



## **Supplemental Material to:**

**Dirk S Paul, Paul Guilhamon, Anna Karpathakis,  
Lee M Butcher, Christina Thirlwell, Andrew Feber,  
and Stephan Beck**

**Assessment of RainDrop BS-seq as a method for large-  
scale, targeted bisulfite sequencing**

**Epigenetics 2013; 9(5)**

**<http://dx.doi.org/10.4161/epi.28041>**

**[http://www.landesbioscience.com/journals/epigenetics/  
article/28041/](http://www.landesbioscience.com/journals/epigenetics/article/28041/)**

# **Assessment of RainDrop BS-seq as a method for large-scale, targeted bisulfite sequencing**

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## **SUPPLEMENTARY INFORMATION**

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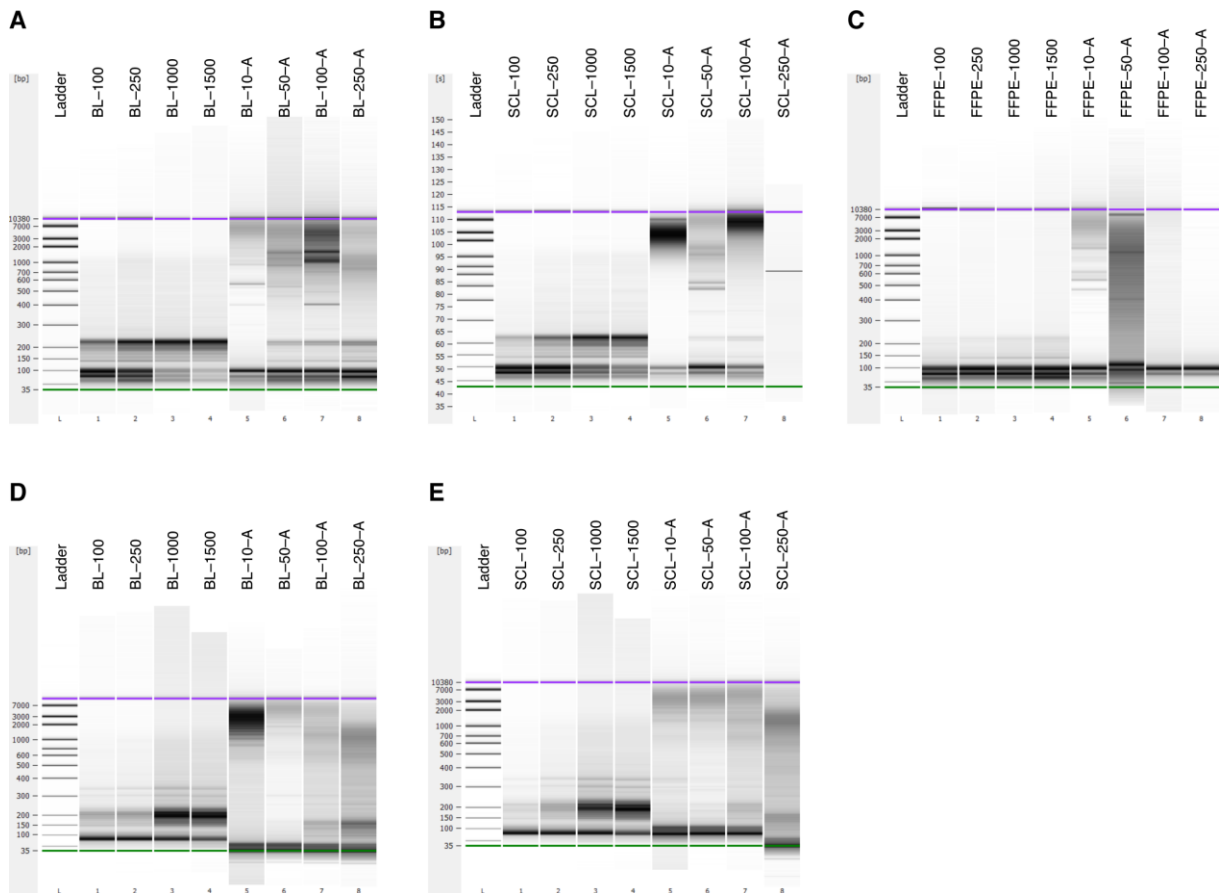
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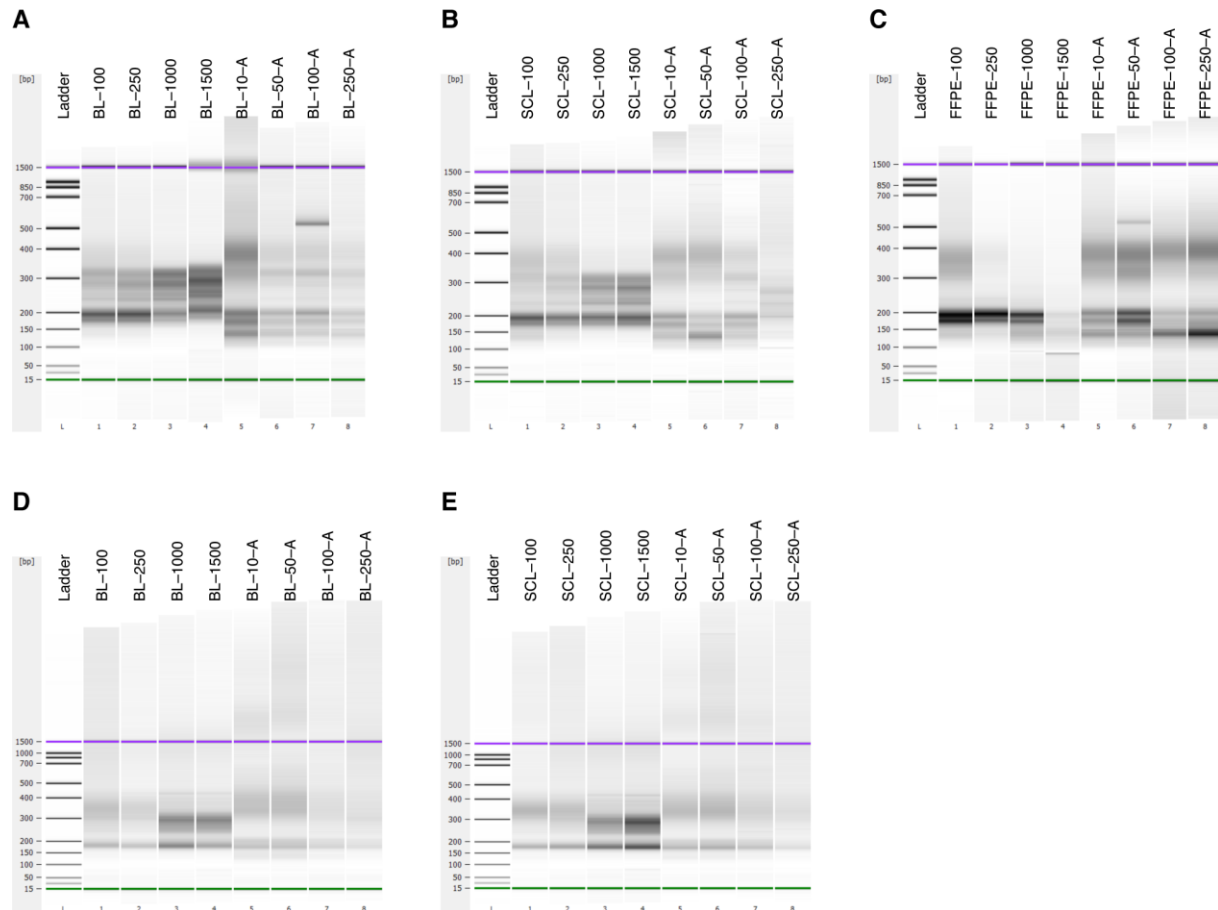
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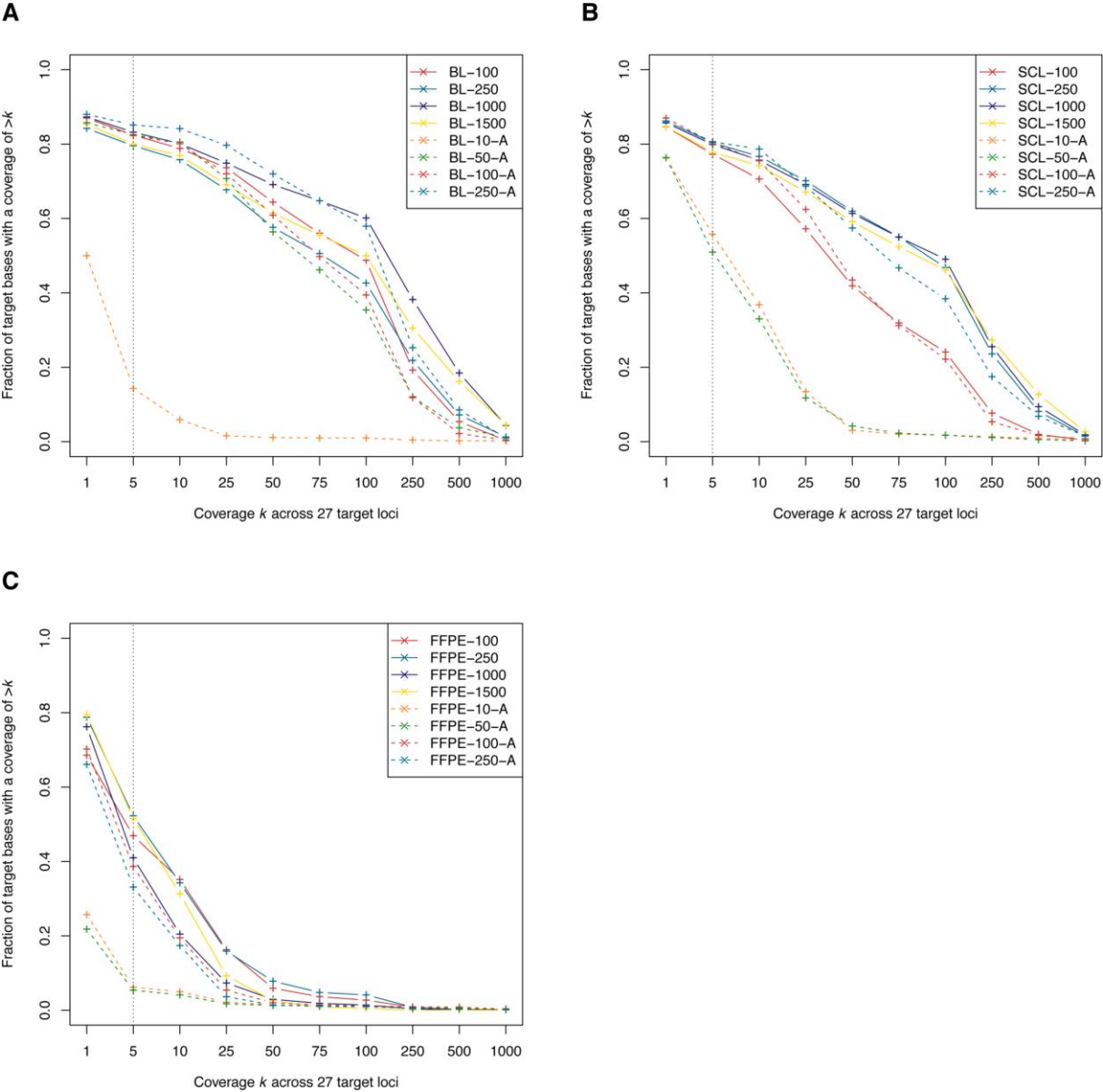
**Figure S1. Digital gel electrophoresis of DNA amplicons following microdroplet PCR and purification.** The PCR amplicons were analyzed using a High Sensitivity DNA Kit [Agilent Technologies]. Gels are shown for (A) BL, (B) SCL and (C) FFPE samples using the SC primer panel, and (D) BL and (E) SCL samples using the BM primer panel. The expected band of the amplified fragments was between 120 and 250 bp. The band indicating primer dimers was below 100 bp. For WGA samples, we observed bands corresponding to unspecific, high-molecular fragments.



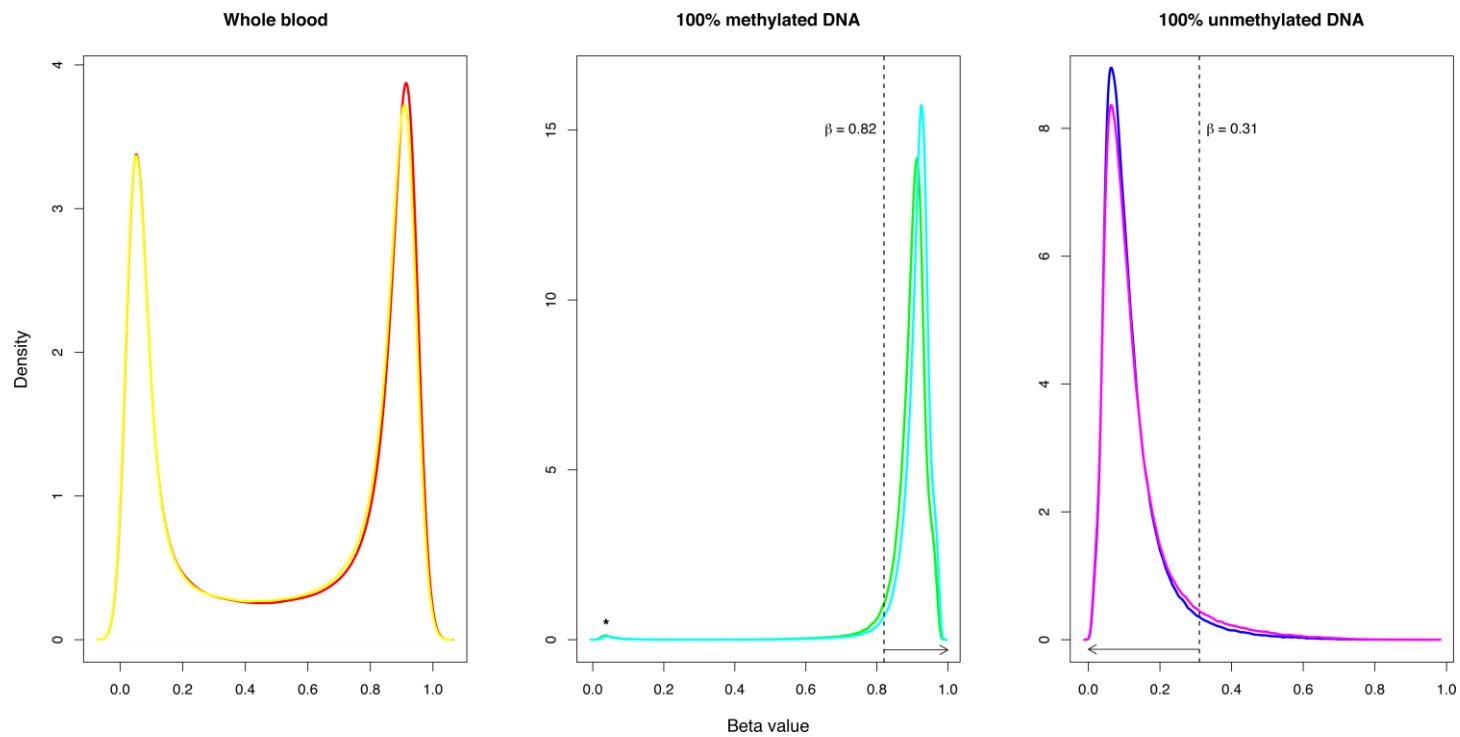
**Figure S2. Digital gel electrophoresis of DNA amplicons following universal PCR and purification.** The PCR amplicons were analyzed using a DNA 1000 Kit [Agilent Technologies]. Gels are shown for (A) BL, (B) SCL and (C) FFPE samples using the SC primer panel, and (D) BL and (E) SCL samples using the BM panel. The expected band of the amplified fragments after integration of Illumina adapters was shifted by +130 bp (Fig. S1 and Table S3A). Primer dimers (<100 bp), which could affect the subsequent DNA sequencing reaction, were not observed.



**Figure S3. Fraction of target bases with a coverage ranging from 1 to 1000 across target loci.** The plots show the fraction of target bases with a coverage ranging from 1 to 1000 at 27 target loci across (A) BL, (B) SCL and (C) FFPE samples, assessed using the SC panel. A coverage of five sequencing reads is indicated with a dotted line. For BL and SCL samples, the coverage profiles for unamplified samples as well as the 100 and 250 ng WGA samples, were relatively similar across different starting DNA amounts.



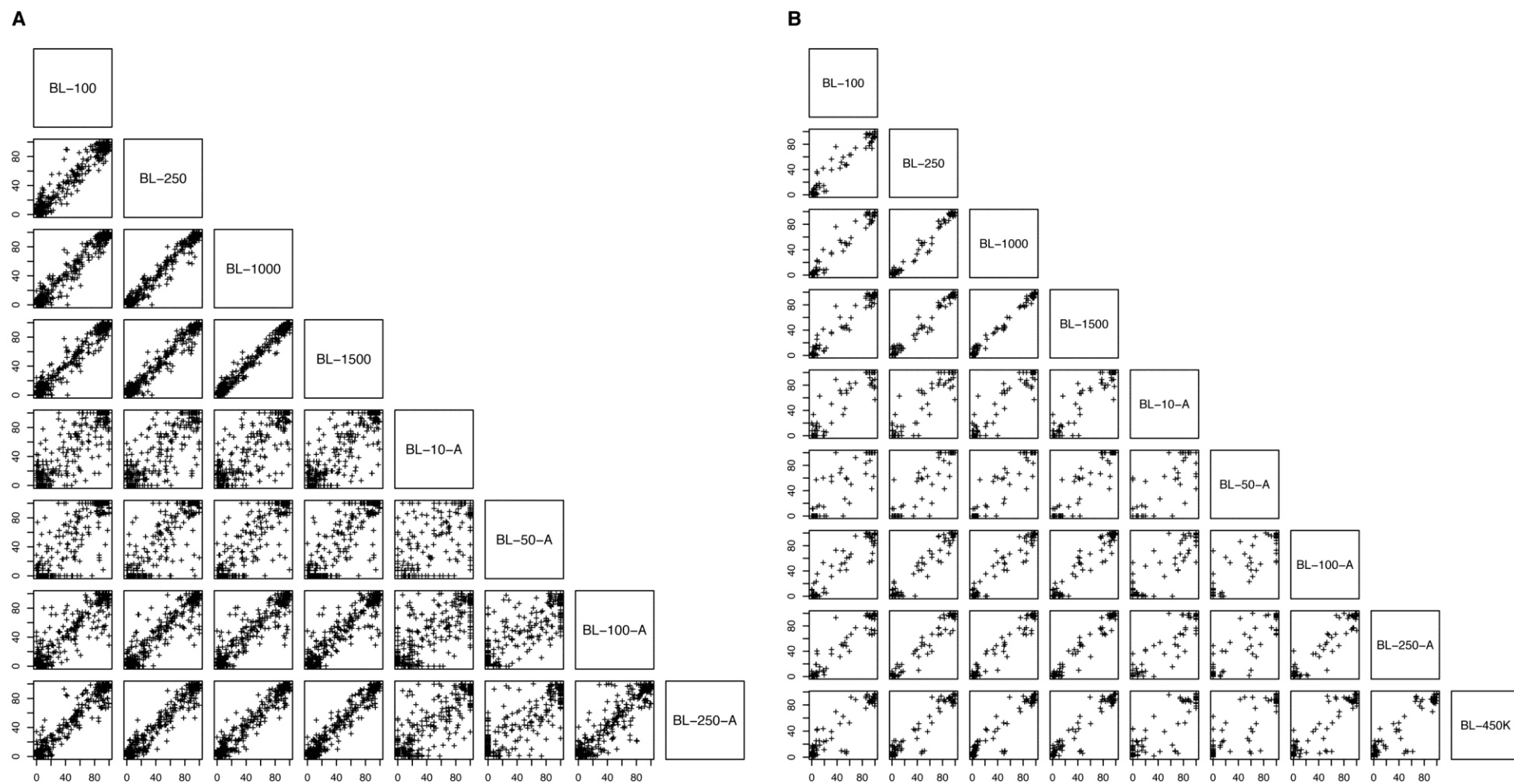
**Figure S4. Assessment of the quantitative resolution of the 450K array platform in comparison to RainDrop BS-seq.** We performed two technical replicates each of *in vitro* methylated and unmethylated whole blood genomic DNA. The *in vitro* methylated DNA was created using SssI methyltransferase. The *in vitro* unmethylated DNA was created by performing two sequential rounds of whole-genome amplification using REPLI-g. Array processing and analysis were performed as described in the Methods section. The figure shows the distribution of beta values for these experiments. Non-CpG probes (n=2800) are indicated with an asterisk. For *in vitro* methylated CpG sites we found that 95% of 450K probes had beta values above 0.82, while *in vitro* unmethylated CpGs had beta values below 0.31. On the basis of these data, the quantitative resolution of RainDrop BS-seq is comparable to 450K arrays to detect ‘methylated’ sites (i.e.  $\beta \geq 0.82$  vs  $\geq 4/5$  [ $\geq 80\%$ ] methylated reads), and actually exceeds the 450K array’s ability to resolve ‘unmethylated’ sites (i.e.  $\beta \leq 0.31$  vs  $\leq 1/5$  [ $\leq 20\%$ ] methylated reads). Therefore, we suggest a quantitative resolution of 20% to be appropriate for the experiments performed in this study.



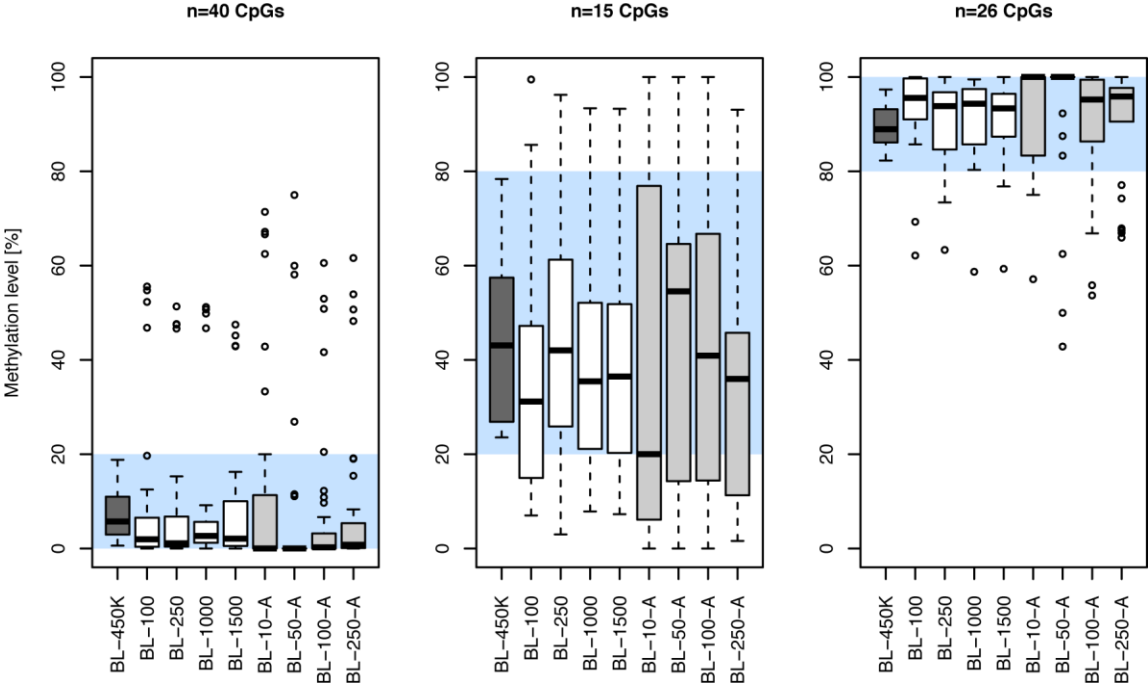




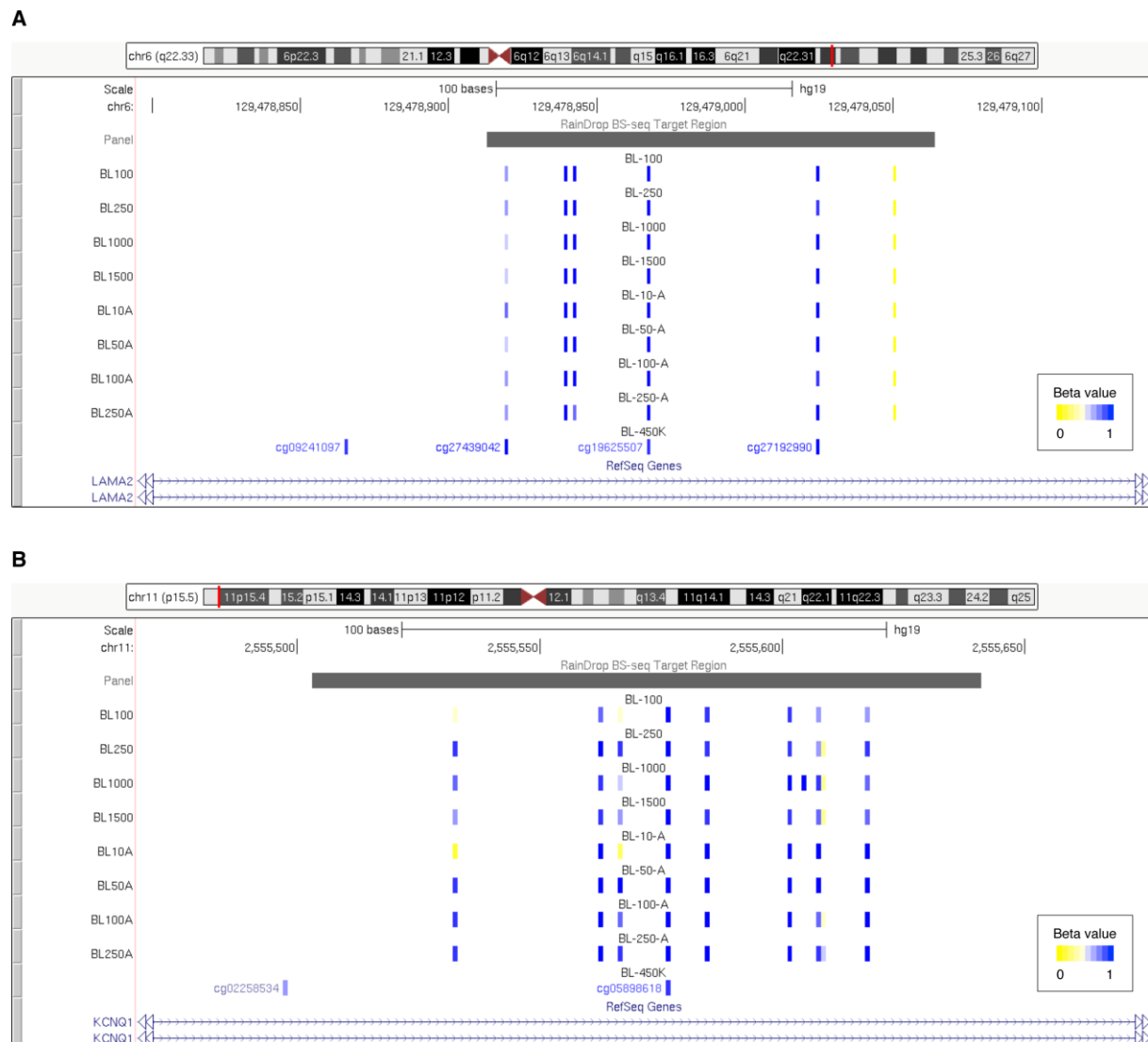
**Figure S6. Scatterplot of DNA methylation levels and comparison to 450K arrays.** (A) The scatterplot matrix shows the correlation of DNA methylation levels across BL samples (n=497 CpG sites), assessed using the BM panel. (B) In addition, we correlated a subset of these CpG sites that are represented on the 450K array (n=81). The assessed CpG sites correspond to those shown in Figure 4A.



**Figure S7. Distribution of DNA methylation levels over the entire dynamic range of the 450K array.** The boxplots show the observed DNA methylation levels for CpG sites that, according to the 450K array, fall into low (0–20%), medium (20–80%) and high (80–100%) methylation bins (indicated with light blue shading). As in Figure S6B, we assessed CpG sites (n=81) in BL samples that are represented on the 450K array.



**Figure S8. Methylation profiling of the (A) *LAMA2* and (B) *KCNQ1* target loci.** The figure shows the *LAMA2* (BM primer panel; Locus ID: 161; Amplicon ID: 175) and *KCNQ1* (BM primer panel; Locus ID: 243; Amplicon ID: 263) gene loci in the UCSC Genome Browser. We annotated the genomic loci with the RainDrop BS-seq target region coordinates in gray, and the methylation levels (color range: yellow–blue) assessed in independent experiments using a range of starting DNA quantities (10–1500 ng) and application of MDA-based WGA of bisulfite-converted DNA (indicated with ‘-A’). We also added the methylation data generated on the 450K array platform.



Supplementary Tables

**Table S1. Selected genomic loci assessed using RainDrop BS-seq.** The table provides genomic coordinates (hg19) of the target loci and PCR amplicons. In addition, CpG sites that are present on the 450K array and located within the target loci are indicated. **(A)** The SC primer panel targeted 27 genomic regions (500 PCR amplicons) containing a total of 212 CpG sites that are represented on the 450K array. **(B)** The BM panel targeted 462 candidate epigenetic biomarker loci (501 PCR amplicons) containing 778 of the 450K CpG sites.

**(A) SC Panel**

**(B) BM Panel**

Locus		Amplicon			450K CpG Site		Locus		Amplicon			450K CpG Site	
ID	ID	Chr	Start	End	ID	Position	ID	ID	Chr	Start	End	ID	Position
1	1	1	3,569,197	3,569,352	NA	NA	1	1	1	949,741	949,920	cg04788999	949,850
1	2	1	3,569,197	3,569,380	NA	NA	1	1	1	949,741	949,920	cg16526047	949,893
1	3	1	3,569,335	3,569,530	cg16741710	3,569,386	2	2	1	1,158,573	1,158,728	cg03467014	1,158,648
1	4	1	3,569,364	3,569,562	cg16741710	3,569,386	3	3	1	2,266,291	2,266,447	cg16600733	2,266,362
1	5	1	3,569,504	3,569,698	cg05924583	3,569,624	3	3	1	2,266,291	2,266,447	cg02687883	2,266,431
1	6	1	3,569,597	3,569,757	cg05924583	3,569,624	4	4	1	3,459,820	3,460,014	cg06511312	3,459,949
1	7	1	3,569,703	3,569,809	NA	NA	5	5	1	3,567,586	3,567,765	cg07382920	3,567,646
1	8	1	3,569,714	3,569,870	NA	NA	5	5	1	3,567,586	3,567,765	cg10038618	3,567,719
1	9	1	3,569,776	3,569,941	cg11504517	3,569,899	5	5	1	3,567,586	3,567,765	cg24678611	3,567,732
1	10	1	3,569,873	3,570,003	cg11504517	3,569,899	5	5	1	3,567,586	3,567,765	cg25731359	3,567,738
1	11	1	3,569,927	3,570,115	NA	NA	6	6	1	10,579,082	10,579,235	cg10801633	10,579,190
1	12	1	3,570,037	3,570,226	NA	NA	7	7	1	12,123,442	12,123,552	NA	NA
1	13	1	3,570,082	3,570,279	NA	NA	7	8	1	12,123,527	12,123,696	cg05121790	12,123,554
1	14	1	3,570,183	3,570,343	NA	NA	8	9	1	14,343,060	14,343,254	cg13686807	14,343,114
1	15	1	3,570,262	3,570,460	NA	NA	9	10	1	21,978,575	21,978,724	cg18150885	21,978,612
2	16	2	74,271,947	74,272,097	NA	NA	9	10	1	21,978,575	21,978,724	cg08892236	21,978,698
2	17	2	74,272,021	74,272,196	cg00694660	74,272,104	10	11	1	21,986,195	21,986,331	cg05120085	21,986,286
2	18	2	74,272,076	74,272,207	cg00694660	74,272,104	11	12	1	22,615,848	22,616,017	cg021053477	22,615,900
2	19	2	74,272,223	74,272,373	NA	NA	12	13	1	25,257,516	25,257,669	cg15014975	25,257,547
2	20	2	74,272,293	74,272,456	NA	NA	12	13	1	25,257,516	25,257,669	cg24019564	25,257,566
2	21	2	74,272,378	74,272,525	NA	NA	12	13	1	25,257,516	25,257,669	cg10993442	25,257,587
2	22	2	74,272,466	74,272,665	NA	NA	12	13	1	25,257,516	25,257,669	cg24842859	25,257,599
2	23	2	74,272,556	74,272,748	NA	NA	12	13	1	25,257,516	25,257,669	cg20695936	25,257,624
2	24	2	74,272,627	74,272,820	cg01355757	74,272,773	12	13	1	25,257,516	25,257,669	cg13106389	25,257,626
2	25	2	74,272,729	74,272,870	cg01355757	74,272,773	12	13	1	25,257,516	25,257,669	cg18087266	25,257,629
2	26	2	74,272,818	74,272,962	cg01244346	74,272,962	13	14	1	47,898,845	47,898,969	cg05658236	47,898,911
2	27	2	74,272,882	74,273,079	cg01244346	74,272,962	14	15	1	55,752,451	55,752,568	cg03847541	55,752,514
2	28	2	74,272,989	74,273,151	NA	NA	15	16	1	65,991,125	65,991,285	cg08234308	65,991,176
2	29	2	74,273,089	74,273,270	NA	NA	16	17	1	87,795,021	87,795,189	cg03129717	87,795,122
2	30	2	74,273,116	74,273,311	NA	NA	17	18	1	91,182,737	91,182,936	cg18322569	91,182,777
2	31	2	74,273,251	74,273,450	NA	NA	17	18	1	91,182,737	91,182,936	cg17241310	91,182,856
2	32	2	74,273,390	74,273,562	NA	NA	18	19	1	110,052,257	110,052,519	cg05947402	110,052,352
2	33	2	74,273,458	74,273,609	NA	NA	18	19	1	110,052,257	110,052,519	cg21646186	110,052,458
3	34	2	106,681,135	106,681,319	cg22948077	106,681,280	18	19	1	110,052,257	110,052,519	cg12419308	110,052,464
3	35	2	106,681,191	106,681,373	cg22948077	106,681,280	18	19	1	110,052,257	110,052,519	cg06764670	110,052,490
3	36	2	106,681,292	106,681,463	NA	NA	19	20	1	111,175,024	111,175,158	cg05656442	111,175,082
3	37	2	106,681,350	106,681,533	NA	NA	20	21	1	115,124,440	115,124,544	cg20145149	115,124,491
3	38	2	106,681,468	106,681,667	NA	NA	21	22	1	121,261,272	121,261,451	cg07794500	121,261,369
3	39	2	106,681,612	106,681,718	NA	NA	21	22	1	121,261,272	121,261,451	cg05827631	121,261,404
3	40	2	106,681,670	106,681,869	cg10735632	106,681,831	22	23	1	144,930,878	144,931,062	cg02854922	144,930,964
3	41	2	106,681,804	106,681,975	cg10735632	106,681,831	23	24	1	146,547,949	146,548,127	cg12406683	146,548,032
3	41	2	106,681,804	106,681,975	cg14535980	106,681,937	24	25	1	147,736,119	147,736,302	cg21167167	147,736,190
3	41	2	106,681,804	106,681,975	cg21838979	106,681,945	25	26	1	155,271,700	155,271,834	cg15490880	155,271,762
3	42	2	106,681,861	106,682,026	cg14535980	106,681,937	26	27	1	161,442,628	161,442,808	cg08527195	161,442,697
3	42	2	106,681,861	106,682,026	cg21838979	106,681,945	26	27	1	161,442,628	161,442,808	cg09846895	161,442,738
3	42	2	106,681,861	106,682,026	cg06499647	106,681,983	27	28	1	171,811,400	171,811,594	cg20800956	171,811,477
3	42	2	106,681,861	106,682,026	cg07510423	106,681,998	27	28	1	171,811,400	171,811,594	cg16515523	171,811,529
3	43	2	106,681,972	106,682,072	cg06499647	106,681,983	28	29	1	177,002,175	177,002,347	cg22506490	177,002,228
3	43	2	106,681,972	106,682,072	cg07510423	106,681,998	29	30	1	202,317,732	202,317,906	cg05798608	202,317,770
3	44	2	106,682,239	106,682,309	NA	NA	29	30	1	202,317,732	202,317,906	cg13976253	202,317,780
3	45	2	106,682,240	106,682,439	cg09661951	106,682,331	30	31	1	209,822,863	209,823,015	cg07168232	209,822,928
3	46	2	106,682,276	106,682,450	cg09661951	106,682,331	31	32	1	210,425,198	210,425,298	cg14561217	210,425,254
3	47	2	106,682,405	106,682,536	NA	NA	31	33	1	210,425,255	210,425,409	NA	NA
3	48	2	106,682,495	106,682,669	cg10885338	106,682,640	32	34	1	224,400,579	224,400,742	cg01553584	224,400,672
3	49	2	106,682,577	106,682,769	cg10885338	106,682,640	33	35	1	227,057,956	227,058,113	cg20000641	227,058,046
3	50	2	106,682,646	106,682,806	NA	NA	33	35	1	227,057,956	227,058,113	cg00479101	227,058,070
3	51	2	106,682,765	106,682,909	NA	NA	34	36	1	230,289,965	230,290,156	cg22153407	230,290,089
4	52	2	121,669,794	121,669,993	NA	NA	35	37	1	231,981,776	231,981,974	cg09186051	231,981,906
4	53	2	121,669,892	121,670,063	NA	NA	36	38	1	232,940,913	232,941,059	cg02191044	232,940,990
4	54	2	121,670,000	121,670,160	NA	NA	36	38	1	232,940,913	232,941,059	cg02903907	232,941,055

















27	495	22	42,828,673	42,828,847	NA	NA	376	406	17	56,084,407	56,084,548	cg14553224	56,084,507
27	496	22	42,828,757	42,828,944	NA	NA	377	407	17	75,368,850	75,369,019	cg06848185	75,368,902
27	497	22	42,828,869	42,829,060	cg07464578	42,828,946	377	408	17	75,368,995	75,369,168	cg19554255	75,369,051
27	498	22	42,828,925	42,829,117	cg07464578	42,828,946	377	408	17	75,368,995	75,369,168	cg16779463	75,369,055
27	499	22	42,829,044	42,829,182	NA	NA	377	408	17	75,368,995	75,369,168	cg17300544	75,369,091
27	500	22	42,829,135	42,829,242	NA	NA	377	409	17	75,369,102	75,369,272	cg03804136	75,369,219
							377	409	17	75,369,102	75,369,272	cg15044248	75,369,224
							377	409	17	75,369,102	75,369,272	cg02884239	75,369,228
							378	410	17	76,220,836	76,220,997	cg19272238	76,220,898
							378	410	17	76,220,836	76,220,997	cg07366188	76,220,929
							378	410	17	76,220,836	76,220,997	cg10070788	76,220,955
							379	411	17	76,348,748	76,348,914	cg17845279	76,348,823
							379	411	17	76,348,748	76,348,914	cg21640526	76,348,849
							380	412	17	76,921,811	76,921,999	cg06641285	76,921,829
							380	412	17	76,921,811	76,921,999	cg05306745	76,921,845
							380	412	17	76,921,811	76,921,999	cg15981475	76,921,948
							381	413	17	76,932,217	76,932,388	cg14779622	76,932,315
							381	413	17	76,932,217	76,932,388	cg17552357	76,932,332
							382	414	17	78,155,158	78,155,313	cg13855862	78,155,264
							383	415	17	79,415,561	79,415,759	cg01810575	79,415,682
							384	416	17	79,971,390	79,971,506	cg06105699	79,971,436
							385	417	17	80,709,099	80,709,345	cg00960700	80,709,150
							386	418	18	12,911,072	12,911,244	cg25356164	12,911,138
							387	419	18	34,327,403	34,327,561	cg21367586	34,327,498
							388	420	18	74,499,448	74,499,560	cg25691825	74,499,511
							389	421	18	74,848,763	74,848,902	cg12635694	74,848,845
							390	422	18	77,793,100	77,793,237	cg04727522	77,793,182
							391	423	19	2,131,225	2,131,338	cg05718393	2,131,293
							391	423	19	2,131,225	2,131,338	cg23103406	2,131,296
							392	424	19	2,643,016	2,643,134	cg15425921	2,643,085
							393	425	19	6,495,792	6,495,950	cg06548442	6,495,872
							393	425	19	6,495,792	6,495,950	cg11915671	6,495,897
							394	426	19	6,746,129	6,746,248	cg00513012	6,746,184
							394	426	19	6,746,129	6,746,248	cg19736654	6,746,221
							395	427	19	8,214,628	8,214,731	cg17909996	8,214,685
							396	428	19	8,273,644	8,273,781	cg24413781	8,273,693
							397	429	19	10,450,412	10,450,532	cg18054725	10,450,472
							398	430	19	31,843,302	31,843,447	cg22079008	31,843,360
							398	430	19	31,843,302	31,843,447	cg09253179	31,843,396
							399	431	19	35,249,109	35,249,273	cg00019275	35,249,217
							400	432	19	37,019,197	37,019,372	cg23949233	37,019,255
							400	432	19	37,019,197	37,019,372	cg16885608	37,019,262
							400	432	19	37,019,197	37,019,372	cg18105335	37,019,307
							400	432	19	37,019,197	37,019,372	cg18882201	37,019,327
							400	432	19	37,019,197	37,019,372	cg19990402	37,019,372
							401	433	19	37,861,428	37,861,557	cg06610641	37,861,546
							401	434	19	37,861,533	37,861,645	cg06610641	37,861,546
							402	435	19	38,085,577	38,085,746	cg27321876	38,085,706
							402	435	19	38,085,577	38,085,746	cg16708785	38,085,709
							403	436	19	45,654,162	45,654,321	cg27052073	45,654,213
							404	437	19	45,901,506	45,901,703	cg08512167	45,901,567
							404	437	19	45,901,506	45,901,703	cg13645732	45,901,649
							405	438	19	46,180,196	46,180,355	cg18642271	46,180,314
							405	439	19	46,180,306	46,180,440	cg18642271	46,180,314
							405	439	19	46,180,306	46,180,440	cg17144108	46,180,412
							406	440	19	46,318,696	46,318,892	cg27118825	46,318,748
							406	440	19	46,318,696	46,318,892	cg04379348	46,318,762
							407	441	19	47,923,301	47,923,461	cg07478208	47,923,331
							407	441	19	47,923,301	47,923,461	cg17910813	47,923,387
							408	442	19	48,918,066	48,918,187	cg15779837	48,918,116
							409	443	19	54,377,144	54,377,342	cg18054632	54,377,302
							409	444	19	54,377,302	54,377,463	cg18691133	54,377,440
							410	445	19	58,514,764	58,514,943	cg05377415	58,514,816
							410	445	19	58,514,764	58,514,943	cg27295481	58,514,839
							411	446	20	271,313	271,469	cg11991627	271,345
							411	446	20	271,313	271,469	cg23989297	271,347
							411	446	20	271,313	271,469	cg17441724	271,417
							411	446	20	271,313	271,469	cg25232745	271,422
							411	446	20	271,313	271,469	cg06440615	271,425
							411	446	20	271,313	271,469	cg25031040	271,432
							412	447	20	304,008	304,179	cg01822050	304,156
							413	448	20	2,508,885	2,509,043	cg07545427	2,508,981
							414	449	20	20,349,139	20,349,565	cg13326227	20,349,168
							414	449	20	20,349,139	20,349,565	cg00648301	20,349,241
							415	450	20	24,449,631	24,449,790	cg05347927	24,449,668
							415	450	20	24,449,631	24,449,790	cg22479299	24,449,704
							415	450	20	24,449,631	24,449,790	cg02997755	24,449,734
							415	450	20	24,449,631	24,449,790	cg22452236	24,449,760
							416	451	20	24,450,260	24,450,404	cg09623400	24,450,353
							416	451	20	24,450,260	24,450,404	cg19654195	24,450,361
							417	452	20	32,378,120	32,378,279	cg01554089	32,378,171
							418	453	20	34,099,423	34,099,569	cg04070427	34,099,511
							419	454	20	36,013,363	36,013,478	cg23635789	36,013,428
							420	455	20	36,153,843	36,153,992	cg03226872	36,153,865
							420	455	20	36,153,843	36,153,992	cg17271585	36,153,947

421	456	20	42,142,967	42,143,118	cg22601123	42,142,995
421	456	20	42,142,967	42,143,118	cg22330467	42,143,015
421	456	20	42,142,967	42,143,118	cg14306330	42,143,045
421	456	20	42,142,967	42,143,118	cg01071811	42,143,080
421	456	20	42,142,967	42,143,118	cg02611863	42,143,096
422	457	20	43,161,193	43,161,315	cg00726046	43,161,260
423	458	20	44,782,043	44,782,220	cg11206526	44,782,126
424	459	20	48,728,592	48,728,758	cg13046524	48,728,642
425	460	20	50,158,137	50,158,289	cg02561912	50,158,202
426	461	20	56,881,887	56,882,037	cg21532801	56,881,949
427	462	20	58,586,763	58,586,907	cg02423105	58,586,819
428	463	20	60,701,221	60,701,380	cg06096175	60,701,350
428	464	20	60,701,310	60,701,504	cg06096175	60,701,350
429	465	20	61,803,852	61,804,017	cg13590979	61,803,906
430	466	20	62,124,844	62,125,003	cg14636268	62,124,947
431	467	21	30,365,345	30,365,502	cg01446372	30,365,398
431	467	21	30,365,345	30,365,502	cg23864210	30,365,465
432	468	21	34,398,760	34,398,899	cg14843922	34,398,849
433	469	21	36,412,349	36,412,507	cg16071713	36,412,456
434	470	21	43,098,798	43,098,952	cg20206277	43,098,901
435	471	21	47,784,037	47,784,223	cg05931989	47,784,119
436	472	22	19,748,761	19,748,948	cg02583938	19,748,777
436	472	22	19,748,761	19,748,948	cg16332936	19,748,910
436	472	22	19,748,761	19,748,948	cg16113681	19,748,925
436	473	22	19,748,917	19,749,020	cg16113681	19,748,925
437	474	22	21,333,564	21,333,718	cg06985578	21,333,706
438	475	22	30,723,129	30,723,265	cg10574851	30,723,212
438	475	22	30,723,129	30,723,265	cg24944736	30,723,217
439	476	22	39,414,416	39,414,543	cg27451450	39,414,471
440	477	22	41,183,411	41,183,603	cg03888645	41,183,474
441	478	22	42,949,775	42,949,924	cg04416895	42,949,810
441	478	22	42,949,775	42,949,924	cg08157579	42,949,829
442	479	22	42,976,088	42,976,208	cg08094784	42,976,149
443	480	22	46,831,936	46,832,056	cg17551295	46,831,988
444	481	22	50,435,357	50,435,476	cg18730511	50,435,413
445	482	X	11,683,507	11,683,626	cg21880156	11,683,548
445	482	X	11,683,507	11,683,626	cg18036967	11,683,625
445	483	X	11,683,601	11,683,783	cg18036967	11,683,625
446	484	X	23,017,880	23,018,046	cg21908878	23,017,887
446	484	X	23,017,880	23,018,046	cg14020146	23,017,980
446	484	X	23,017,880	23,018,046	cg26009591	23,017,991
446	484	X	23,017,880	23,018,046	cg08264725	23,018,003
446	484	X	23,017,880	23,018,046	cg27124742	23,018,006
446	484	X	23,017,880	23,018,046	cg22052586	23,018,036
447	485	X	30,904,301	30,904,451	cg05204193	30,904,380
448	486	X	66,765,192	66,765,368	cg03096488	66,765,289
449	487	X	69,355,989	69,356,149	cg00713642	69,356,087
450	488	X	69,509,620	69,509,767	cg18030003	69,509,686
450	488	X	69,509,620	69,509,767	cg17206029	69,509,694
450	488	X	69,509,620	69,509,767	cg00213957	69,509,697
450	488	X	69,509,620	69,509,767	cg13622893	69,509,727
451	489	X	69,673,743	69,673,865	cg01448525	69,673,805
452	490	X	70,316,329	70,316,487	cg18288715	70,316,444
453	491	X	73,513,315	73,513,500	cg10575170	73,513,412
453	491	X	73,513,315	73,513,500	cg07823797	73,513,418
453	491	X	73,513,315	73,513,500	cg19487868	73,513,453
453	491	X	73,513,315	73,513,500	cg04667267	73,513,464
454	492	X	75,249,487	75,249,685	cg08103876	75,249,519
454	492	X	75,249,487	75,249,685	cg03236853	75,249,539
455	493	X	117,629,107	117,629,286	cg27062326	117,629,163
456	494	X	118,750,201	118,750,354	cg17385225	118,750,273
457	495	X	135,333,537	135,333,704	cg07446674	135,333,561
457	495	X	135,333,537	135,333,704	cg27047283	135,333,567
457	495	X	135,333,537	135,333,704	cg24347720	135,333,661
457	495	X	135,333,537	135,333,704	cg13304035	135,333,674
457	495	X	135,333,537	135,333,704	cg13243544	135,333,698
458	496	X	136,115,950	136,116,119	cg14463432	136,115,991
459	497	X	138,287,387	138,287,578	cg06981242	138,287,529
459	497	X	138,287,387	138,287,578	cg08349204	138,287,578
460	498	X	139,587,254	139,587,372	cg23862713	139,587,270
460	498	X	139,587,254	139,587,372	cg26369916	139,587,279
460	498	X	139,587,254	139,587,372	cg07929406	139,587,304
460	498	X	139,587,254	139,587,372	cg03202526	139,587,311
460	498	X	139,587,254	139,587,372	cg11638117	139,587,335
460	498	X	139,587,254	139,587,372	cg11409998	139,587,372
460	499	X	139,587,337	139,587,500	cg11409998	139,587,372
460	499	X	139,587,337	139,587,500	cg24432916	139,587,479
461	500	X	144,903,592	144,903,733	cg24880787	144,903,661
461	500	X	144,903,592	144,903,733	cg19332075	144,903,691
462	501	X	151,143,173	151,143,343	cg18748981	151,143,213
462	501	X	151,143,173	151,143,343	cg25528646	151,143,302

**Table S2. Summary sequencing and methylation statistics for the (A) SC panel and (B) BM panel.** Summary statistics regarding trimming and mapping of sequencing reads, as well as methylation levels are reported. The data sets were analyzed as described in the Methods section.

**(A) SC Panel**

	BL 100	BL 250	BL 1000	BL 1500	BL 10-A	BL 50-A	BL 100-A	BL 250-A	SCL 100	SCL 250	SCL 1000	SCL 1500	SCL 10-A	SCL 50-A	SCL 100-A	SCL 250-A
<b>Raw sequencing read quality filtering (fastq-mcf)</b>																
Total number of sequencing reads	305,590	253,255	273,603	215,406	190,386	448,017	466,972	535,264	324,934	404,680	234,005	243,583	381,979	372,326	425,036	456,764
Number of discarded low-quality reads	20,128	15,497	13,564	7,548	33,127	64,864	64,978	49,528	23,697	30,245	13,826	11,846	68,043	46,823	68,224	14,009
<b>Final alignment report (Bismark)</b>																
Total number of sequencing reads analyzed	285,462	237,758	260,039	207,858	157,259	383,153	401,994	485,736	301,237	374,435	220,179	231,737	313,936	325,503	356,812	442,755
Sequence pairs with a unique best hit	50,950	52,449	88,158	77,987	5,537	50,661	47,885	72,498	30,035	59,954	61,605	65,520	14,519	11,948	36,301	52,171
CT/GA/CT (converted top strand)	25,841	26,950	45,388	40,886	2,990	24,954	24,425	38,147	16,140	29,269	30,902	33,186	8,445	5,933	18,655	26,929
GA/CT/CT (compl. to converted top strand)	812	571	1,146	586	238	2,212	2,048	3,018	360	897	656	517	603	981	2,205	1,413
GA/CT/GA (compl. to converted bottom strand)	23,766	24,498	41,158	36,164	1,422	22,489	20,505	30,283	13,101	29,143	29,572	31,403	4,048	3,733	14,339	23,367
CT/GA/GA (converted bottom strand)	531	430	466	351	887	1,006	907	1,050	434	645	475	414	1,423	1,301	1,102	462
Mapping efficiency	17.80%	22.10%	33.90%	37.50%	3.50%	13.20%	11.90%	14.90%	10.00%	16.00%	28.00%	28.30%	4.60%	3.70%	10.20%	11.80%
Sequence pairs with no alignments under any condition	230,296	182,088	169,151	128,073	147,169	323,119	345,266	403,760	265,673	308,019	155,755	163,526	289,682	305,764	311,638	387,963
Sequence pairs that did not map uniquely	4,216	3,221	2,730	1,798	4,553	9,373	8,843	9,478	5,529	6,462	2,819	2,691	9,735	7,791	8,873	2,621
Sequence pairs that were discarded, because genomic sequence could not be extracted	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Targeted sequencing statistics (TEQC)</b>																
Number of sequencing reads used	101,900	104,898	176,316	155,974	11,074	101,322	95,770	144,996	60,070	119,908	123,210	131,040	29,038	23,896	72,602	104,342
Fraction of aligned reads that overlap with any target region	0.947	0.958	0.961	0.974	0.660	0.871	0.869	0.876	0.930	0.943	0.962	0.968	0.743	0.642	0.819	0.918
<b>Final cytosine methylation report (Bismark)</b>																
Total number of C's analyzed	1,755,662	1,809,211	3,142,878	2,791,484	88,718	1,486,819	1,440,186	2,304,494	938,487	1,979,953	2,138,844	2,322,030	283,795	245,177	968,769	1,726,513
Total methylated C's in CpG context	60,894	64,307	112,329	99,370	2,006	50,213	54,278	80,600	96,638	208,548	239,126	266,576	11,008	9,712	61,519	138,007
Total methylated C's in CHG context	4,521	4,703	7,691	6,379	352	2,870	4,833	5,066	2,686	5,171	4,082	4,707	747	811	1,760	4,370
Total methylated C's in CHH context	23,504	21,285	25,009	19,679	19,638	41,267	36,018	42,676	20,708	29,434	16,916	18,296	40,594	37,655	34,924	22,195
Total C to T conversions in CpG context	264,855	285,653	472,920	457,113	4,648	159,028	157,429	268,765	69,294	142,078	158,502	174,856	17,444	13,312	55,533	133,988
Total C to T conversions in CHG context	515,501	540,786	937,339	849,569	16,818	424,062	410,229	659,168	280,052	587,904	638,813	701,058	65,738	55,388	270,147	510,946
Total C to T conversions in CHH context	886,387	892,477	1,587,590	1,359,374	45,256	809,379	777,399	1,248,219	469,109	1,006,818	1,081,405	1,156,537	148,264	128,299	544,886	917,007
C methylated in CpG context	18.70%	18.40%	19.20%	17.90%	30.10%	24.00%	25.60%	23.10%	58.20%	59.50%	60.10%	60.40%	38.70%	42.20%	52.60%	50.70%
C methylated in CHG context	0.90%	0.90%	0.80%	0.70%	2.10%	0.70%	1.20%	0.80%	0.90%	0.90%	0.60%	0.70%	1.10%	1.40%	0.60%	0.80%
C methylated in CHH context	2.60%	2.30%	1.60%	1.40%	30.30%	4.90%	4.40%	3.30%	4.20%	2.80%	1.50%	1.60%	21.50%	22.70%	6.00%	2.40%

	FFPE 100	FFPE 250	FFPE 1000	FFPE 1500	FFPE 10-A	FFPE 50-A	FFPE 100-A	FFPE 250-A
<b>Raw sequencing read quality filtering (fastq-mcf)</b>								
Total number of sequencing reads	197,172	190,892	97,956	54,414	320,858	274,304	452,992	296,896
Number of discarded low-quality reads	18,754	14,777	8,778	5,222	50,972	51,514	49,175	29,535
<b>Final alignment report (Bismark)</b>								
Total number of sequencing reads analyzed	178,418	176,115	89,178	49,192	269,886	222,790	403,817	267,361
Sequence pairs with a unique best hit	8,607	8,394	4,377	3,912	9,570	8,974	9,914	6,443
CT/GA/CT (converted top strand)	5,156	4,800	2,419	2,039	5,752	5,716	5,703	3,363

GA/CT/CT (compl. to converted top strand)	180	220	100	115	267	211	519	642
GA/CT/GA (compl. to converted bottom strand)	2,856	3,046	1,637	1,615	2,594	2,305	2,702	1,706
CT/GA/GA (converted bottom strand)	415	328	221	143	957	742	990	732
Mapping efficiency	4.80%	4.80%	4.90%	8.00%	3.50%	4.00%	2.50%	2.40%
Sequence pairs with no alignments under any condition	164,887	163,706	82,577	44,172	251,252	205,615	385,703	255,696
Sequence pairs that did not map uniquely	4,924	4,015	2,224	1,108	9,064	8,201	8,200	5,222
Sequence pairs that were discarded, because genomic sequence could not be extracted	0	0	0	0	0	0	0	0
<b>Targeted sequencing statistics (TEQC)</b>								
Number of sequencing reads used	17,214	16,788	8,754	7,824	19,140	17,948	19,828	12,886
Fraction of aligned reads that overlap with any target region	0.837	0.841	0.814	0.845	0.713	0.748	0.666	0.598
<b>Final cytosine methylation report (Bismark)</b>								
Total number of C's analyzed	221,077	229,836	115,732	115,309	137,560	120,540	178,147	124,523
Total methylated C's in CpG context	8,041	8,729	4,522	4,097	2,786	2,777	8,295	6,532
Total methylated C's in CHG context	507	722	341	274	399	346	642	647
Total methylated C's in CHH context	14,591	11,803	7,604	4,325	31,410	24,993	34,373	27,797
Total C to T conversions in CpG context	21,305	23,207	13,108	13,443	4,359	3,785	7,168	5,151
Total C to T conversions in CHG context	57,517	64,522	31,638	32,685	27,563	24,942	36,690	23,436
Total C to T conversions in CHH context	119,116	120,853	58,519	60,485	71,043	63,697	90,979	60,960
C methylated in CpG context	27.40%	27.30%	25.60%	23.40%	39.00%	42.30%	53.60%	55.90%
C methylated in CHG context	0.90%	1.10%	1.10%	0.80%	1.40%	1.40%	1.70%	2.70%
C methylated in CHH context	10.90%	8.90%	11.50%	6.70%	30.70%	28.20%	27.40%	31.30%

## (B) BM Panel

	BL 100	BL 250	BL 1000	BL 1500	BL 10-A	BL 50-A	BL 100-A	BL 250-A	SCL 100	SCL 250	SCL 1000	SCL 1500	SCL 10-A	SCL 50-A	SCL 100-A	SCL 250-A
<b>Raw sequencing read quality filtering (fastq-mcf)</b>																
Total number of sequencing reads	582,276	804,368	429,851	378,921	854,499	430,215	759,866	1,053,732	476,191	673,353	453,915	283,451	364,617	600,335	605,800	1,054,642
Number of discarded low-quality reads	22,617	30,032	11,855	7,929	69,394	34,255	52,774	59,765	24,612	29,870	14,475	7,983	37,056	58,337	52,120	76,018
<b>Final alignment report (Bismark)</b>																
Total number of sequencing reads analyzed	559,659	774,336	417,996	370,992	785,105	395,960	707,092	993,967	451,579	643,483	439,440	275,468	327,561	541,998	553,680	978,624
Sequence pairs with a unique best hit	65,766	101,257	121,647	134,812	24,239	13,951	57,757	142,385	30,992	83,346	116,942	90,278	15,944	26,081	46,530	112,189
CT/GA/CT (converted top strand)	31,448	48,727	59,434	65,413	5,595	4,170	23,418	64,652	13,517	38,180	54,973	43,087	4,977	8,732	18,851	50,189
GA/CT/CT (compl. to converted top strand)	1,867	2,762	1,582	1,478	2,965	1,339	2,482	3,563	1,408	2,474	1,614	987	997	1,752	1,936	3,581
GA/CT/GA (compl. to converted bottom strand)	27,997	43,923	57,794	65,587	8,469	5,066	25,116	64,919	12,284	37,522	57,082	44,245	5,952	9,316	19,908	48,365
CT/GA/GA (converted bottom strand)	4,454	5,845	2,837	2,334	7,210	3,376	6,741	9,251	3,783	5,170	3,273	1,959	4,018	6,281	5,835	10,054
Mapping efficiency	11.80%	13.10%	29.10%	36.30%	3.10%	3.50%	8.20%	14.30%	6.90%	13.00%	26.60%	32.80%	4.90%	4.80%	8.40%	11.50%
Sequence pairs with no alignments under any condition	480,871	655,652	288,720	229,817	733,782	368,840	630,936	828,445	408,373	543,673	313,012	180,031	301,577	498,888	491,209	841,144
Sequence pairs that did not map uniquely	13,022	17,427	7,629	6,363	27,084	13,169	18,399	23,137	12,214	16,464	9,486	5,159	10,040	17,029	15,941	25,291
Sequence pairs that were discarded, because genomic sequence could not be extracted	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Targeted sequencing statistics (TEQC)</b>																
Number of sequencing reads used	131,532	202,514	243,294	269,624	48,478	27,902	115,514	284,770	61,984	166,692	233,884	180,556	31,888	52,162	93,060	224,378
Fraction of aligned reads that overlap with any target region	0.730	0.760	0.888	0.912	0.115	0.180	0.590	0.750	0.546	0.742	0.873	0.899	0.301	0.286	0.583	0.687
<b>Final cytosine methylation report (Bismark)</b>																
Total number of C's analyzed	2,231,032	3,475,046	4,274,365	4,801,645	482,071	297,007	1,797,890	4,706,481	940,575	2,774,485	4,042,875	3,165,998	392,178	625,554	1,401,229	3,537,416
Total methylated C's in CpG context	136,827	211,382	248,228	272,026	8,619	8,548	107,597	280,668	68,985	225,261	331,199	264,391	15,469	26,771	101,189	252,659

Total methylated C's in CHG context	4,556	6,707	7,749	8,034	2,857	1,631	3,554	9,194	2,173	5,677	6,565	4,660	1,161	1,716	2,691	6,356
Total methylated C's in CHH context	88,335	123,030	59,833	48,119	224,813	107,940	156,072	189,735	96,218	123,836	71,269	39,661	112,347	172,607	142,900	220,610
Total C to T conversions in CpG context	243,117	375,397	493,633	576,172	10,774	10,426	157,013	441,440	63,987	212,005	333,917	265,317	20,322	27,843	89,924	237,358
Total C to T conversions in CHG context	593,709	922,917	1,168,209	1,320,134	38,430	37,076	445,782	1,250,708	222,187	727,832	1,106,767	871,985	61,944	105,902	339,741	900,274
Total C to T conversions in CHH context	1,164,488	1,835,613	2,296,713	2,577,160	196,578	131,386	927,872	2,534,736	487,025	1,479,874	2,193,158	1,719,984	180,935	290,715	724,784	1,920,159
C methylated in CpG context	36.00%	36.00%	33.50%	32.10%	44.40%	45.10%	40.70%	38.90%	51.90%	51.50%	49.80%	49.90%	43.20%	49.00%	52.90%	51.60%
C methylated in CHG context	0.80%	0.70%	0.70%	0.60%	6.90%	4.20%	0.80%	0.70%	1.00%	0.80%	0.60%	0.50%	1.80%	1.60%	0.80%	0.70%
C methylated in CHH context	7.10%	6.30%	2.50%	1.80%	53.40%	45.10%	14.40%	7.00%	16.50%	7.70%	3.10%	2.30%	38.30%	37.30%	16.50%	10.30%



**Table S3. Primer sequences for the (A) universal PCR and (B) Illumina sequencing reaction.** In the universal PCR, the reverse primers harbored a sample-specific 8-bp barcode. The column ‘Barcode’ provides the reverse complementary DNA sequences of these barcodes, which were used for demultiplexing in the subsequent Illumina sequencing reaction. A total of 96 barcodes (based on Kozarewa I & Turner DJ. *Methods Mol Biol* 2011; 733:279-98) are shown. For the universal PCR using the SC and BM panel, we used the primers M\_rev\_25–48 and M\_rev\_25–40, respectively. For the sequencing reaction on a MiSeq DNA sequencer [Illumina], we used custom Read1 and Read2 primers.

**(A) Universal PCR**

ID	Sequence	Barcode
U_fwd	5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTG	NA
U_rev_1	5'-CAAGCAGAAGACGGCATAACGAGAT-ACAAGCTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAGCTTGT
U_rev_2	5'-CAAGCAGAAGACGGCATAACGAGAT-AAACATCG-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	CGATGTTT
U_rev_3	5'-CAAGCAGAAGACGGCATAACGAGAT-ACATTGGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCCAATGT
U_rev_4	5'-CAAGCAGAAGACGGCATAACGAGAT-ACCCTGTG-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	ACAGTGGT
U_rev_5	5'-CAAGCAGAAGACGGCATAACGAGAT-AACGTGAT-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	ATCACGTT
U_rev_6	5'-CAAGCAGAAGACGGCATAACGAGAT-CGCTGATC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GATCAGCG
U_rev_7	5'-CAAGCAGAAGACGGCATAACGAGAT-CAGATCTG-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	CAGATCTG
U_rev_8	5'-CAAGCAGAAGACGGCATAACGAGAT-ATGCTTAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTAGGCAT
U_rev_9	5'-CAAGCAGAAGACGGCATAACGAGAT-CTGTAGCC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GGCTACAG
U_rev_10	5'-CAAGCAGAAGACGGCATAACGAGAT-AGTACAAG-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	CTTGTACT
U_rev_11	5'-CAAGCAGAAGACGGCATAACGAGAT-CATCAAGT-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	ACTTGTATG
U_rev_12	5'-CAAGCAGAAGACGGCATAACGAGAT-AGTGGTCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGACCACT
U_rev_13	5'-CAAGCAGAAGACGGCATAACGAGAT-AAACAACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGGTGTGT
U_rev_14	5'-CAAGCAGAAGACGGCATAACGAGAT-AACCGAGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCTCGGTT
U_rev_15	5'-CAAGCAGAAGACGGCATAACGAGAT-AACGCTTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAAGCGTT
U_rev_16	5'-CAAGCAGAAGACGGCATAACGAGAT-AAGACGGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCCGCTTT
U_rev_17	5'-CAAGCAGAAGACGGCATAACGAGAT-AAGGTACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGTACCTT
U_rev_18	5'-CAAGCAGAAGACGGCATAACGAGAT-ACTATGCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTCTGTGT
U_rev_19	5'-CAAGCAGAAGACGGCATAACGAGAT-ACAGCAGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCTGCTGT
U_rev_20	5'-CAAGCAGAAGACGGCATAACGAGAT-ACCTCAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGGAGGT
U_rev_21	5'-CAAGCAGAAGACGGCATAACGAGAT-ACGCTCGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCGAGCGT
U_rev_22	5'-CAAGCAGAAGACGGCATAACGAGAT-ACGTATCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGATACGT
U_rev_23	5'-CAAGCAGAAGACGGCATAACGAGAT-ACTATGCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGCATAGT
U_rev_24	5'-CAAGCAGAAGACGGCATAACGAGAT-AGAGTCAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGACTCT
U_rev_25	5'-CAAGCAGAAGACGGCATAACGAGAT-AGATCGCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGCGATCT
U_rev_26	5'-CAAGCAGAAGACGGCATAACGAGAT-AGCAGGAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTCTGCTT
U_rev_27	5'-CAAGCAGAAGACGGCATAACGAGAT-AGTCACTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAGTGACT
U_rev_28	5'-CAAGCAGAAGACGGCATAACGAGAT-ATCCTGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TACAGGAT
U_rev_29	5'-CAAGCAGAAGACGGCATAACGAGAT-ATTGAGGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCCTCAAT
U_rev_30	5'-CAAGCAGAAGACGGCATAACGAGAT-CAACCACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGTGGTTG
U_rev_31	5'-CAAGCAGAAGACGGCATAACGAGAT-CAAGACTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAGTCTTG
U_rev_32	5'-CAAGCAGAAGACGGCATAACGAGAT-CAATGGAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTCCATTG
U_rev_33	5'-CAAGCAGAAGACGGCATAACGAGAT-CACTTCGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCGAGGTG
U_rev_34	5'-CAAGCAGAAGACGGCATAACGAGAT-CAGCGTTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAACGCTG
U_rev_35	5'-CAAGCAGAAGACGGCATAACGAGAT-CATAACAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGGTATG
U_rev_36	5'-CAAGCAGAAGACGGCATAACGAGAT-CCAGTTCG-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGAAGTGG
U_rev_37	5'-CAAGCAGAAGACGGCATAACGAGAT-CCGAAGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TACTCCGG
U_rev_38	5'-CAAGCAGAAGACGGCATAACGAGAT-CCGTGAGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCTCACGG
U_rev_39	5'-CAAGCAGAAGACGGCATAACGAGAT-CCTCTGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCAGGAGG
U_rev_40	5'-CAAGCAGAAGACGGCATAACGAGAT-CGAAGTCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAAGTTCG
U_rev_41	5'-CAAGCAGAAGACGGCATAACGAGAT-CGACTGGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCCAGTCG
U_rev_42	5'-CAAGCAGAAGACGGCATAACGAGAT-CGCATACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGTATGCG
U_rev_43	5'-CAAGCAGAAGACGGCATAACGAGAT-CTCAATGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCATTGAG
U_rev_44	5'-CAAGCAGAAGACGGCATAACGAGAT-CGTAGACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGGCTCAG
U_rev_45	5'-CAAGCAGAAGACGGCATAACGAGAT-CTGGCATA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TATGCCAG
U_rev_46	5'-CAAGCAGAAGACGGCATAACGAGAT-GAATCTGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCAGATTC
U_rev_47	5'-CAAGCAGAAGACGGCATAACGAGAT-GACTAGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TACTAGTC
U_rev_48	5'-CAAGCAGAAGACGGCATAACGAGAT-GAGCTGAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGACGTC
U_rev_49	5'-CAAGCAGAAGACGGCATAACGAGAT-GATAGACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGCTATATC
U_rev_50	5'-CAAGCAGAAGACGGCATAACGAGAT-GCCACATA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TATGTGGC
U_rev_51	5'-CAAGCAGAAGACGGCATAACGAGAT-GCGAGTAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTACTFCG
U_rev_52	5'-CAAGCAGAAGACGGCATAACGAGAT-GCTAACCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCGTTAGC

U_rev_53	5'-CAAGCAGAAGACGGCATAACGAGAT-GCTCGGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TACCGAGC
U_rev_54	5'-CAAGCAGAAGACGGCATAACGAGAT-GGAGAACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGTTCTCC
U_rev_55	5'-CAAGCAGAAGACGGCATAACGAGAT-GGTGCGAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTCCGACC
U_rev_56	5'-CAAGCAGAAGACGGCATAACGAGAT-GTACGCAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGCGTAC
U_rev_57	5'-CAAGCAGAAGACGGCATAACGAGAT-GTCTGTAGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCTACGAC
U_rev_58	5'-CAAGCAGAAGACGGCATAACGAGAT-GTCTGTCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGACAGAC
U_rev_59	5'-CAAGCAGAAGACGGCATAACGAGAT-GTGTCTTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAGAACAC
U_rev_60	5'-CAAGCAGAAGACGGCATAACGAGAT-TAGGATGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCATCCTA
U_rev_61	5'-CAAGCAGAAGACGGCATAACGAGAT-TATCAGCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGCTGATA
U_rev_62	5'-CAAGCAGAAGACGGCATAACGAGAT-TCCGTCTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TAGACGGA
U_rev_63	5'-CAAGCAGAAGACGGCATAACGAGAT-TCTTCACA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGTGAAGA
U_rev_64	5'-CAAGCAGAAGACGGCATAACGAGAT-TGAAGAGA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TCTCTTCA
U_rev_65	5'-CAAGCAGAAGACGGCATAACGAGAT-TGGAACAA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TTGTTCCA
U_rev_66	5'-CAAGCAGAAGACGGCATAACGAGAT-TGGCTTCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGAAGCCA
U_rev_67	5'-CAAGCAGAAGACGGCATAACGAGAT-TGGTGGTA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TACCACCA
U_rev_68	5'-CAAGCAGAAGACGGCATAACGAGAT-TTACGCA-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	TGCGTGAA
U_rev_69	5'-CAAGCAGAAGACGGCATAACGAGAT-AACTCACC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GGTGAGTT
U_rev_70	5'-CAAGCAGAAGACGGCATAACGAGAT-AAGAGATC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GATCTCTT
U_rev_71	5'-CAAGCAGAAGACGGCATAACGAGAT-AAGGACAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTGTCTTT
U_rev_72	5'-CAAGCAGAAGACGGCATAACGAGAT-AATCCGTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GACGGATT
U_rev_73	5'-CAAGCAGAAGACGGCATAACGAGAT-AATGTGTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCAACATT
U_rev_74	5'-CAAGCAGAAGACGGCATAACGAGAT-ACACGACC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GGTCGTGT
U_rev_75	5'-CAAGCAGAAGACGGCATAACGAGAT-ACAGATTTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GATCTGTG
U_rev_76	5'-CAAGCAGAAGACGGCATAACGAGAT-AGATGTAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTACATCT
U_rev_77	5'-CAAGCAGAAGACGGCATAACGAGAT-AGCACCTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GAGGTGCT
U_rev_78	5'-CAAGCAGAAGACGGCATAACGAGAT-AGCCATGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCATGGCT
U_rev_79	5'-CAAGCAGAAGACGGCATAACGAGAT-AGGTAAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTTAGCCT
U_rev_80	5'-CAAGCAGAAGACGGCATAACGAGAT-ATAGCGAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTGCTAT
U_rev_81	5'-CAAGCAGAAGACGGCATAACGAGAT-ATCATTCC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GGAAATGAT
U_rev_82	5'-CAAGCAGAAGACGGCATAACGAGAT-ATTGGCTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GAGCCAAT
U_rev_83	5'-CAAGCAGAAGACGGCATAACGAGAT-CAAGGAGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCTCCTTG
U_rev_84	5'-CAAGCAGAAGACGGCATAACGAGAT-CACCTTAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTAAGGTG
U_rev_85	5'-CAAGCAGAAGACGGCATAACGAGAT-CCATCTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GAGGATGG
U_rev_86	5'-CAAGCAGAAGACGGCATAACGAGAT-CCGACAAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTTGTGCG
U_rev_87	5'-CAAGCAGAAGACGGCATAACGAGAT-CCTAATCC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GGATTAGG
U_rev_88	5'-CAAGCAGAAGACGGCATAACGAGAT-CCTCTATC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GATAGAGG
U_rev_89	5'-CAAGCAGAAGACGGCATAACGAGAT-CGACACAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTGTGTGCG
U_rev_90	5'-CAAGCAGAAGACGGCATAACGAGAT-CGGATTGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCAATCCG
U_rev_91	5'-CAAGCAGAAGACGGCATAACGAGAT-CTAAGGTC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GACCTTAG
U_rev_92	5'-CAAGCAGAAGACGGCATAACGAGAT-GAACAGGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCCTGTTC
U_rev_93	5'-CAAGCAGAAGACGGCATAACGAGAT-GACAGTGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCACGTGC
U_rev_94	5'-CAAGCAGAAGACGGCATAACGAGAT-GAGTTAGC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GCTAACTC
U_rev_95	5'-CAAGCAGAAGACGGCATAACGAGAT-GATGAATC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GATTCATC
U_rev_96	5'-CAAGCAGAAGACGGCATAACGAGAT-GCCAAGAC-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	GTCTTGGC

**(B) Illumina sequencing reaction**

ID	Sequence	Barcode
Read_1	5'-ACACTCTTCCCTACACGACGCTCTTCCGATCTCTG	NA
Read_2	5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAC	NA