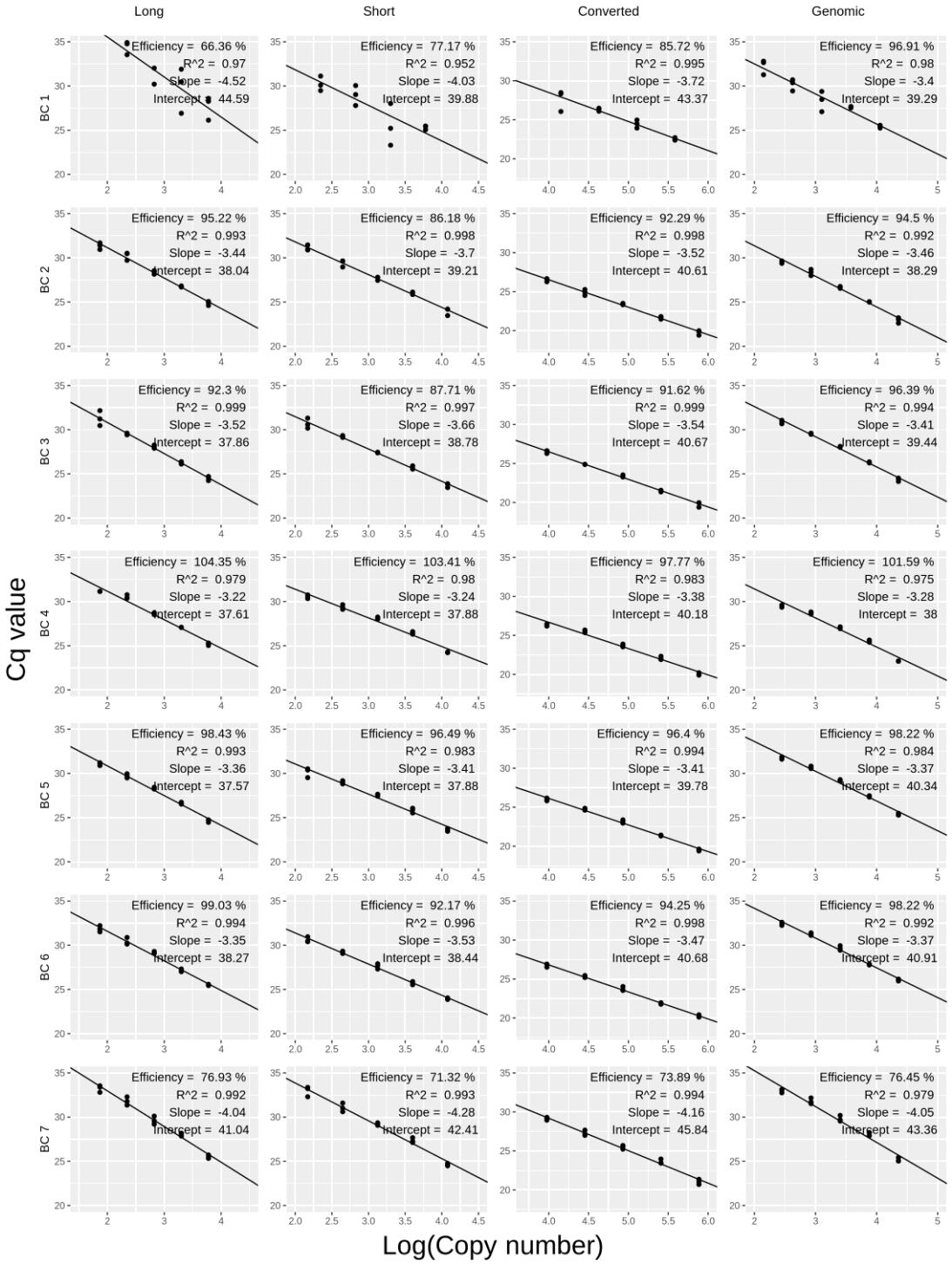
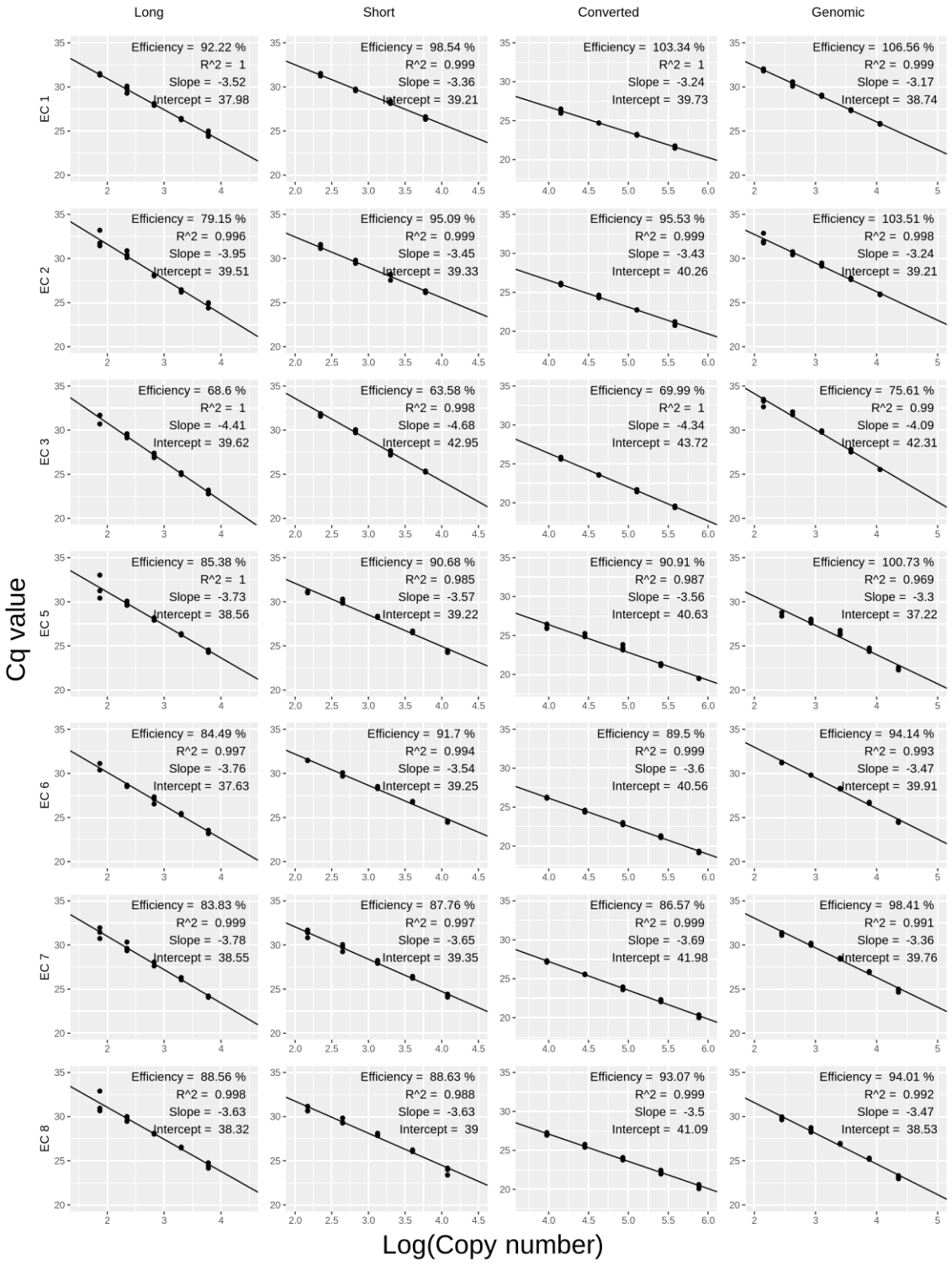
Additional file 1 – qBiCo standard curves and study design

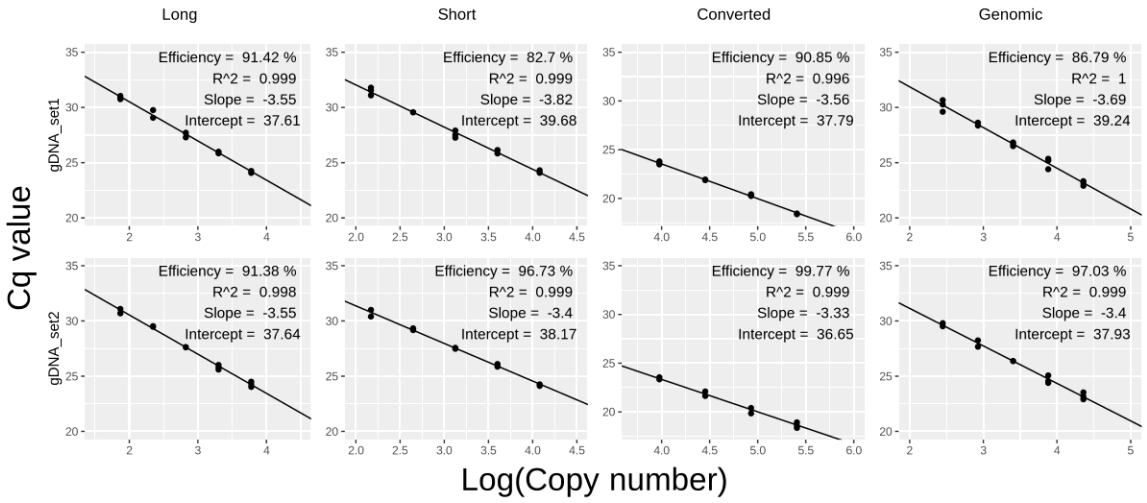
The qBiCo standard curve was created by using a DNA mixture containing synthetic DNA fragments as targets for each of the four assays. The copy number of each synthetic DNA fragment in the first standard of each qBiCo run can be seen in supplementary table 4. The four consecutive standards were created by 3-fold serial dilution in TE buffer. Supplementary Figure 1 and 2 show the standard curves of each separate conversion experiment for the bisulfite and enzymatic conversions of 100 ng gDNA samples. Supplementary Figure 3 shows the qBiCo standard curves for the set of 22 10 ng samples. The summary statistics of each qBiCo assay fit can be seen in Supplementary Table 1, Supplementary Table 2 and Supplementary Table 3. For an overview of the study design, indicating which samples were converted for which developmental validation parameter see Supplementary Table 5.



Supplementary Figure 1. qBiCo standard curves of the four assays per BC conversion experiment. Each column indicates the qBiCo assay and each row indicates a conversion experiment.



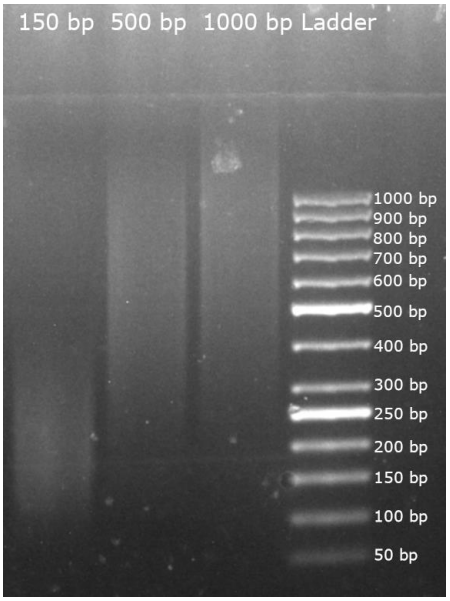
Supplementary Figure 2. qBiCo standard curves of the four assays per EC conversion experiment. Each column indicates the qBiCo assay and each row indicates a conversion experiment.



Supplementary Figure 3. qBiCo standard curves of the four assays per qBiCo assay for the BC and EC conversion of 22 10 ng gDNA samples. Each column indicates the qBiCo assay and each row indicates a conversion experiment.

Sonication assessment

To test the robustness of the conversion methods, conversions with degraded DNA input were performed. Genomic DNA samples were sheared to ~150 bp, ~500 bp and ~1000 bp by sonication with the Covaris S220 instrument and E220 intensifier in 55 µl AFA Fiber Snap-Cap microTUBES (Covaris). To assess this degradation, the fragment lengths were confirmed by 1 \% agarose gel electrophoresis (Supplementary Figure 4). The incremental fragmentation of all three sonication levels can be seen. The obtained fragment length ranges are as expected.



Supplementary Figure 4. Degradation assessment of sonicated samples on a 1% agarose gel. Genomic DNA samples were sheared to ~150 bp, ~500 bp and ~1000 bp by sonication

Supplementary Table 1. qBiCo standard curve parameters of the four assays per BC conversion experiment.

Experiment	Assay	Fluorophore	PCR efficiency (%)	r_squared	intercept	slope
BC_1	Long	FAM	66.4	0.970	44.6	-4.52
	Short	HEX	77.2	0.952	39.9	-4.03
	Converted	TEX615	85.7	0.995	43.4	-3.72
	Genomic	Cy5	96.9	0.980	39.3	-3.40
BC_2	Long	FAM	95.2	0.993	38.0	-3.44
	Short	HEX	86.2	0.998	39.2	-3.70
	Converted	TEX615	92.3	0.998	40.6	-3.52
	Genomic	Cy5	94.5	0.992	38.3	-3.46
BC_3	Long	FAM	92.3	0.999	37.9	-3.52
	Short	HEX	87.7	0.997	38.8	-3.66
	Converted	TEX615	91.6	0.999	40.7	-3.54
	Genomic	Cy5	96.4	0.994	39.4	-3.41
BC_4	Long	FAM	104.3	0.979	37.6	-3.22
	Short	HEX	103.4	0.980	37.9	-3.24
	Converted	TEX615	97.8	0.983	40.2	-3.38
	Genomic	Cy5	101.6	0.975	38.0	-3.28
BC_5	Long	FAM	98.4	0.993	37.6	-3.36
	Short	HEX	96.5	0.983	37.9	-3.41
	Converted	TEX615	96.4	0.994	39.8	-3.41
	Genomic	Cy5	98.2	0.984	40.3	-3.37
BC_6	Long	FAM	99.0	0.994	38.3	-3.35
	Short	HEX	92.2	0.996	38.4	-3.53
	Converted	TEX615	94.2	0.998	40.7	-3.47
	Genomic	Cy5	98.2	0.992	40.9	-3.37
BC_7	Long	FAM	76.9	0.992	41.0	-4.04
	Short	HEX	71.3	0.993	42.4	-4.28
	Converted	TEX615	73.9	0.994	45.8	-4.16
	Genomic	Cy5	76.5	0.979	43.4	-4.05

Supplementary Table 2. qBiCo standard curve parameters of the four assays per EC conversion experiment.

Experiment	Assay	Fluorophore	PCR efficiency (%)	r_squared	intercept	slope
EC_1	Long	FAM	92.2	1.000	38.0	-3.52
	Short	HEX	98.5	0.999	39.2	-3.36
	Converted	TEX615	103.3	1.000	39.7	-3.24
	Genomic	Cy5	106.6	0.999	38.7	-3.17
EC_2	Long	FAM	79.2	0.996	39.5	-3.95
	Short	HEX	95.1	0.999	39.3	-3.45
	Converted	TEX615	95.5	0.999	40.3	-3.43
	Genomic	Cy5	103.5	0.998	39.2	-3.24
EC_3	Long	FAM	68.6	1.000	39.6	-4.41
	Short	HEX	63.6	0.998	42.9	-4.68
	Converted	TEX615	70.0	1.000	43.7	-4.34
	Genomic	Cy5	75.6	0.990	42.3	-4.09
EC_5	Long	FAM	85.4	1.000	38.6	-3.73
	Short	HEX	90.7	0.985	39.2	-3.57
	Converted	TEX615	90.9	0.987	40.6	-3.56
	Genomic	Cy5	100.7	0.969	37.2	-3.30
EC_6	Long	FAM	84.5	0.997	37.6	-3.76
	Short	HEX	91.7	0.994	39.3	-3.54
	Converted	TEX615	89.5	0.999	40.6	-3.60
	Genomic	Cy5	94.1	0.993	39.9	-3.47
EC_7	Long	FAM	83.8	0.999	38.6	-3.78
	Short	HEX	87.8	0.997	39.4	-3.65
	Converted	TEX615	86.6	0.999	42.0	-3.69
	Genomic	Cy5	98.4	0.991	39.8	-3.36
EC_8	Long	FAM	88.6	0.998	38.3	-3.63
	Short	HEX	88.6	0.988	39.0	-3.63
	Converted	TEX615	93.1	0.999	41.1	-3.50
	Genomic	Cy5	94.0	0.992	38.5	-3.47

Supplementary Table 3. qBiCo standard curve parameters of the four assays per qBiCo assay for the BC and EC conversion of 22 10 ng gDNA samples.

Experiment	Assay	Fluorophore	PCR efficiency (%)	r_squared	intercept	slope
Set_1	Long	FAM	91.4	0.999	37.6	-3.55
	Short	HEX	82.7	0.999	39.7	-3.82
	Converted	TEX615	90.8	0.996	37.8	-3.56
	Genomic	Cy5	86.8	1.000	39.2	-3.69
Set_2	Long	FAM	91.4	0.998	37.6	-3.55
	Short	HEX	96.7	0.999	38.2	-3.40
	Converted	TEX615	99.8	0.999	36.6	-3.33
	Genomic	Cy5	97.0	0.999	37.9	-3.40

Supplementary Table 4. qBiCo gBlock amount per assay for each conversion experiment.

Experiment	Copies_Long	Copies_Short	Copies_Converted	Copies_Genomic
BC_1	6000	6000	384000	11400
BC_2	6000	12000	768000	22800
BC_3	6000	12000	768000	22800
BC_4	6000	12000	768000	22800
BC_5	6000	12000	768000	22800
BC_6	6000	12000	768000	22800
BC_7	6000	12000	768000	22800
EC_1	6000	6000	384000	11400
EC_2	6000	6000	384000	11400
EC_3	6000	6000	384000	11400
EC_5	6000	12000	768000	22800
EC_6	6000	12000	768000	22800
EC_7	6000	12000	768000	22800
EC_8	6000	12000	768000	22800
Set_1	6000	12000	768000	22800
Set_2	6000	12000	768000	22800

Supplementary table 5. Study design overview. Conversions performed per developmental validation parameter. Three gDNA samples were used in this study.

Parameter	Description	Conversions per gDNA sample
Repeatability	DNA amount (100,20,10,5,1 ng)	3 for 100 ng and lowest repeatable amount (BC: 5 ng, EC: 10 ng); 2 for other amounts
Reproducibility and Sensitivity	DNA amount (100,20,10,5,1 ng)	5 for 100 ng and lowest repeatable amount (BC: 5 ng, EC: 10 ng); 2 for other amounts
Recovery: Elution	1 and 5 minutes binding time, double elution (BC only)	1
Incubation time	BC (12 and 20 hours); EC (TET: 1.5 and APOBEC 4 hours)	1
Artificial methylation	100, 75, 50, 25 and 0 % methylation	2 (artificial DNA)
Robustness: UV	0, 30, 60 and 120 seconds UV	1
Robustness: Sonication	150, 500, 1000 bp and non sonicated control	1
Inhibition: Hematin	0, 100 and 200 µM and solute control	1
Inhibition: Proteinase	0, 0.05, 0.1 and 0.2 mAU	1
Stability: Storage	0 and 4 weeks	1
Stability: Freeze-thaw cycles	0, 5 and 10 FT-cycles	1

Enzymatic conversion trial

The effect of shearing the gDNA to 300 bp by sonication prior to enzymatic conversion was tested. With the denaturation adjustment to the enzymatic conversion protocol, it was possible to perform enzymatic conversion without fragmentation. Recovery and conversion efficiency were higher for the samples which were not fragmented. As expected the fragmented samples showed a higher fragmentation level than the non-fragmented samples.

Supplementary Table 6. Effect of fragmentation on enzymatic conversion performance measured by qBiCo.

Duplicate	Fragmentation	Input (ng)	Output (ng)	Recovery	Conversion efficiency	Fragmentation
1	-	106.4	69.8	66%	99.1%	2.19
2	-	106.4	56.7	53%	99.1%	2.16
1	300 bp Covaris	83.2	47.2	57%	97.8%	5.14
2	300 bp Covaris	83.2	38.9	47%	98.4%	6.23