	1,106	41	59				
			A>T/T>A	173	156	17	90.2	9.8				
		P3	A>G/T>C	1,199	1,082	117	90.2	9.8				
			A>C/T>G	275	238	37	86.5	13.5	4,964	2,677	65	35
			C>T/G>A	3,182	2,067	1,115	65	35				
			C>G/G>C	505	412	93	81.6	18.4				
			C>A/G>T	2,702	778	1,924	28.8	71.2				
		P4	A>T/T>A	203	165	38	81.3	18.7				
			A>G/T>C	1,277	1,068	209	83.6	16.4				
			A>C/T>G	294	227	67	77.2	22.8	4,717	3,446	57.8	42.2
			C>T/G>A	1,875	1,732	143	92.4	7.6				
			C>G/G>C	399	382	17	95.7	4.3				
	Fresh frozen	F1	C>A/G>T	1,715	760	955	44.3	55.7				
			A>T/T>A	164	155	9	94.5	5.5				
			A>G/T>C	933	859	74	92.1	7.9				
			A>C/T>G	316	267	49	84.5	15.5	4,155	1,247	76.9	23.1
			C>T/G>A	1,858	1,551	307	83.5	16.5				
		F2	C>G/G>C	372	343	29	92.2	7.8				
			C>A/G>T	1,432	559	873	39	61				
			A>T/T>A	152	134	18	88.2	11.8				
			A>G/T>C	892	804	88	90.1	9.9				
			A>C/T>G	239	208	31	87	13	3,599	1,346	72.8	27.2
		F3	C>T/G>A	1,764	1,667	97	94.5	5.5				
			C>G/G>C	390	382	8	97.9	2.1				
			C>A/G>T	1,099	748	351	68.1	31.9				
			A>T/T>A	173	170	3	98.3	1.7				
			A>G/T>C	1,013	972	41	96	4				
		F4	A>C/T>G	267	258	9	96.6	3.4	4,197	509	89.2	10.8
			C>T/G>A	1,710	1,634	76	95.6	4.4				
			C>G/G>C	368	355	13	96.5	3.5				
			C>A/G>T	386	345	41	89.4	10.6				
			A>T/T>A	157	144	13	91.7	8.3				
	Agilent (previous study)	FFPE	A>G/T>C	1,002	970	32	96.8	3.2				
			A>C/T>G	236	225	11	95.3	4.7	3,673	186	95.2	4.8
			C>T/G>A	1,692	1,621	71	95.8	4.2				
			C>G/G>C	383	371	12	96.9	3.1				
			C>A/G>T	471	412	59	87.5	12.5				
			A>T/T>A	156	151	5	96.8	3.2				
			A>G/T>C	989	953	36	96.4	3.6				
			A>C/T>G	248	237	11	95.6	4.4	3,745	194	95.1	4.9
			C>T/G>A	1,552	1,499	53	96.6	3.4				
			C>G/G>C	388	372	16	95.9	4.1				
			C>A/G>T	289	273	16	94.5	5.5				
			A>T/T>A	157	150	7	95.5	4.5				
			A>G/T>C	804	773	31	96.1	3.9				
			A>C/T>G	306	291	15	95.1	4.9	3,358	138	96.1	3.9
			C>T/G>A	3,150	2,055	1,095	65.2	34.8				
			C>G/G>C	355	336	19	94.6	5.4				
			C>A/G>T	412	326	86	79.1	20.9				
			A>T/T>A	241	170	71	70.5	29.5				
			A>G/T>C	1,004	941	63	93.7	6.3				
			A>C/T>G	237	211	26	89	11	4,039	1360	74.8	25.2
		P2	C>T/G>A	2,156	1,746	410	81	19				
			C>G/G>C	413	387	26	93.7	6.3				
			C>A/G>T	428	322	106	75.2	24.8				
			A>T/T>A	299	178	121	59.5	40.5				
			A>G/T>C	1,071	994	77	92.8	7.2				
		P3	A>C/T>G	227	207	20	91.2	8.8	3,834	760	83.5	16.5
			C>T/G>A	2,113	1,930	183	91.3	8.7				
			C>G/G>C	463	439	24	94.8	5.2				
			C>A/G>T	480	411	69	85.6	14.4				
			A>T/T>A	257	214	43	83.3	16.7				
		P4	A>G/T>C	1,106	1,035	71	93.6	6.4				
			A>C/T>G	286	259	27	90.6	9.4	4,288	417	91.1	8.9
			C>T/G>A	1,800	1,674	126	93	7				
			C>G/G>C	399	373	26	93.5	6.5				
			C>A/G>T	421	369	52	87.6	12.4				
	Fresh frozen	F1	A>T/T>A	209	185	24	88.5	11.5				
			A>G/T>C	883	818	65	92.6	7.4				
			A>C/T>G	205	190	15	92.7	7.3	3,609	308	92.1	7.9
			C>T/G>A	1,417	1,330	87	93.9	6.1				
			C>G/G>C	368	349	19	94.8	5.2				
		F2	C>A/G>T	392	284	108	72.4	27.6				
			A>T/T>A	157	145	12	92.4	7.6				
			A>G/T>C	797	750	47	94.1	5.9				
			A>C/T>G	352	339	13	96.3	3.7	3,197	286	91.8	8.2
			C>T/G>A	1,433	1,359	74	94.8	5.2				
		F3	C>G/G>C	381	357	24	93.7	6.3				
			C>A/G>T	388	285	103	73.5	26.5				
			A>T/T>A	172	162	10	94.2	5.8				
			A>G/T>C	803	757	46	94.3	5.7				
			A>C/T>G	382	367	15	96.1	3.9	3,287	272	92.4	7.6
			C>T/G>A	1,462	1394	68	95.3	4.7				
			C>G/G>C	367	346	21	94.3	5.7				
			C>A/G>T	314	278	36	88.5	11.5				

			A>T/T>A	189	171	18	90.5	9.5				
			A>G/T>C	813	769	44	94.6	5.4				
			A>C/T>G	187	173	14	92.5	7.5	3,131	201	94	6
		F4	C>T/G>A	1,394	1320	74	94.7	5.3				
			C>G/G>C	354	340	14	96	4				
			C>A/G>T	322	281	41	87.3	12.7				
			A>T/T>A	168	152	16	90.5	9.5				
			A>G/T>C	801	758	43	94.6	5.4				
			A>C/T>G	187	177	10	94.7	5.3	3,028	198	93.9	6.1
			C>T/G>A	1,532	1398	134	91.3	8.7				
		F5	C>G/G>C	402	367	35	91.3	8.7				
			C>A/G>T	385	326	59	84.7	15.3				
			A>T/T>A	204	176	28	86.3	13.7				
			A>G/T>C	911	800	111	87.8	12.2				
			A>C/T>G	245	215	30	87.8	12.2	3,282	397	89.2	10.8

Table S4. Proportion of Agilent (previous study) autosomal SNV calls (depth ≥ 20) validated in Twist (current study) according to allele frequency and filtering step

Sample	Allele frequency of alternate allele	No filtering	Filtering with Mutect2 (FilterMutectCalls)			Filtering with Mutect2 (FilterMutectCalls) & SOBDetector			Filtering with Mutect2 (FilterMutectCalls), SOBDetector, & FFPolish			Filtering with Mutect2 (FilterMutectCalls), SOBDetector, FFPolish, & microSEC		
		Total number of Agilent SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls
P1	>0	5,331	1,244	689	55.4	1,022	689	67.4	713	684	95.9	693	672	97.0
	≥ 0.05	3,816	888	689	77.6	821	689	83.9	711	684	96.2	693	672	97.0
	≥ 0.1	3,158	731	684	93.6	723	684	94.6	699	682	97.6	683	670	98.1
	≥ 0.15	2,970	691	674	97.5	691	674	97.5	685	673	98.2	673	663	98.5
	≥ 0.2	2,841	660	652	98.8	660	652	98.8	660	652	98.8	652	644	98.8
	≥ 0.25	2,741	636	629	98.9	636	629	98.9	636	629	98.9	628	621	98.9
P2	>0	4,477	620	484	78.1	557	484	86.9	498	478	96.0	485	470	96.9
	≥ 0.05	3,825	573	479	83.6	525	479	91.2	497	477	96.0	484	469	96.9
	≥ 0.1	3,342	512	472	92.2	495	472	95.4	480	471	98.1	471	463	98.3
	≥ 0.15	3,170	467	460	98.5	467	460	98.5	466	460	98.7	459	453	98.7
	≥ 0.2	3,089	457	451	98.7	457	451	98.7	457	451	98.7	450	444	98.7
	≥ 0.25	2,989	447	441	98.7	447	441	98.7	447	441	98.7	442	436	98.6
P3	>0	4,583	998	715	71.6	939	711	75.7	743	658	88.6	725	648	89.4
	≥ 0.05	3,958	841	681	81.0	815	678	83.2	741	658	88.8	725	648	89.4
	≥ 0.1	3,401	667	622	93.3	660	620	93.9	642	618	96.3	633	610	96.4
	≥ 0.15	3,181	613	596	97.2	613	596	97.2	610	596	97.7	604	590	97.7
	≥ 0.2	3,037	587	578	98.5	587	578	98.5	587	578	98.5	581	572	98.5
	≥ 0.25	2,940	570	562	98.6	570	562	98.6	570	562	98.6	565	557	98.6
P4	>0	3,863	773	680	88.0	752	676	89.9	680	652	95.9	672	644	95.8
	≥ 0.05	3,400	706	665	94.2	702	665	94.7	676	651	96.3	668	643	96.3
	≥ 0.1	3,024	637	626	98.3	637	626	98.3	633	624	98.6	627	618	98.6
	≥ 0.15	2,834	616	612	99.4	616	612	99.4	616	612	99.4	611	607	99.3
	≥ 0.2	2,709	605	601	99.3	605	601	99.3	605	601	99.3	601	597	99.3
	≥ 0.25	2,610	601	597	99.3	601	597	99.3	601	597	99.3	597	593	99.3
F1	>0	3,439	382	370	96.9	382	370	96.9	371	366	98.7	364	361	99.2
	≥ 0.05	3,015	379	369	97.4	379	369	97.4	371	366	98.7	364	361	99.2
	≥ 0.1	2,716	374	368	98.4	374	368	98.4	371	366	98.7	364	361	99.2
	≥ 0.15	2,525	367	365	99.5	367	365	99.5	366	364	99.5	360	359	99.7
	≥ 0.2	2,379	365	363	99.5	365	363	99.5	364	362	99.5	358	357	99.7
	≥ 0.25	2,291	359	358	99.7	359	358	99.7	358	357	99.7	355	354	99.7
F2	>0	3,519	483	453	93.8	482	453	94.0	453	443	97.8	443	435	98.2
	≥ 0.05	3,056	464	449	96.8	464	449	96.8	453	443	97.8	443	435	98.2
	≥ 0.1	2,738	441	432	98.0	441	432	98.0	435	431	99.1	427	424	99.3
	≥ 0.15	2,549	429	426	99.3	429	426	99.3	427	425	99.5	421	419	99.5
	≥ 0.2	2,397	417	415	99.5	417	415	99.5	416	414	99.5	412	410	99.5
	≥ 0.25	2,292	412	411	99.8	412	411	99.8	411	410	99.8	408	407	99.8
F3	>0	3,249	505	468	92.7	503	467	92.8	469	461	98.3	462	455	98.5
	≥ 0.05	2,880	487	467	95.9	486	466	95.9	469	461	98.3	462	455	98.5
	≥ 0.1	2,556	466	460	98.7	466	460	98.7	462	458	99.1	456	453	99.3
	≥ 0.15	2,369	459	458	99.8	459	458	99.8	458	457	99.8	452	452	100
	≥ 0.2	2,247	458	457	99.8	458	457	99.8	457	456	99.8	452	452	100
	≥ 0.25	2,179	454	454	100.0	454	454	100.0	453	453	100.0	449	449	100
F4	>0	3,134	475	463	97.5	475	463	97.5	462	460	99.6	455	455	100
	≥ 0.05	2,827	472	463	98.1	472	463	98.1	462	460	99.6	455	455	100
	≥ 0.1	2,532	464	461	99.4	464	461	99.4	460	459	99.8	455	455	100
	≥ 0.15	2,374	460	459	99.8	460	459	99.8	459	458	99.8	455	455	100
	≥ 0.2	2,249	456	456	100.0	456	456	100.0	456	456	100.0	453	453	100
	≥ 0.25	2,163	452	452	100.0	452	452	100.0	452	452	100.0	449	449	100
F5	>0	3,596	699	670	95.9	698	670	96.0	681	663	97.4	666	655	98.3
	≥ 0.05	3,034	690	668	96.8	689	668	97.0	680	663	97.5	665	655	98.5
	≥ 0.1	2,670	664	657	98.9	664	657	98.9	659	652	98.9	648	644	99.4
	≥ 0.15	2,497	650	646	99.4	650	646	99.4	648	644	99.4	640	637	99.5
	≥ 0.2	2,358	634	631	99.5	634	631	99.5	633	630	99.5	630	627	99.5
	≥ 0.25	2,210	591	588	99.5	591	588	99.5	590	587	99.5	588	585	99.5

Table S5. Proportion of Twist (current study) autosomal SNV calls (depth ≥ 20) validated in Agilent (previous study) according to allele frequency and filtering step

Sample	Allele frequency of alternate allele	No filtering	Filtering with Mutect2 (FilterMutectCalls)			Filtering with Mutect2 (FilterMutectCalls) & SOBDetector			Filtering with Mutect2 (FilterMutectCalls), SOBDetector, & FFPolish			Filtering with Mutect2 (FilterMutectCalls), SOBDetector, & FFPolish, & microSEC		
		Total number of Agilent SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls	No. of Agilent SNV calls after filtering	No. of Agilent SNV calls validated in Twist	% of validated SNV calls
P1	>0	7,261	1,127	466	41.3	927	464	50.1	500	451	90.2	482	443	91.9
	≥ 0.05	6,817	1,092	466	42.7	905	464	51.3	500	451	90.2	482	443	91.9
	≥ 0.1	4,395	733	461	62.9	638	459	71.9	465	449	96.6	454	442	97.4
	≥ 0.15	3,282	514	453	88.1	486	451	92.8	450	444	98.7	441	437	99.1
	≥ 0.2	3,047	446	436	97.8	442	435	98.4	434	430	99.1	427	425	99.5
	≥ 0.25	2,907	400	397	99.2	400	397	99.2	397	394	99.2	391	390	99.7
P2	>0	7,504	862	316	36.7	774	314	40.6	376	304	80.9	341	283	83.0
	≥ 0.05	6,613	780	316	40.5	703	314	44.7	376	304	80.9	341	283	83.0
	≥ 0.1	4,108	399	306	76.7	376	305	81.1	318	298	93.7	295	279	94.6
	≥ 0.15	3,441	313	296	94.6	311	296	95.2	302	293	97.0	281	274	97.5
	≥ 0.2	3,287	290	281	96.9	290	281	96.9	289	280	96.9	268	261	97.4
	≥ 0.25	3,191	275	271	98.5	275	271	98.5	274	270	98.5	258	254	98.4
P3	>0	7,113	1,068	641	60.0	900	625	69.4	633	594	93.8	594	570	96.0
	≥ 0.05	6,353	1,041	636	61.1	883	621	70.3	633	594	93.8	594	570	96.0
	≥ 0.1	4,552	754	566	75.1	671	560	83.5	565	550	97.3	542	532	98.2
	≥ 0.15	3,538	565	533	94.3	546	529	96.9	527	522	99.1	513	510	99.4
	≥ 0.2	3,280	513	508	99.0	510	506	99.2	503	500	99.4	492	491	99.8
	≥ 0.25	3,152	478	477	99.8	477	476	99.8	473	472	99.8	467	466	99.8
P4	>0	5,372	847	612	72.3	800	609	76.1	614	578	94.1	605	573	94.7
	≥ 0.05	4,888	822	611	74.3	782	608	77.7	614	578	94.1	605	573	94.7
	≥ 0.1	3,281	592	569	96.1	590	569	96.4	566	559	98.8	559	554	99.1
	≥ 0.15	3,060	547	542	99.1	547	542	99.1	540	537	99.4	535	533	99.6
	≥ 0.2	2,963	535	530	99.1	535	530	99.1	530	527	99.4	525	523	99.6
	≥ 0.25	2,844	524	521	99.4	524	521	99.4	522	519	99.4	517	515	99.6
F1	>0	4,868	791	336	42.5	600	335	55.8	342	322	94.2	333	318	95.5
	≥ 0.05	4,737	775	336	43.4	591	335	56.7	342	322	94.2	333	318	95.5
	≥ 0.1	3,285	446	332	74.4	388	331	85.3	326	322	98.8	321	318	99.1
	≥ 0.15	2,847	338	328	97.0	332	328	98.8	320	320	100.0	317	317	100.0
	≥ 0.2	2,739	329	325	98.8	326	325	99.7	319	319	100.0	316	316	100.0
	≥ 0.25	2,663	318	318	100.0	318	318	100.0	313	313	100.0	312	312	100.0
F2	>0	4,655	703	430	61.2	622	429	69.0	427	406	95.1	412	395	95.9
	≥ 0.05	4,238	664	428	64.5	600	427	71.2	427	406	95.1	412	395	95.9
	≥ 0.1	3,485	469	409	87.2	459	409	89.1	398	394	99.0	388	384	99.0
	≥ 0.15	3,257	411	401	97.6	410	401	97.8	391	389	99.5	381	379	99.5
	≥ 0.2	3,130	391	388	99.2	391	388	99.2	381	379	99.5	372	370	99.5
	≥ 0.25	3,045	382	380	99.5	382	380	99.5	375	373	99.5	369	367	99.5
F3	>0	3,777	683	372	54.5	640	371	58.0	375	354	94.4	361	346	95.8
	≥ 0.05	3,688	663	372	56.1	631	371	58.8	375	354	94.4	361	346	95.8
	≥ 0.1	3,134	430	364	84.7	430	364	84.7	358	350	97.8	347	342	98.6
	≥ 0.15	2,921	365	357	97.8	365	357	97.8	350	349	99.7	342	341	99.7
	≥ 0.2	2,781	351	350	99.7	351	350	99.7	348	347	99.7	341	340	99.7
	≥ 0.25	2,677	341	340	99.7	341	340	99.7	339	338	99.7	333	332	99.7
F4	>0	3,915	682	397	58.2	646	397	61.5	400	383	95.8	393	377	95.9
	≥ 0.05	3,685	627	396	63.2	605	396	65.5	400	383	95.8	393	377	95.9
	≥ 0.1	3,135	427	393	92.0	425	393	92.5	386	383	99.2	380	377	99.2
	≥ 0.15	2,965	391	389	99.5	390	389	99.7	384	383	99.7	378	377	99.7
	≥ 0.2	2,880	386	385	99.7	386	385	99.7	383	382	99.7	377	376	99.7
	≥ 0.25	2,778	376	375	99.7	376	375	99.7	375	374	99.7	370	369	99.7
F5	>0	3,478	599	519	86.6	594	519	87.4	510	503	98.6	500	494	98.8
	≥ 0.05	3,307	574	517	90.1	573	517	90.2	510	503	98.6	500	494	98.8
	≥ 0.1	2,894	515	509	98.8	515	509	98.8	500	498	99.6	491	489	99.6
	≥ 0.15	2,731	497	495	99.6	497	495	99.6	488	487	99.8	480	479	99.8
	≥ 0.2	2,613	475	474	99.8	475	474	99.8	472	471	99.8	465	464	99.8
	≥ 0.25	2,469	443	443	100.0	443	443	100.0	443	443	100.0	437	437	100.0

Table S6. Estimated bisulfite conversion rate from cytosine methylation in CHG and CHH context

Sample	Cytosine methylation level (%)			Estimated bisulfite conversion rate
	CHG	CHH	Average	
NA12878	0.2%	0.2%	0.20%	99.80%
HCT116_100	0.6%	0.3%	0.45%	99.55%
HCT116_70	0.5%	0.3%	0.40%	99.60%
HCT116_30	0.4%	0.2%	0.30%	99.70%
HCT116_0	0.2%	0.2%	0.20%	99.80%
P1	0.5%	0.6%	0.55%	99.45%
P2	0.5%	0.5%	0.50%	99.50%
P3	0.6%	0.6%	0.60%	99.40%
P4	0.4%	0.5%	0.45%	99.55%
F1	0.3%	0.3%	0.30%	99.70%
F2	0.4%	0.5%	0.45%	99.55%
F3	0.3%	0.3%	0.30%	99.70%
F4	0.3%	0.3%	0.30%	99.70%
F5	0.5%	0.6%	0.55%	99.45%

Table S7. Statistics of methylome sequencing data

Sample	No. of raw reads	No. of uniquely aligned reads (%)	No. of uniquely aligned bases	No. of on-target bases	No. of near-target bases (±100 bp)	No. of off-target bases (%)	No. of duplicated reads (%)	Mean target coverage	Median insert size (bases)	Fold 80 base penalty
NA12878	144,814,736	123,609,136 (85.4%)	14,538,548,306	10,872,527,734	2,644,067,726	1,021,952,846 (7.0%)	17,796,070 (14.4%)	41.8	129	2.49
HCT116_100	223,101,852	181,768,388 (81.5%)	20,268,149,557	15,455,739,037	2,723,299,655	2,089,110,865 (10.3%)	24,532,164 (13.5%)	58.4	117	1.96
HCT116_70	213,511,904	168,392,644 (78.9%)	20,217,234,427	14,250,245,147	3,487,413,764	2,479,575,516 (12.3%)	67,097,524 (39.9%)	35.9	133	1.77
HCT116_30	179,289,748	146,954,058 (82.0%)	17,652,025,676	12,385,132,078	3,111,874,162	2,155,019,436 (12.2%)	46,377,530 (31.6%)	36.8	134	1.93
HCT116_0	143,381,030	118,388,630 (82.6%)	13,819,764,343	8,535,128,527	2,066,338,925	3,218,296,891 (23.3%)	13,126,216 (11.1%)	34.3	129	2.32
P1	143,295,114	116,595,340 (81.4%)	12,590,623,760	9,808,966,133	1,836,574,361	945,083,266 (7.5%)	47,968,800 (41.1%)	22.3	105	1.91
P2	43,468,480	33,645,812 (77.4%)	3,298,546,119	2,565,574,211	419,568,192	313,403,716 (9.5%)	8,466,074 (25.2%)	7.57	94	2.38
P3	135,606,898	111,224,504 (82.0%)	11,121,256,941	8,863,920,859	1,362,098,552	895,237,530 (8.0%)	31,851,058 (28.6%)	25.1	97	1.81
P4	131,855,532	110,097,620 (83.5%)	12,838,343,056	9,081,609,610	2,223,553,169	1,533,180,277 (11.9%)	17,886,206 (16.3%)	34.3	129	1.69
F1	121,774,790	100,946,022 (82.9%)	11,449,624,671	8,723,523,169	1,872,691,350	853,410,152 (7.5%)	11,448,610 (11.3%)	33.9	121	1.89
F2	187,420,258	144,678,994 (77.2%)	17,565,555,801	10,699,958,367	3,305,614,070	3,559,983,364 (20.3%)	25,994,150 (18.0%)	40.5	140	2.73
F3	196,465,634	162,654,214 (82.8%)	18,998,856,587	14,121,137,500	3,272,939,119	1,604,779,968 (8.4%)	20,466,838 (12.6%)	55.2	127	1.94
F4	358,590,154	284,654,028 (79.4%)	31,453,429,921	16,942,881,581	4,101,704,119	10,408,844,221 (33.1%)	38,675,614 (13.6%)	63.3	116	1.86
F5	184,860,650	146,935,312 (79.5%)	18,117,038,527	10,264,149,018	3,191,421,428	4,661,468,081 (25.7%)	21,647,342 (14.7%)	42.6	150	1.65

Table S8. Number of detected CpG sites within target region of the Twist Human Methylome Panel targeting approximately 3.98M CpG sites in 123Mb of human genome

Depth	Sample	Number of detected CpG sites	Average	
1x	NA12878	3,921,589	3,935,231	3,921,130
	HCT116_100	3,955,758		
	HCT116_70	3,949,087		
	HCT116_30	3,942,251		
	HCT116_0	3,907,468		
	P1	3,931,729	3,913,296	
	P2	3,791,568		
	P3	3,943,417		
	P4	3,949,936		
	F1	3,915,609		
	F2	3,846,086		
	F3	3,932,828		
	F4	3,946,572		
	F5	3,961,919		
5x	NA12878	3,552,013	3,716,934	3,621,726
	HCT116_100	3,934,138		
	HCT116_70	3,868,563		
	HCT116_30	3,774,065		
	HCT116_0	3,455,891		
	P1	3,665,170	3,568,832	
	P2	2,499,328		
	P3	3,822,343		
	P4	3,868,103		
	F1	3,632,335		
	F2	3,115,501		
	F3	3,744,776		
	F4	3,841,603		
	F5	3,930,329		
10x	NA12878	2,968,341	3,357,181	3,217,067
	HCT116_100	3,895,330		
	HCT116_70	3,672,184		
	HCT116_30	3,378,255		
	HCT116_0	2,871,796		
	P1	3,117,982	3,139,226	
	P2	1,006,686		
	P3	3,511,938		
	P4	3,671,123		
	F1	3,273,106		
	F2	2,627,608		
	F3	3,502,122		
	F4	3,687,711		
	F5	3,854,755		