Supplementary Figure 1

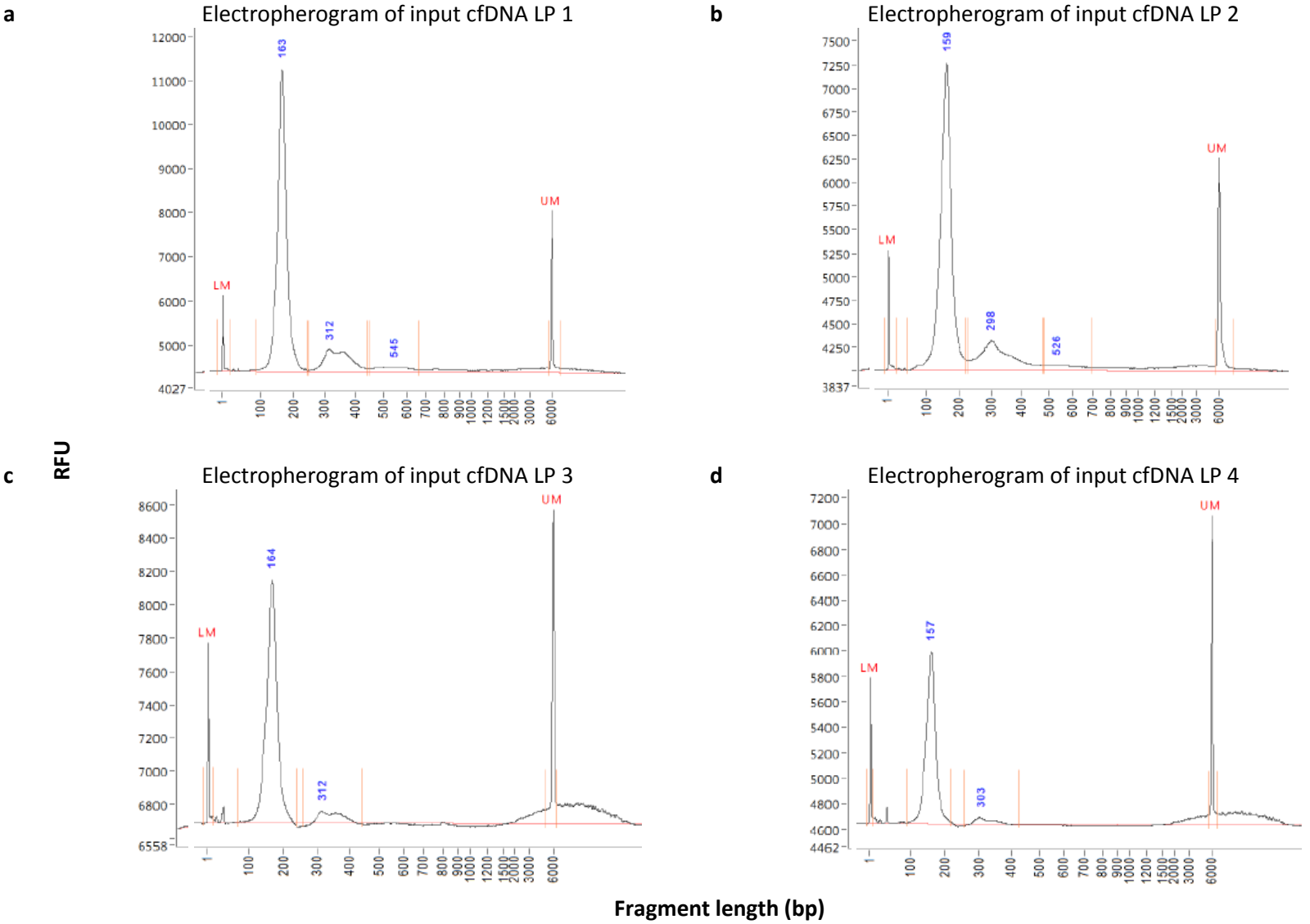


FIG S1: Electropherograms of the input cfDNA samples of four patients with hepatocellular carcinoma, used for cf-RRBS method development. Electropherograms are generated by the FEMTO Pulse (AATI). (a) Liver patient 1 (LP1), (b) LP 2, (c) LP 3 and (d) LP 4. Peak around 160bp is a mononucleosomal DNA fragment, peak around 300bp is a dinucleosomal fragment, peak around 500bp is a trinucleosomal fragment; high molecular weight DNA runs broadly above 1500 bp. RFU = relative fluorescence units. LM =lower marker, UM = upper marker.

Supplementary Figure 2

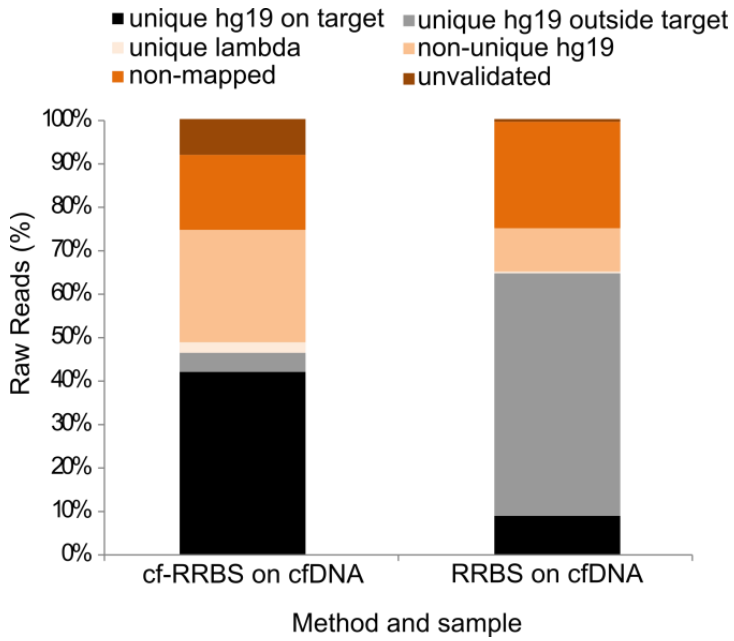


Fig S2: Read mapping characteristics of the cf-RRBS method versus the RRBS method on the same cfDNA sample. About 7 times more of raw reads originate from MspI/MspI-fragments when performing cf-RRBS on cfDNA, as compared to RRBS on cfDNA (allowing ± 1 order of magnitude reduced sequencing depth for RRBS).The bar graph shows the percentage of raw reads which are invalid (shorter than 20 bp and Phred score lower than 20), unmapped to the human reference genome (hg19), not uniquely mapped, mapped to the phage lambda genome, mapped to the hg19 but outside the MspI/MspI-target (20-165 bp) or mapped to the hg19 on the MspI/MspI-target.

Supplementary Figure 3

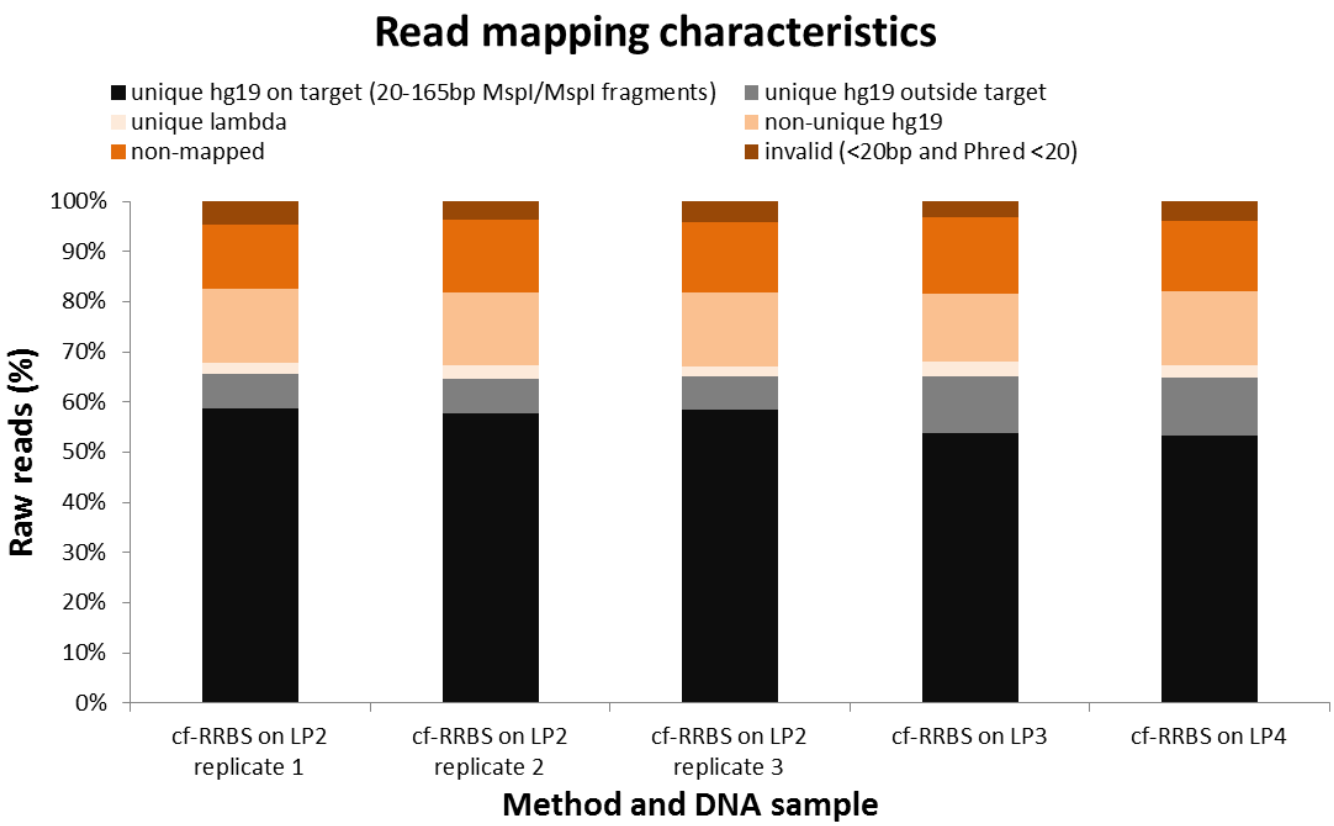


Fig S3: Read mapping characteristics of the cf-RRBS method. The mapping performance of the three technical cf-RRBS replicates on liver patient 2 (LP2) and the cf-RRBS performed on liver patient 3 and 4 (LP3 and LP4), is visualized by a bar graph showing the percentage of raw reads which are invalid (shorter than 20 bp and Phred score lower than 20), unmapped to the human reference genome (hg19), not uniquely mapped, mapped to the lambda genome, mapped to the hg19 but outside the single-nucleosomal winding MspI/MspI-target (20-165 bp) and mapped to the hg19 on the MspI/MspI-target.

Supplementary Figure 4

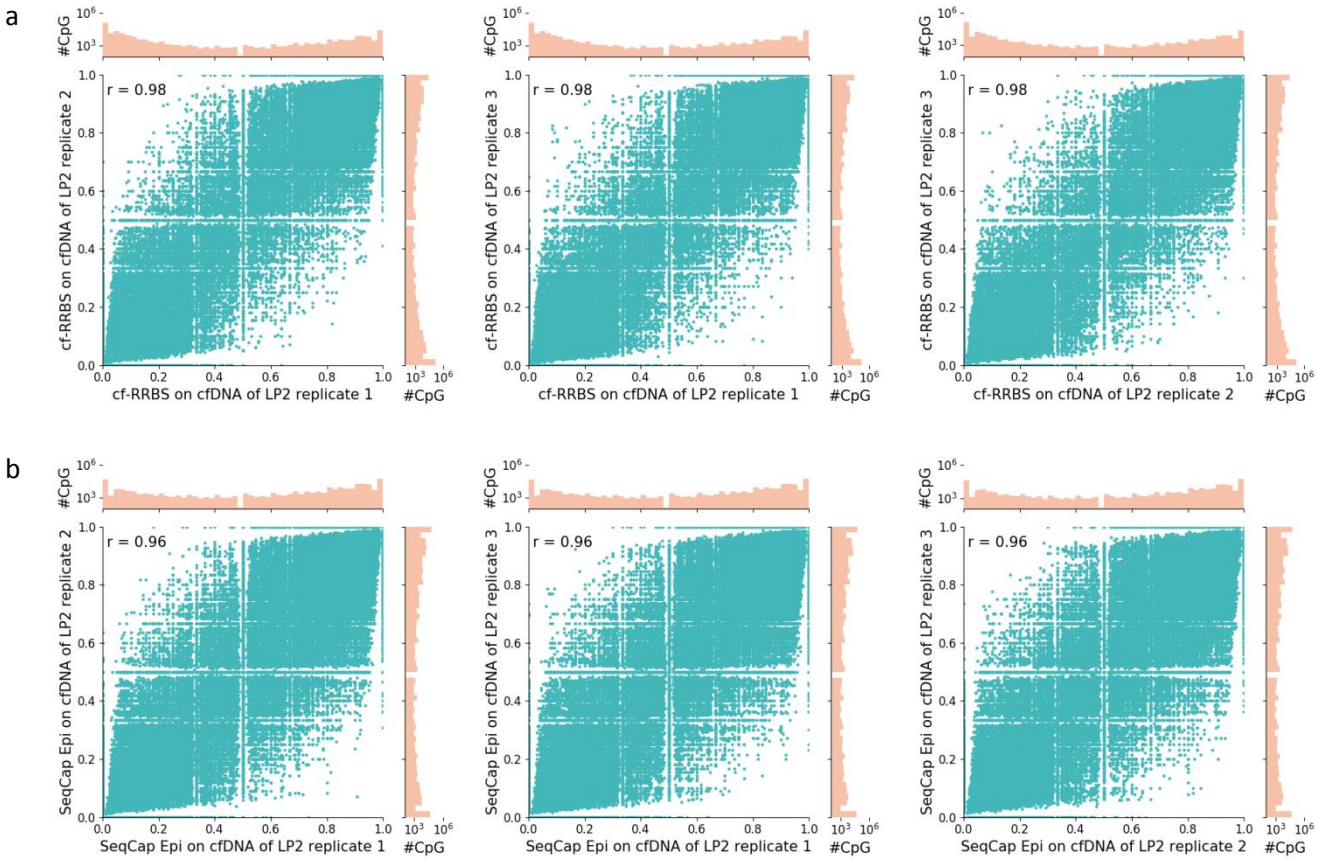


FIG S4: cf-RRBS and SeqCap Epi methods both yield highly replicable CpG methylation calls. Pairwise correlation plots of CpGs covered >=10X in all three cf-RRBS or SeqCap Epi technical replicates on cfDNA of liver patient 2 (LP2). Histograms show the amount of CpGs at certain methylation status. Pearson r-value is also given.

Supplementary Figure 5

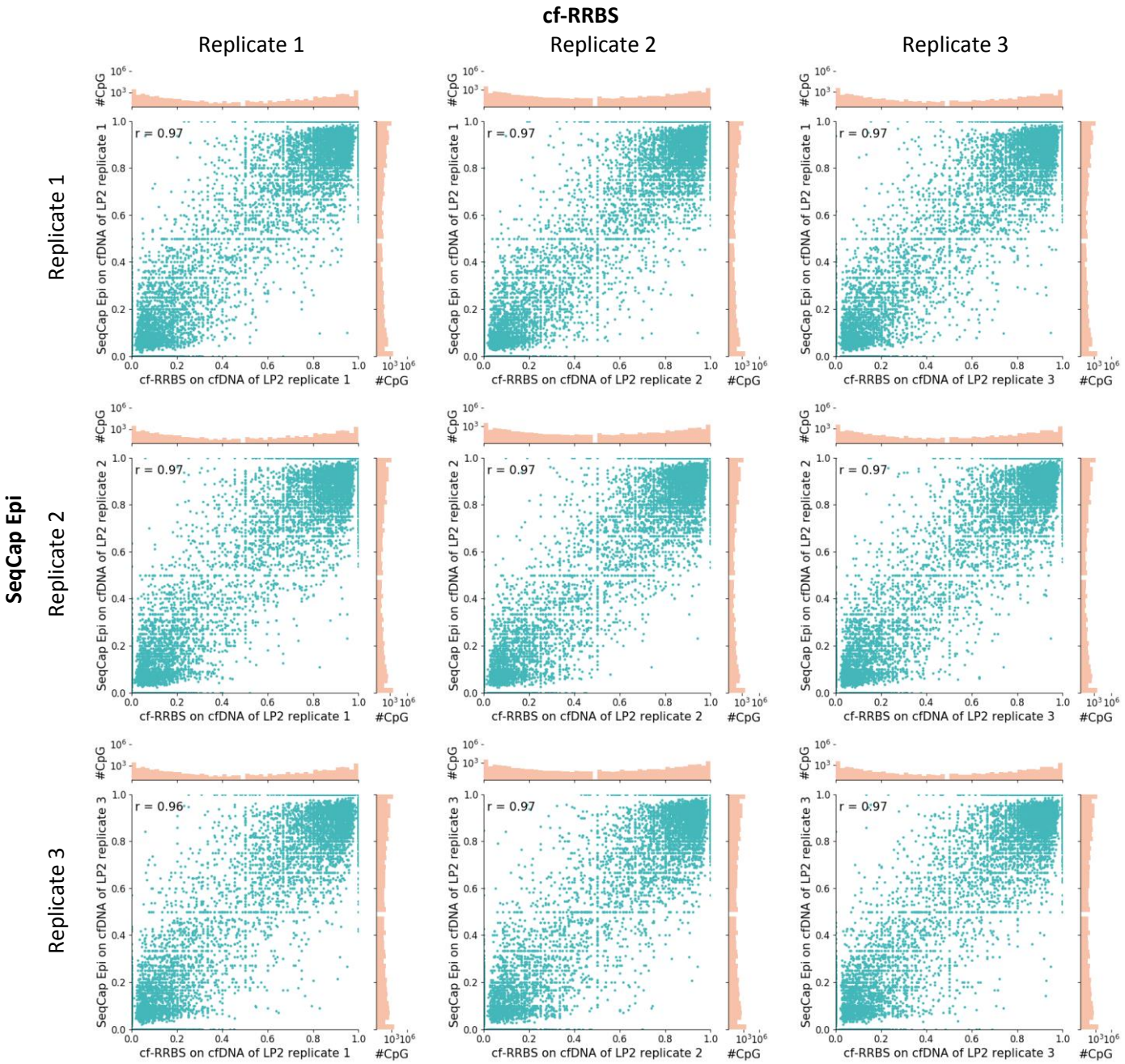
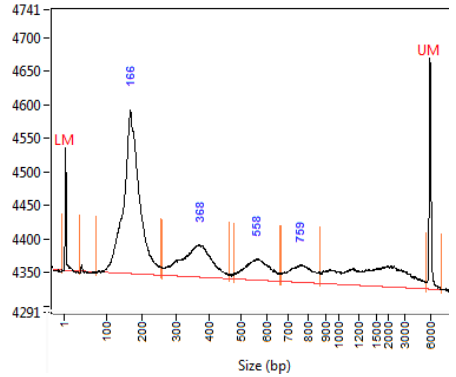


FIG S5: Across replicate measurements cf-RRBS and SeqCap Epi yield highly correlated methylation calls on CpGs that are assayed by both methods. Pairwise correlation plots of CpGs covered >=10X in all three cf-RRBS and all three SeqCap Epi technical replicates on cfDNA of liver patient 2 (LP2). Histograms show the amount of CpGs at certain methylation status. Pearson r-value is also given.

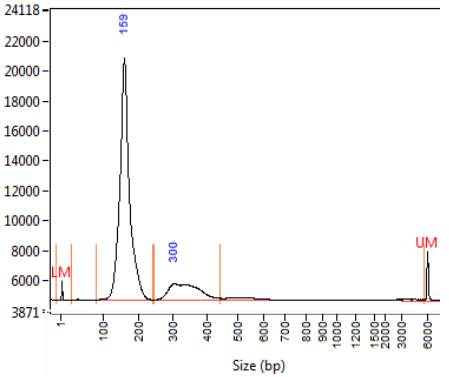
Supplementary Figure 6

Fig S6: Electropherograms of the input cfDNA samples of ten neuroblastoma patients. Electropherograms are generated by the FEMTO Pulse (AATI). RFU = relative fluorescence units. LM =lower marker, UM = upper marker. Peak around 160bp is a mononucleosomal DNA fragment, peak around 300bp is a dinucleosomal fragment, peak around 500bp is a trinucleosomal fragment, etcetera. NBL1 and 2 is serum cfDNA, NBL3-10 is plasma cfDNA.

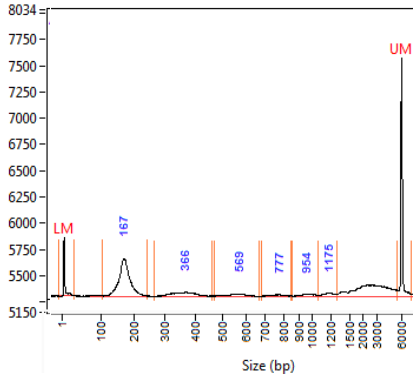
Electropherogram of input cfDNA NBL1



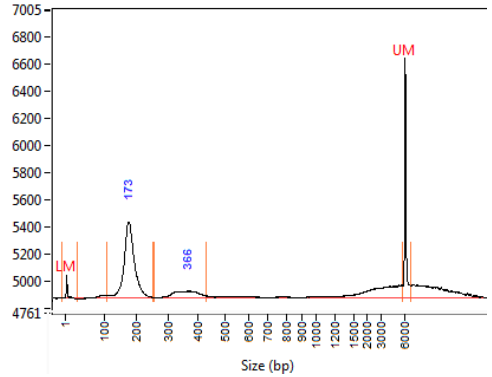
Electropherogram of input cfDNA NBL2



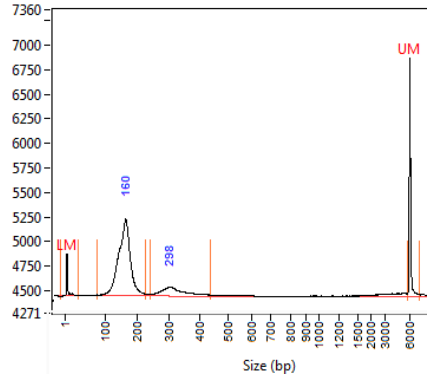
Electropherogram of input cfDNA NBL3 (CFD 1600812)



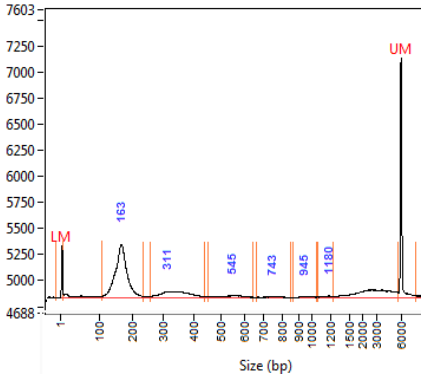
Electropherogram of input cfDNA NBL4 (CFD 1600418)



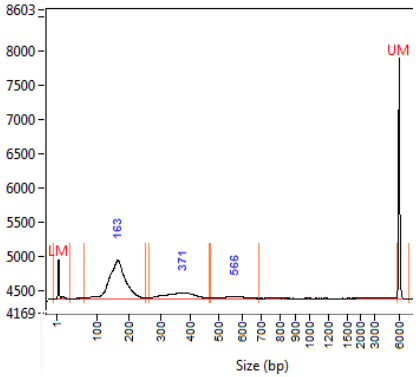
Electropherogram of input cfDNA NBL5 (CFD 1600417)



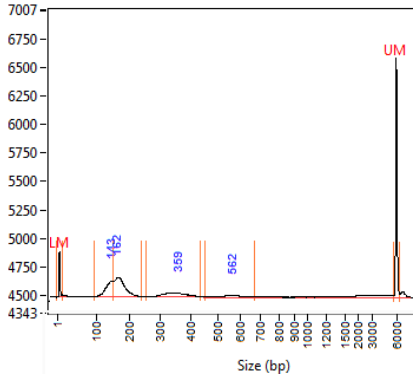
Electropherogram of input cfDNA NBL6 (CFD 1601067)



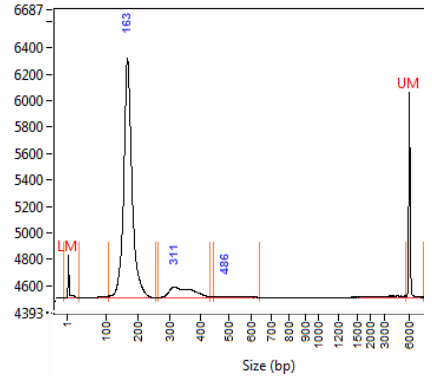
Electropherogram of input cfDNA NBL7 (CFD 1601214)



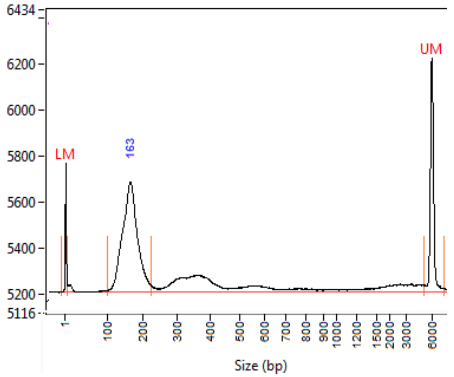
Electropherogram of input cfDNA NBL8 (CFD 1601224)



Electropherogram of input cfDNA NBL9 (CFD 1600374)



Electropherogram of input cfDNA NBL10 (CFD 1600416)



RFU

Fragment length (bp)