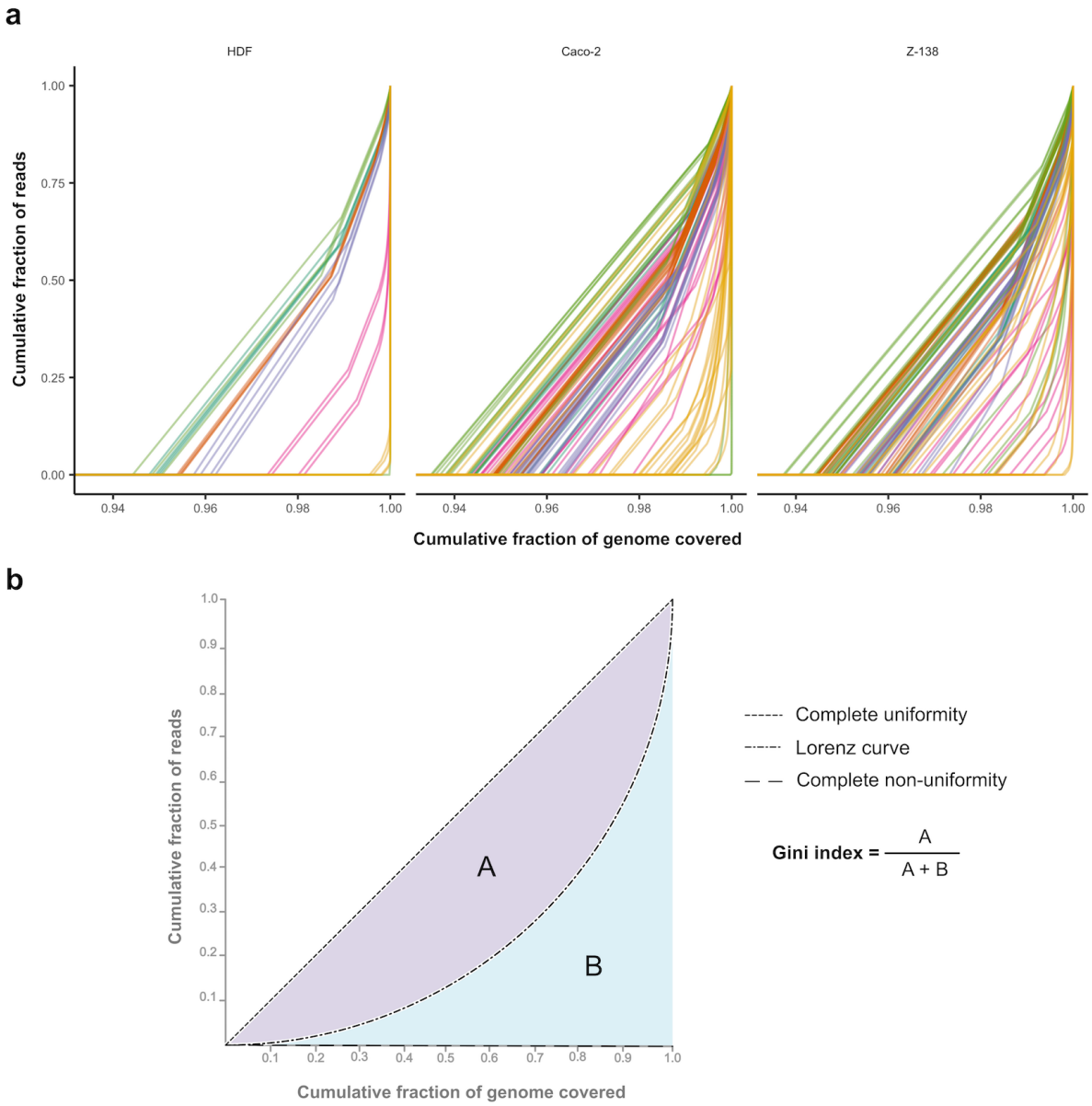
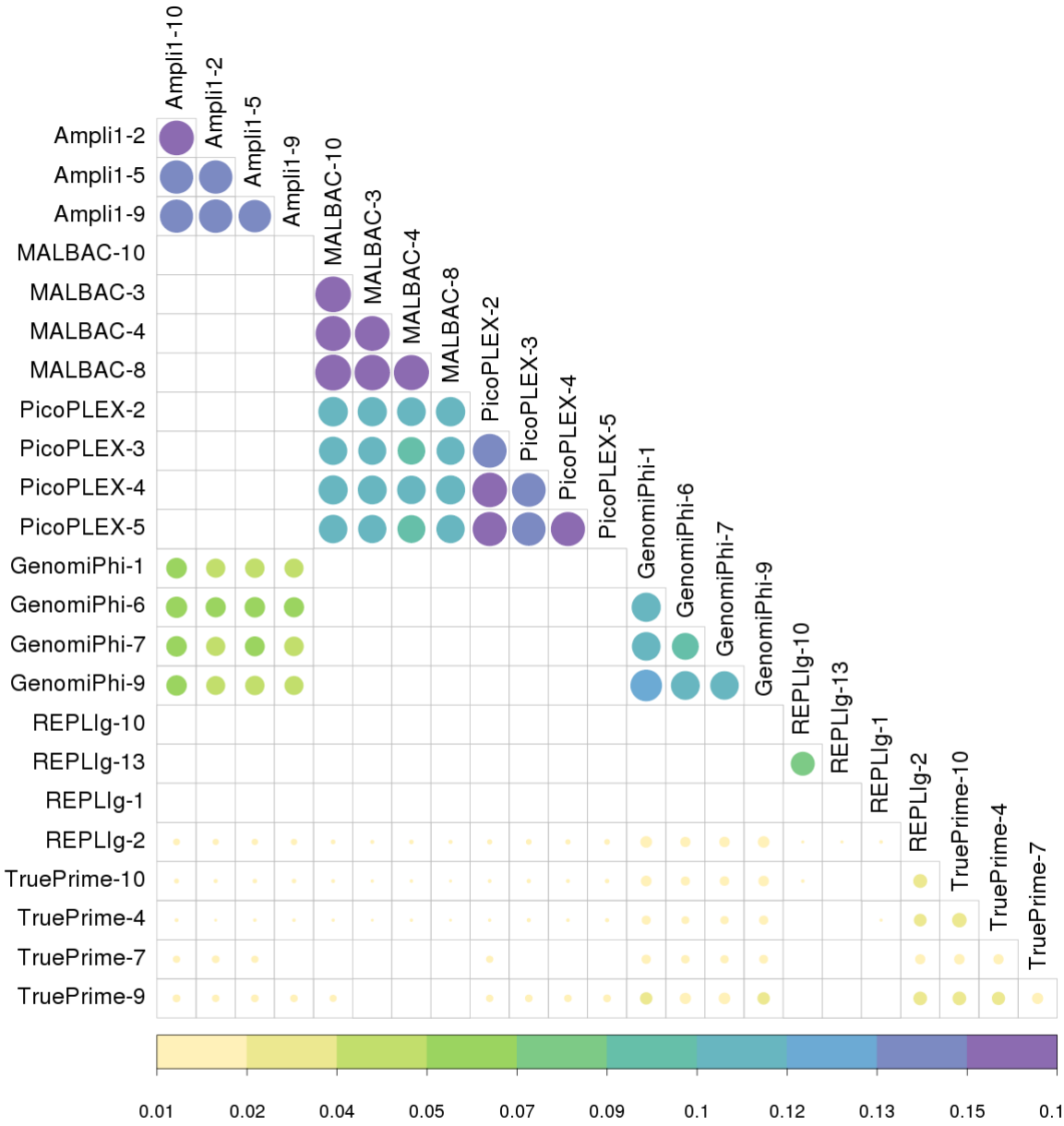
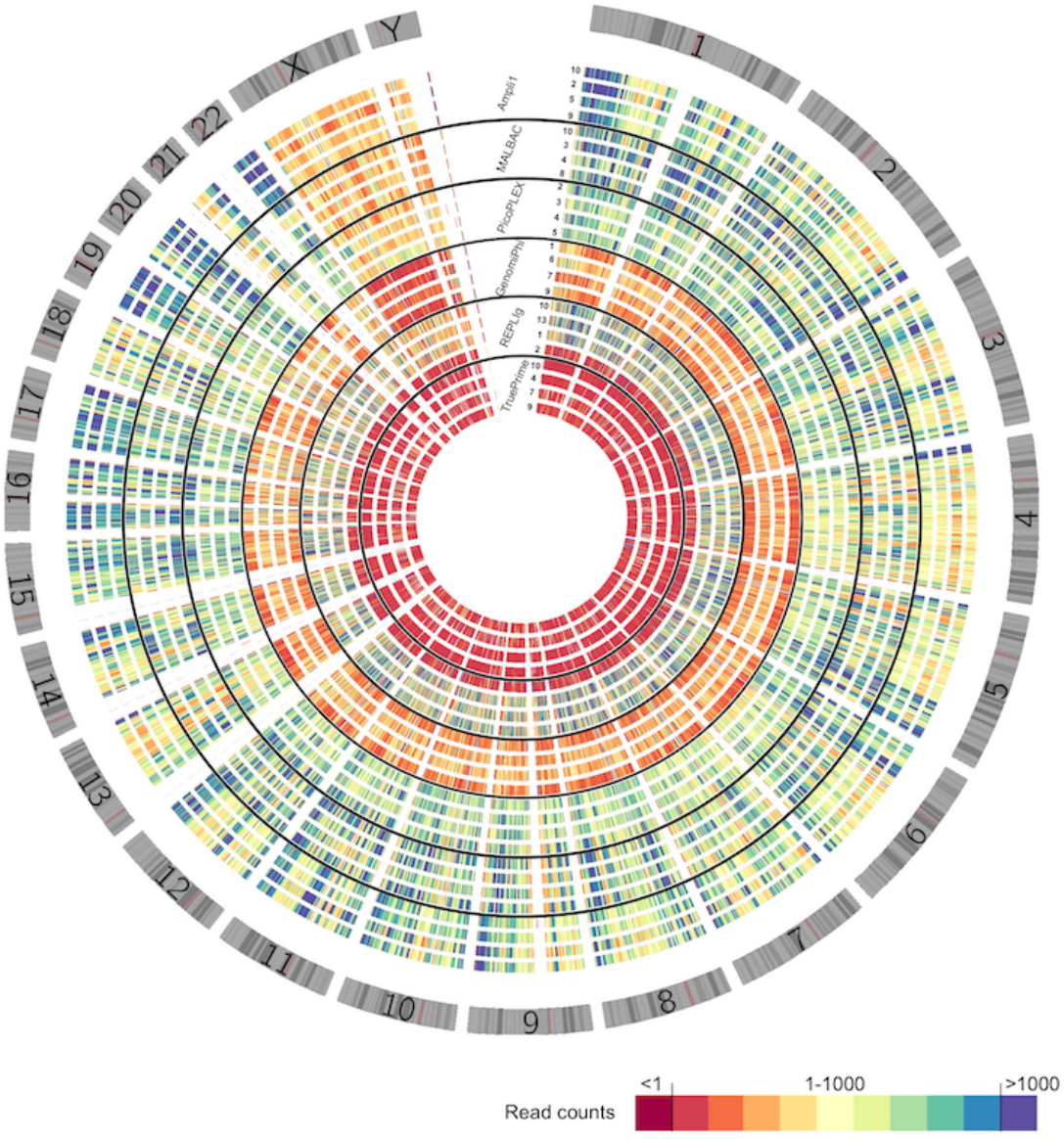


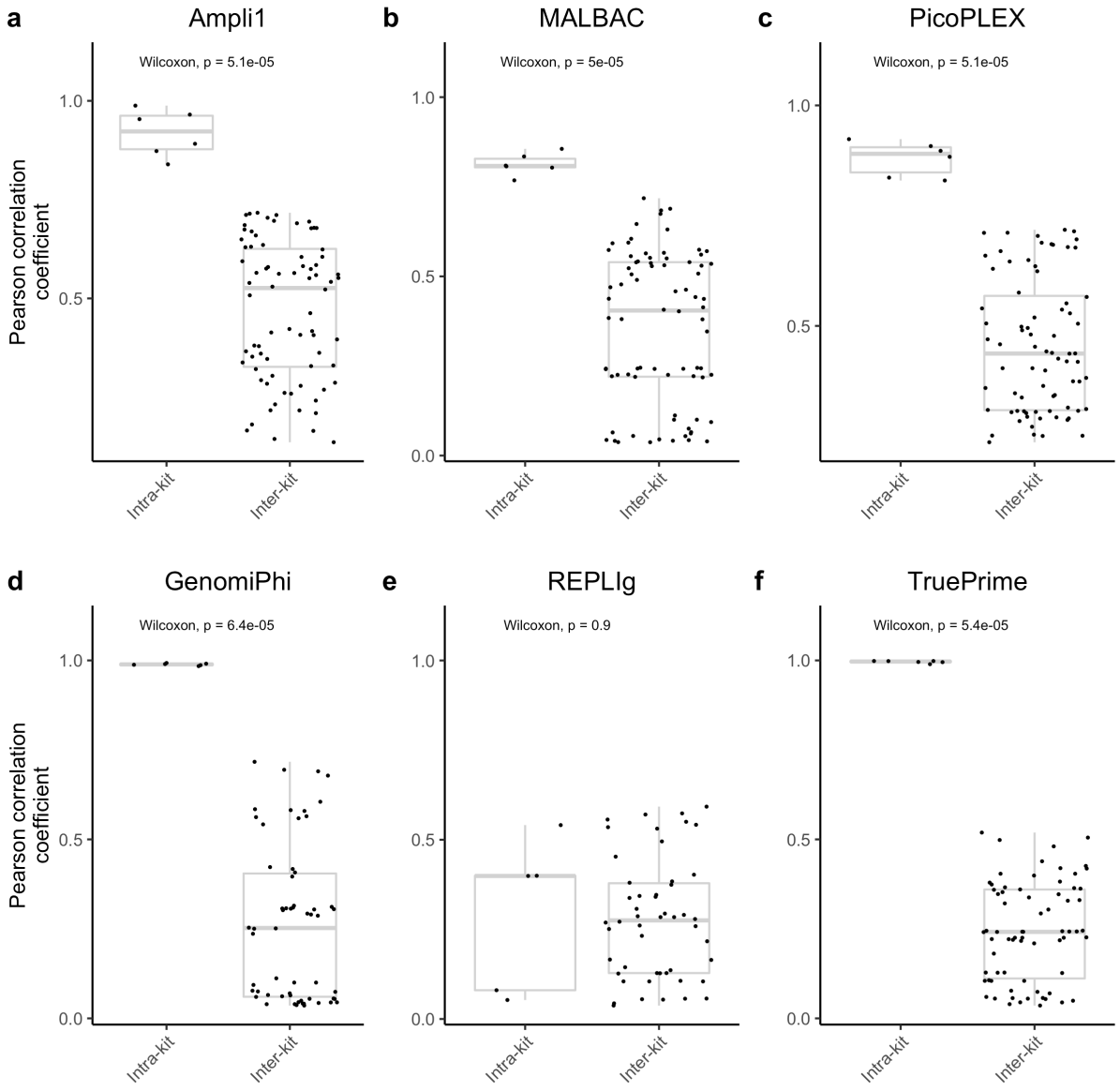
Supplementary Fig. 1 | Lorenz curves and Gini indexes. **a,** Lorenz curves of the 24, 104 and 102 single-cells of HDF, Caco-2 and Z-138, respectively. Colors of the curves match with the scWGA kit. Note that x axis has the right limit around 0.94 as Lorenz curves were constructed from low-pass sequencing data and most of the genome is uncovered. **b,** the Gini index summarizes the Lorenz curve and it is computed dividing the area contained between the diagonal and the Lorenz curve by the area comprised below the diagonal. High Gini indexes (up to 1) reflect high amplification bias. In all our figures we report 1-Gini index for easier interpretation.



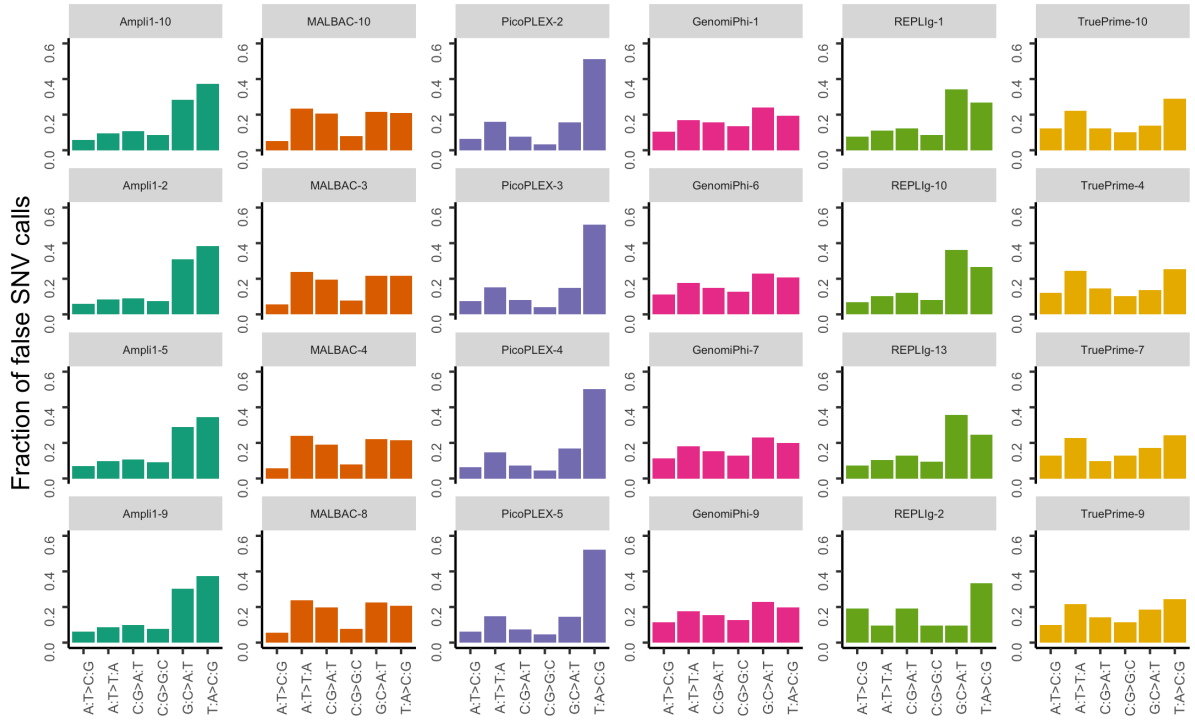
Supplementary Fig. 2 | Coverage Jaccard similarity coefficients between pairs of HDF single-cells. Jaccard coefficients were computed from the arrays of coverage presence/absence along the genome. The Jaccard index spans from 0 to 1 corresponding 1 to the maximum possible similarity, in which both single-cells compared show exactly the same coverage presence/absence pattern along the genome. Here, a maximum of 10% of the covered intervals for one single-cell are also covered in another one (max. jaccard index of 0.1). The highest percentages are recovered for the non-MDA kits as it is expected given the use of non-random primers and amplification chemistry based on PCR. We note also that MALBAC and PicoPLEX are more similar between them than compared to other scWGA kits, which could be explained by similarity of both approaches. Only significant Jaccard indexes after permutation are shown.



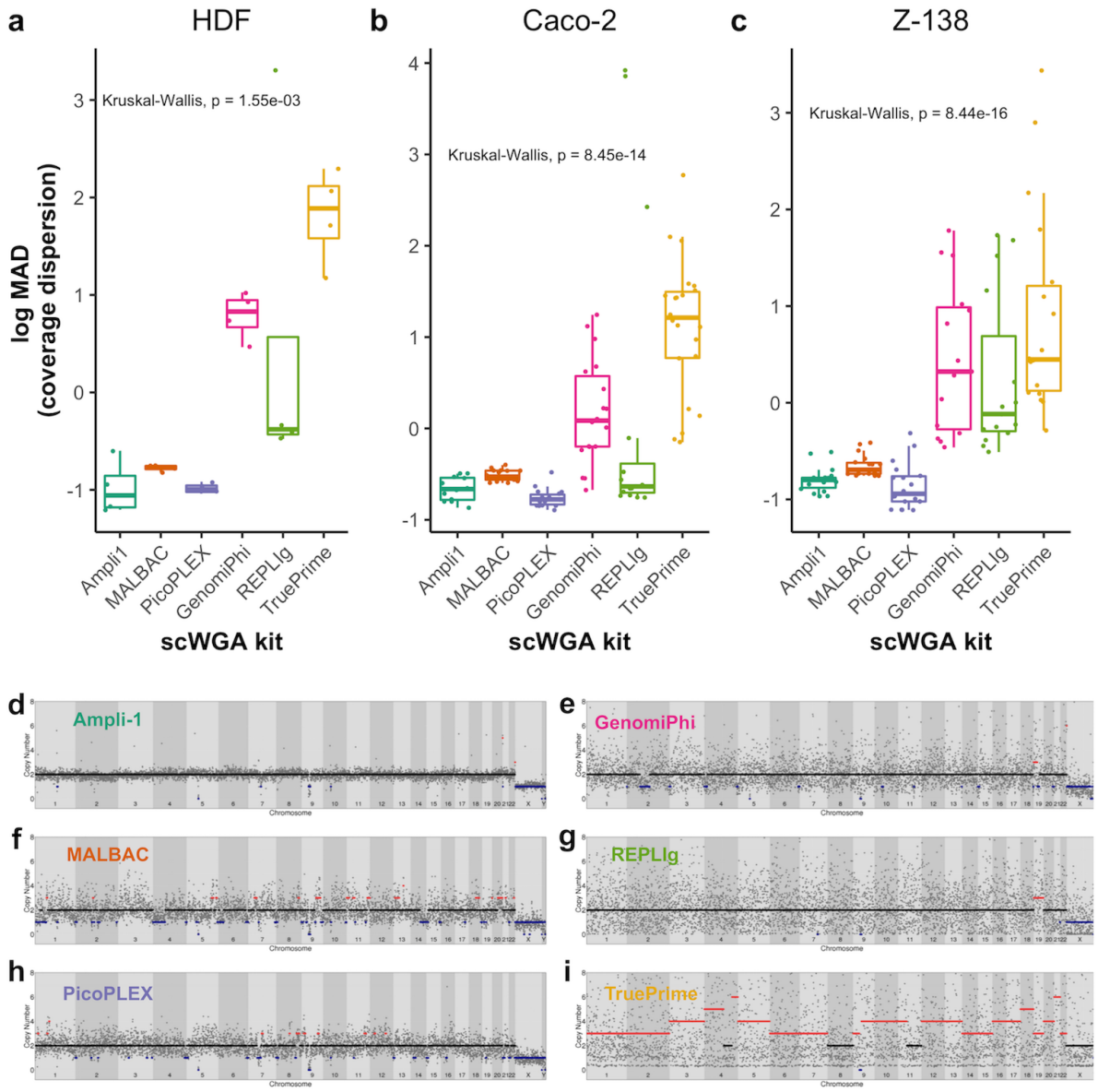
Supplementary Fig. 3 | Circos plot of read counts within 1Mb windows along the genome. The outer segments represent the chromosomes and each concentric heatmap corresponds with one HDF single-cell. Colors of the heatmaps represent read counts as specified in the legend. All files were downsampled to approximately the same number of reads before counting, however, coverages seem in average lower for TruePrime cells due to a high amount of reads mapping to the mitochondria (not shown). REPLIg-2 is the single-cell whose amplification failed also in all the other aspects.



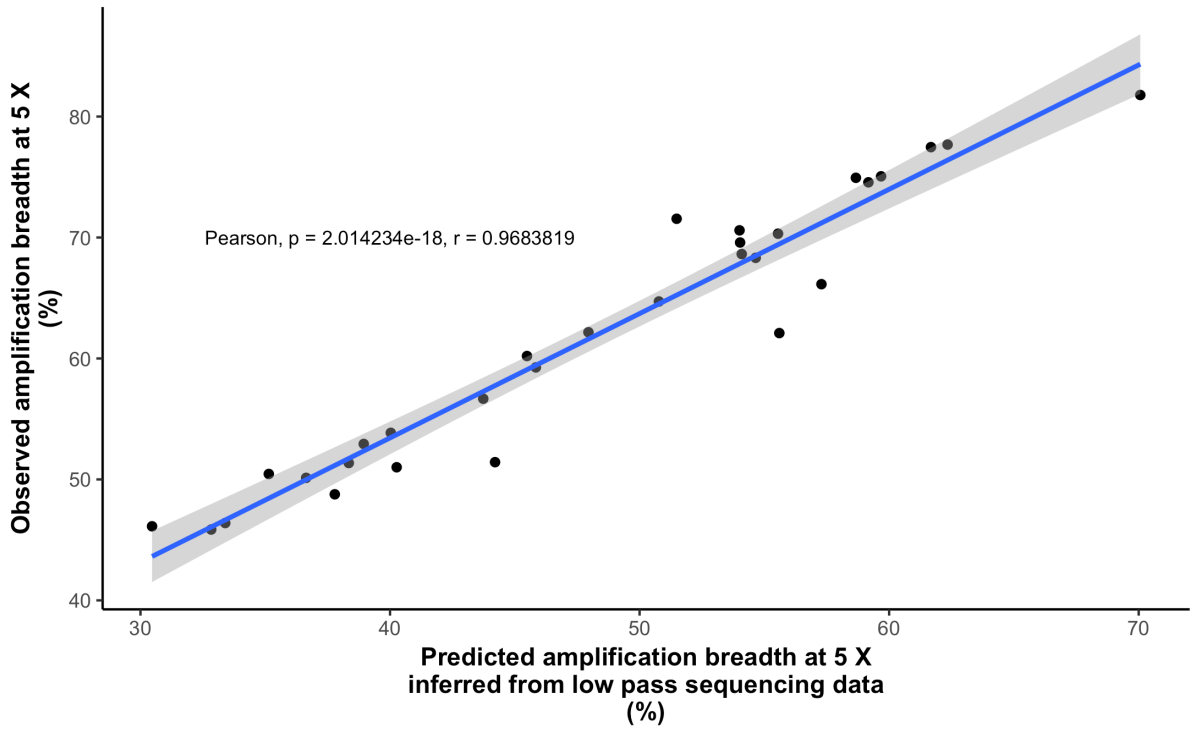
Supplementary Fig. 4 | Distribution of significant correlation coefficients within and among scWGA kits. Two cells amplified with the same scWGA kit (intra-kit) show a more similar read count pattern than any of these two cells compared to a single-cell amplified with a different scWGA kit (inter-kit), except for REPLIg.



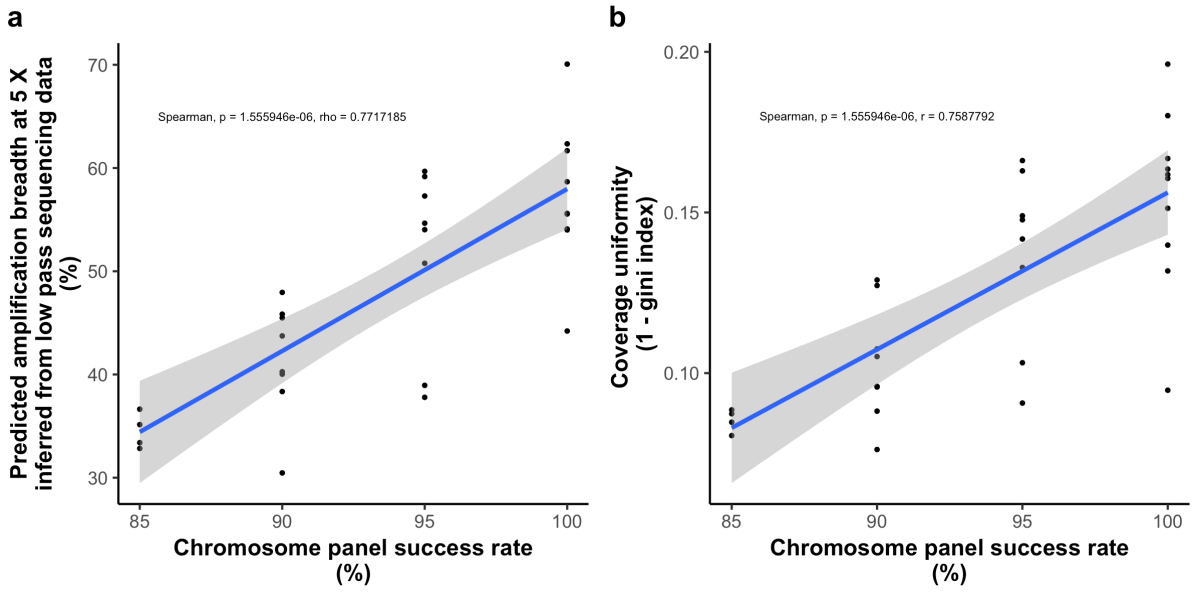
Supplementary Fig. 5 | HDF single-cell mutation signatures reconstructed from the false SNV calls. Each false SNV (according to the marginal calling procedure) was assigned to a transversion (A:T>C:G, A:T>T:A, C:G>A:T, C:G>A:T) or transition (G:C>A:T, T:A>C:G).



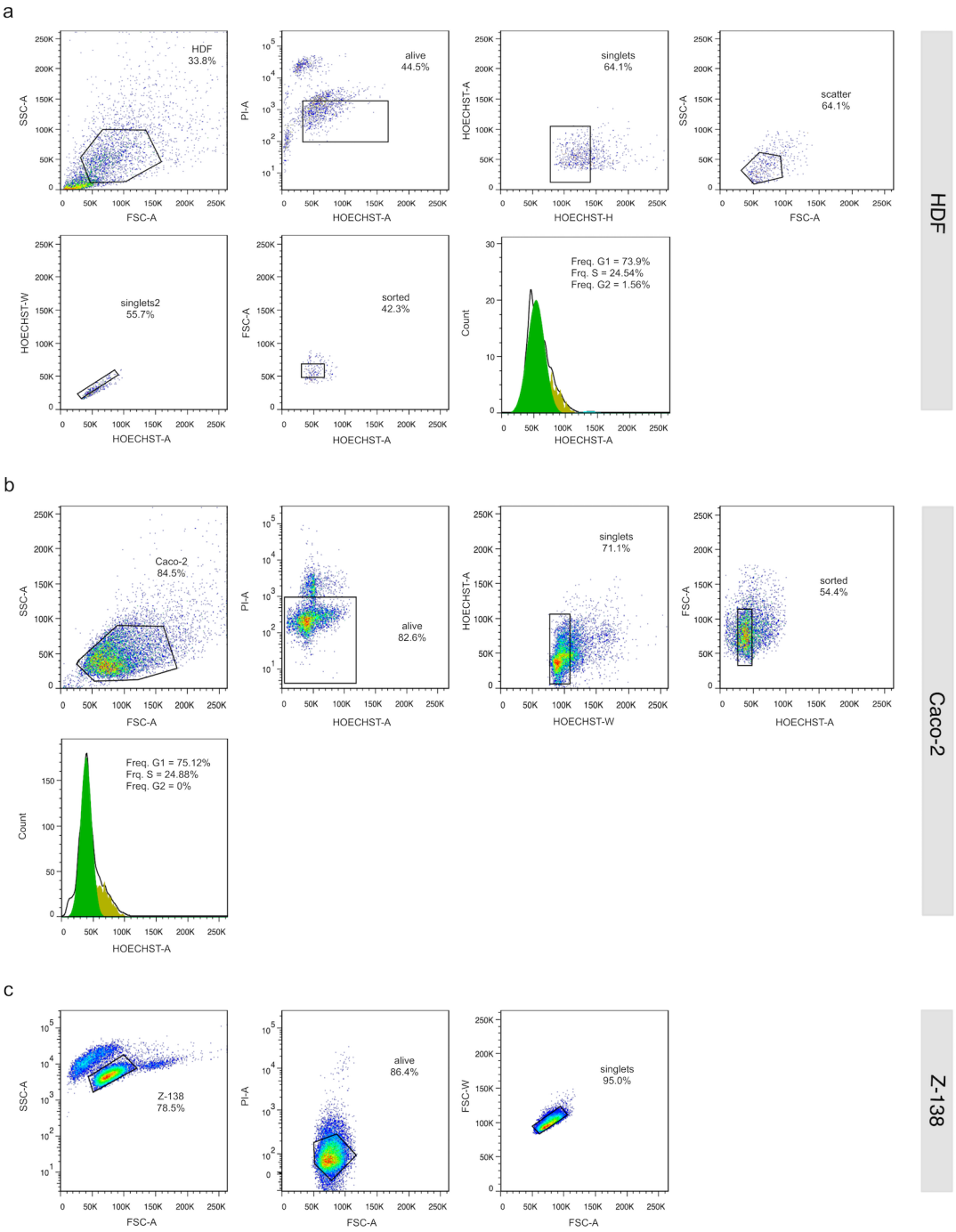
Supplementary Fig. 6 | Coverage dispersion for the three cell lines and representative copy-number profiles in HDF cells. **a-c**, Coverage dispersion (MAD) is a measure implemented in Ginkgo related with reliability of accurate copy-number calls and its resolution. Values are shown independently for each cell line. We have transformed the values to log scale to clearly distinguish among scWGA kits. **d-i,** Copy number profiles for six HDF cells arbitrarily chosen, one for each of the six scWGA kits evaluated. Non-MDA methods (**d,f,h**) show much less dispersion than MDA methods (**e,g,i**). Despite the dispersion in the read counts observed in these examples all methods recovered the expected diploid copy-number profile except TruePrime (**i**), which lead to erroneous copy number variant inferences. The HDF cell line was established from a male individual, which explains the presence of a single copy for both sex chromosomes.



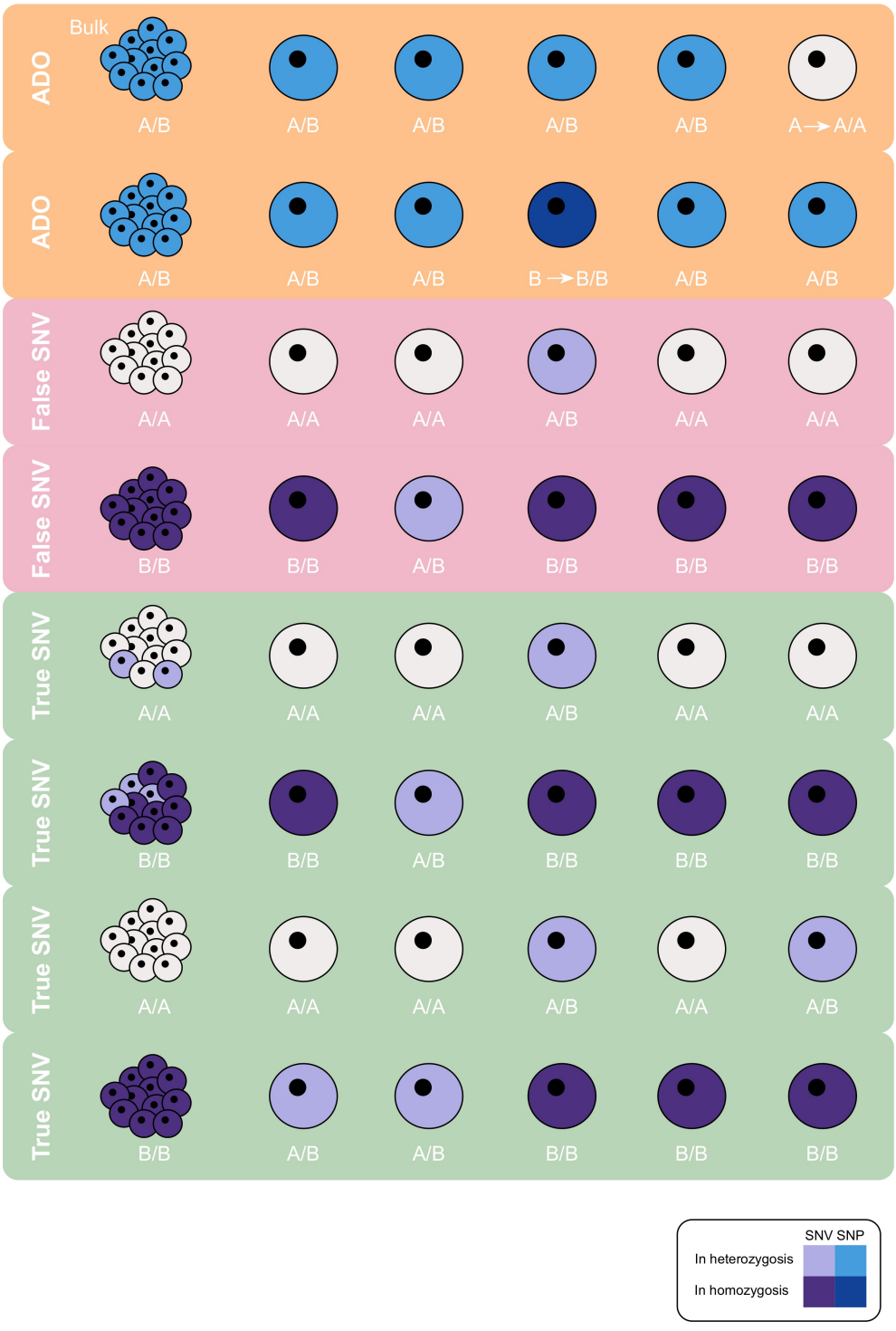
Supplementary Fig. 7 | Relationship between predicted and observed coverage breadth. Linear regression model where the observed coverage breadth at 5X (response variable) is predicted by Preseq *gc_extrap* using 0.1X data (explanatory variable). Values correspond to 30 single-cell libraries from a patient with Chronic Lymphocytic Leukemia (CLL) which were amplified with REPLIg.



Supplementary Fig. 8 | Relationship between the NGS/PCR amplification breadth estimates. a,b, Linear regression model where (**a**) the Preseq *gc extrap* breadth predicted at 5X, or (**b**) 1 minus the Gini index (response variables) is predicted by the percentage of positive PCR amplifications (20 markers in different chromosomes) (explanatory variable). Values correspond to 30 single-cell libraries from a patient with Chronic Lymphocytic Leukemia (CLL) which were amplified with REPLIg.



Supplementary Fig. 9 | Single-cell sorting strategies. a, HDF. We gated the HDF population based on size and complexity (FSC-A vs SSC-A). Then, we selected alive cells based on Hoechst and PI area signal (PI- cells) and removed cell aggregates in three steps, one based on Hoechst height and area signal, other based on size and complexity and the last one facing Hoechst area against width. Finally, we sorted cells in G0/G1 phase (Hoechst-A histogram). **b,** Caco-2. We gated the Caco-2 population based on its size and complexity (FSC-A vs SSC-A), then we selected alive cells based on Hoechst and PI area signal (PI- cells) and removed cell aggregates based on Hoechst area and width. Finally, we sorted cells in G0/G1 phase (Hoechst-A histogram). **c,** Z-138. We gated the Z-138 population based on its size and complexity (FSC-A vs SSC-A) and then based on the PI signal we selected alive cells (PI- cells). We removed aggregates based on size area and width (FSC-A vs FSC-W) and sorted single cells directly from this population. We performed these analyses with the FlowJo v7.6.2 (FlowJo, LLC, Ashland, OR, USA) software.



Supplementary Fig. 10 | Computation of ADO events and false SNV calls due to amplification errors. We counted an ADO event when an alternative allele present in the bulk was absent in a single cell. We only counted as false SNVs mutations in the single-cells that were not detected in the bulk or were called by Monovar in at least in two cells. As our sequencing coverage was low, we only considered sites covered by at least 6 reads in the single cells to reduce the effects of incomplete read sampling.

Supplementary Table 1 | Studies comparing scWGA kits. WES: whole-exome sequencing. WGS: whole-genome sequencing.

Reference	Number of kits compared	Number of cells	SCS method
Babayan et al. 2016	3	120	WES
Borgström et al. 2017	4	8	WES
Voet et al. 2013	2	8	WGS
Chen et al. 2014	2	6	WGS
de Bourcy et al. 2014	3	22	WGS
Ning et al. 2015	3	17	WGS
Hou et al. 2015	7	37	WGS
Huang et al. 2015	5	15	WGS
Li et al. 2015	3	3 and 15 pg of DNA with each WGA kit.	WGS
Deleye et al. 2015	2	6 and triplicates of amplification of 3 and 5 cells.	WGS
Deleye et al. 2016	1 TruePrime comparing with results from Deleye et al. 2015	3 and triplicates of 3 and 5 cells.	WGS
Deleye et al. 2017	4	12 and triplicates of amplifications of 3 and 5 cells	WGS
Biezuner et al. 2017	7	125	targeted sequencing
Deleye et al. 2018	4	14 groups of 3 cells	targeted sequencing

Supplementary Table 2 | **Single-cell WGA kits evaluated**. WGA approach: LM-PCR, Ligation-Mediated PCR; MDA, Multiple Displacement Amplification; MALBAC, Multiple Annealing and Looping Based Amplification Cycles. Lysis type: A, Alkaline; E, Enzymatic. Lysis tem.: Lysis temperature (ºC). Lysis vol.: lysis volume (µl). Template prep., template preparation. Amp. vol.: amplification volume (µl). Amp. PCR-cycles: amplification PCR-cycles. Amp. time: amplification time (min). Purification: Beads, Agencourt AMPure XP kit (Beckman Coulter); Columns, QiAquick PCR purification kit (Qiagen). Avg. Ampl. size (Kb), average amplicon size in kilobases.

scWGA method	***Ampli1***	***MALBAC***	***PicoPLEX***	***GenomiPhi***	***REPLIg***	***TruePrime***
Full Name	Ampli1 WGA Kit	MALBAC Single Cell WGA Kit	PicoPLEX WGA Kit	Illustra Single Cell GenomiPhi DNA Amplification Kit	REPLI-g Single Cell Kit	TruePrime Single Cell WGA Kit (v1)
WGA approach	LM-PCR	Hybrid	Hybrid	MDA	MDA	MDA
Company	Silicon Biosystems	Yikon Genomics	Rubicon Genomics	GE Healthcare	Qiagen	SYGNIS
Reference	WG 001R	YK001B	R30050	29-1081-07	150345	350100
Lysis type	E	E	E	A	A	A
Lysis temp. (ºC)	42, 65, 80	50, 80	75, 95	65	65	on ice
Lysis time (min)	45, 30, 15	50, 10	10, 4	10	10	10
Lysis vol. (µl)	3	5	10	2	7	5
Pre-amplification	Yes	Yes	Yes	No	No	No
Template prep.	Yes	Yes	Yes	No	No	No
Amp. vol. (µl)	50	65	75	20	50	50
Amp. PCR-cycles	44	17	16	-	-	-
Amp. time (min)	180	80	40	120	480	360
DNA yield (µg)	4	2-4	2-5	4-7	~ 40	>5
Purification	Beads	Columns	Columns	-	-	-
Avg. Ampl. size (Kb)	0.2-2	0.3-2	0.6	> 10	> 10	> 10
HDF cells	4	4	4	4	4	4
Caco-2 cells	12	18	18	18	16	22
Z-138 cells	18	18	18	15	15	18
Total cells	34	40	40	37	35	44

Supplementary Table 3 | Whole-genome sequencing library kits compared. Sequencing platform: machines used for library sequencing. Shearing: M, Mechanically (Focused ultrasonicator, Covaris); E, Enzymatically. PCR-free protocol: Y, Yes; N, No.

Library Kit	*Nextera*	*SureSelect*	*KAPA*	*Ion Plus*	*NxSeq*
Full name	Nextera DNA Library Preparation Kit	SureSelectQXT Whole Genome Library Prep Kit	KAPA Library Preparation Kit	Ion Plus Fragment Library Kit	NxSeq AmpFREE Low DNA Library Kit
Company	Illumina	Agilent Technologies	Kapa Biosystems	Life Technologies	Lucigen
Sequencing Platform	HiSeq 2000. Illumina	HiSeq 2000. Illumina	HiSeq 2000. Illumina	Ion Proton. Ion Torrent	HiSeq 4000. Illumina
Reference	FC-121-1031	G9682B	KK8201	4471252	14000-1
Input DNA (ng)	50	50	500-2000	100	75-1000
Shearing	E	E	M	M	M
Library size (bp)	200-2000	300-900	220-550	200-300	500-900
PCR-free protocol	N	N	Y	Y	Y
PCR cycles	5	5	-	-	-
HDF cells	-	-	-	-	24
Caco-2 cells	26	28	27	23	-
Z-138	29	29	27	17	-
Bulk libraries	-	-	-	-	1 HDF
Total libraries	55	57	55	40	25

Supplementary Table 4 | DNA yield, amplicon size, NGS output, amplification breadth and uniformity for Ampli1 and MALBAC. DNA: DNA yielded (µg). DIN values were only calculated for MDA-based kits. Reads: raw reads (millions). Mt reads: reads mapping to the mitochondrial genome (%). Breadth: Amplification breadth (Predicted coverage at 5X (%). Uniformity: Amplification uniformity (1 - Gini index). Reads were mapped against the human reference genome (hs37d5). Values in each cell are mean ± SD.

scWGA kit	Cell line	Cells	DNA (µg)	DIN	Amplicon Size (bp)	Reads (M)	Mapped reads (%)	Duplicated reads (%)	Mt reads (%)	Breadth (%)	Uniformity
Ampli1	All	36	2.98 ± 2.03	*NA*	674.94 ± 60.85	14.1 ± 7.9	99.43 ± 0.56	9.55 ± 10.53	0.44 ± 0.4	37.77 ± 6.83	0.04 ± 0.01
	HDF	4	0.47 ± 0.22	*NA*	774.75 ± 60.04	31.4 ± 2.3	98.12 ± 0.12	31.25 ± 4.2	1.38 ± 0.46	46.64 ± 2.23	0.04 ± 0.00
	Caco-2	12	1.11 ± 0.64	*NA*	716.42 ± 18.29	11.7 ± 4.5	99.72 ± 0.22	7.23 ± 7.00	0.43 ± 0.18	38.89 ± 7.00	0.04 ± 0.01
	Z-138	18	4.78 ± 0.56	*NA*	625.11 ± 12.15	11.8 ± 5.1	99.53 ± 0.31	6.28 ± 7.38	0.24 ± 0.08	35.05 ± 5.54	0.04 ± 0.01
MALBAC	All	40	1.74 ± 0.73	*NA*	1109.38 ± 168.79	12.0 ± 4.8	99.36 ± 0.77	3.46 ± 5.69	0.05 ± 0.07	41.37 ± 8.39	0.04 0.01
	HDF	4	0.48 ± 0.11	*NA*	951.75 ± 29.85	6.6 ± 1.1	97.25 ± 0.59	9.65 ± 0.41	0.26 ± 0.01	28.54 ± 2.29	0.04 ± 0.00
	Caco-2	18	1.43 ± 0.06	*NA*	962.72 ± 17.87	11.9 ± 4.8	99.67 ± 0.23	2.80 ± 5.71	0.03 ± 0.01	39.40 ± 6.74	0.04 ± 0.00
	Z-138	18	2.34 ± 0.61	*NA*	1291.06 ± 36.29	13.3 ± 4.3	99.53 ± 0.26	2.76 ± 5.63	0.01 ± 0.00	46.19 ± 6.92	0.04 ± 0.01

Supplementary Table 5 | DNA yield, amplicon size, NGS output, amplification breadth and uniformity for PicoPLEX and GenomiPhi. DNA: DNA yielded (µg). DIN values were only calculated for MDA-based kits. Reads: raw reads (millions). Mt reads: reads mapping to the mitochondrial genome (%). Breadth: Amplification breadth (Predicted coverage at 5X (%). Uniformity: Amplification uniformity (1 - Gini index). Reads were mapped against the human reference genome (hs37d5). Values in each cell are mean ± SD.

scWGA kit	Cell line	Cells	DNA (µg)	DIN	Amplicon Size (bp)	Reads (M)	Mapped reads (%)	Duplicated reads (%)	Mt reads (%)	Breadth (%)	Uniformity
PicoPLEX	All	40	3.30 ± 1.54	*NA*	441.18 ± 48.95	9.9 ± 3.8	98.89 ± 2.26	4.19 ± 4.53	0.10 ± 0.20	39.81 ± 7.75	0.03 ± 0.00
	HDF	4	2.55 ± 0.05	*NA*	519.00 ± 2.00	11.9 ± 4.7	92.38 ± 1.05	8.71 ± 0.36	0.66 ± 0.18	40.52 ± 3.25	0.03 ± 0.00
	Caco-2	18	2.31 ± 0.18	*NA*	463.56 ± 10.46	9.1 ± 3.8	99.58 ± 0.51	4.1 ± 4.4	0.06 ± 0.03	39.98 ± 7.38	0.03 ± 0.00
	Z-138	18	4.46 ± 1.67	*NA*	401.50 ± 42.00	10.2 ± 3.6	99.66 ± 0.40	3.27 ± 4.68	0.02 ± 0.01	39.49 ± 9.02	0.03 ± 0.01
GenomiPhi	All	37	3.54 ± 2.16	5.49 ± 0.25	10218.54 ± 1764.39	14.6 ± 6.1	99.61 ± 0.44	8.24 ± 14.65	0.08 ± 0.10	43.13 ± 21.07	0.02 ± 0.01
	HDF	4	4.80 ± 1.97	5.50 ± 0.18	9508.50 ± 509.70	23.7 ± 2.4	98.46 ± 0.13	7.21 ± 0.19	0.12 ± 0.08	38.15 ± 5.59	0.01 ± 0.00
	Caco-2	18	3.07 ± 2.62	5.48 ± 0.23	11539.44 ± 1161.65	13.0 ± 5.1	99.72 ± 0.18	4.09 ± 7.28	0.08 ± 0.10	47.50 ± 17.03	0.03 ± 0.01
	Z-138	15	3.76 ± 1.43	5.50 ± 0.29	8822.80 ± 1355.07	14.1 ± 6.0	99.79 ± 0.16	13.50 ± 20.87	0.06 ± 0.09	39.20 ± 27.10	0.02 ± 0.01

Supplementary Table 6 | DNA yield, amplicon size, NGS output, amplification breadth and uniformity for REPLIg and TruePrime. DNA: DNA yielded (µg). DIN values were only calculated for MDA-based kits. Reads: raw reads (millions). Mt reads: reads mapping to the mitochondrial genome (%). Breadth: Amplification breadth (Predicted coverage at 5X (%). Uniformity: Amplification uniformity (1 - Gini index). Reads were mapped against the human reference genome (hs37d5). Values in each cell are mean ± SD.

scWGA kit	Cell line	Cells	DNA (µg)	DIN	Amplicon Size (bp)	Reads (M)	Mapped reads (%)	Duplicated reads (%)	Mt reads (%)	Breadth (%)	Uniformity
REPLIg	All	35	34.89 ± 9.75	6.87 ± 0.38	32326.29 ± 15154.66	21.0 ± 12.2	97.66 ± 11.64	11.00 ± 24.68	0.05 ± 0.12	50.32 ± 27.87	0.04 ± 0.02
	HDF	4	35.88 ± 7.82	6.48 ± 0.10	11459.75 ± 1352.40	26.1 ± 4.8	81.49 ± 33.79	24.65 ± 29.08	0.13 ± 0.04	42.38 ± 27.79	0.03 ± 0.02
	Caco-2	16	37.18 ± 13.26	6.75 ± 0.12	39776.69 ± 14657.47	22.0 ± 13.8	99.75 ± 0.19	15.09 ± 32.55	0.06 ± 0.17	61.29 ± 31.39	0.04 ± 0.02
	Z-138	15	32.17 ± 3.93	7.11 ± 0.45	29943.60 ± 11543.44	18.5 ± 11.8	99.74 ± 0.21	3.00 ± 4.69	0.01 ± 0.01	40.73 ± 20.27	0.04 ± 0.02
TruePrime	All	44	6.55 ± 2.52	6.09 ± 0.22	11937.39 ± 1727.75	24.0 ± 15.4	99.63 ± 0.43	15.53 ± 13.3	8.11 ± 23.05	15.36 ± 15.09	0.02 ± 0.02
	HDF	4	3.92 ± 0.23	5.63 ± 0.05	9652.75 ± 423.09	16.3 ± 4.6	98.39 ± 0.20	25.87 ± 3.92	79.72 ± 5.71	2.47 ± 0.55	0.00 ± 0.00
	Caco-2	22	7.64 ± 3.10	6.16 ± 0.18	12611.82 ± 1781.44	27.3 ± 18.0	99.76 ± 0.15	15.43 ± 14.27	1.43 ± 2.5	14.63 ± 15.84	0.01 ± 0.02
	Z-138	18	5.80 ± 0.66	6.11 ± 0.17	11620.78 ± 1294.55	21.8 ± 12.9	99.74 ± 0.18	13.35 ± 12.77	0.37 ± 1.5	19.11 ± 14.55	0.02 ± 0.01

Supplementary Table 7 | Chimeras, ADO and false SNVs. All calculations were carried out using the HDF cell line. Reads with supplementary alignments are shown as split reads, and paired-end reads mapping at more than 1 kb are shown as discordant reads. We used two different approaches for measuring ADO. With joint calling we aggregated the 24 HDF cells and the bulk, while with marginal calling we combined each single cell with the bulk separately. We also applied these calling approaches for the false SNVs estimation. Values in each cell are mean ± SD.

	Ampli1	***MALBAC***	***PicoPLEX***	***GenomiPhi***	***REPLIg***	***TruePrime***
% split reads	0.86 ± 0.05	0.62 ± 0.04	2.59 ± 0.40	16.60 ± 1.95	1.88 ± 0.93	12.55 ± 0.98
% paired-end discordant reads	2.97 ± 0.27	1.43 ± 0.10	9.13 ± 1.39	33.80 ± 4.10	5.67 ± 1.60	31.38 ± 4.00
% ADO after joint calling	38.34 ± 3.99	50.78 ± 3.82	53.04 ± 5.59	59.40 ± 3.22	60.78 ± 8.03	90.29 ± 4.77
% ADO after marginal calling	37.69 ± 3.99	50.68 ± 3.82	52.55 ± 5.45	57.57 ± 3.45	59.93 ± 6.99	89.50 ± 5.07
% False SNVs after joint calling	11.95 ± 1.98	25.10 ± 1.43	22.07 ± 8.41	12.60 ± 0.54	7.22 ± 3.81	5.15 ± 0.30
% False SNVs after marginal calling	9.50 ± 0.88	57.34 ± 1.22	51.99 ± 6.84	39.06 ± 2.95	7.28 ± 5.31	17.73 ± 3.96

Supplementary Table 8 | Covaris parameters for DNA fragmentation. We used the Covaris S2 and LE220 focused-ultrasonicators. Duty cycle indicates the percentage of active burst time in the acoustic treatment and cycles/burst the number of acoustic oscillations per burst.

	Sample	Volume (ul)	Nº of cycles	Duty cycle (%)	Intensity	Cycles/burst	Treatment time (s)
S2 Focused-ultrasonicator							
NxSeq libraries (300 bp)	Bulk	130	2	10	4	200	40
	MDA amplicons	50	1	10	5	200	50
	non-MDA amplicons	50	1	10	5	200	30
Ion Plus libraries (200 bp)	MDA amplicons	130	6	10	5	100	60
	non-MDA amplicons	130	5	10	5	100	60
LE220 Focused-ultrasonicator							
KAPA libraries (250 bp)	All amplicons	55	1	15	450 W	200	100