

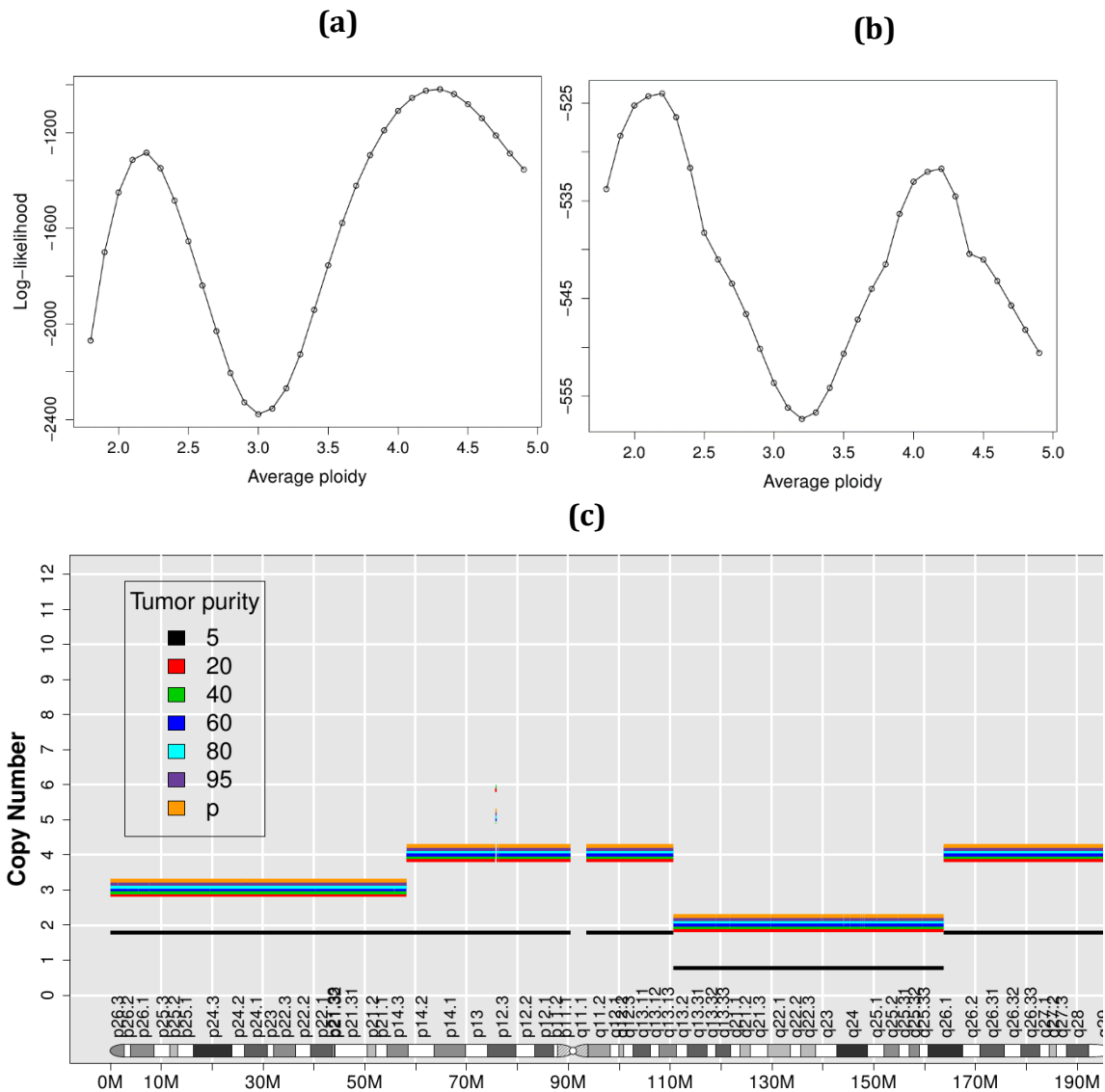
Supplementary Table S1.

A list of tools designed for copy number profile for impure tumor samples.

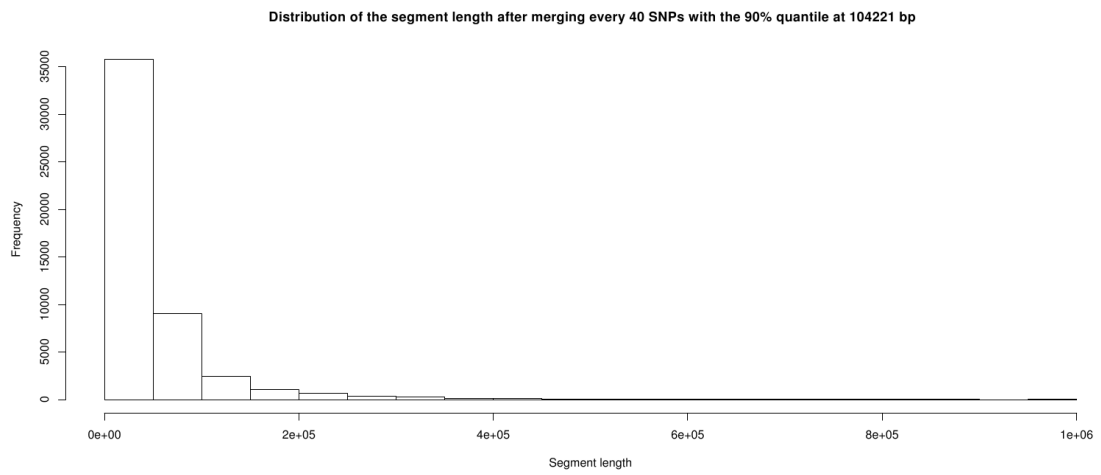
Tool	TC	Ploidy parameter	Paired Normal	GC-bias corr.	LOH	Data type	Model-free pre-segmentation	References
Absolute	Y	Y	Y	-	Y	Array NGS	Based on HAPSEG	(1,2)
ASCAT	Y	Y	Opt	Opt	Y	Array NGS	Itself	(3)
PICNIC	N	Y				Array	No	
OncoSNP	Y	N	Y	Y	Y	Array	No	(4)
PyLOH	Y	N	Y	Y	Y	NGS	BIC-seq	(5,6)
CLImAT	Y	N	N	Y	Y	NGS	No	(7)
THetA2	Y	N	Y	Y	Y	NGS	BIC-seq	(8)
OncoSNP-Seq	Y	N	Y	Y	Y	NGS	No	(9)
CNAnorm	Y	Y	Y	Y	N	NGS	No	(10)

Supplementary Table S2. The source of the mixture samples.

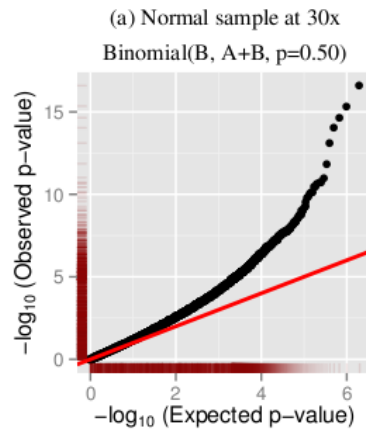
Tumor purity	HCC1143 (TCGA)	HCC1954 (TCGA)	HCC1187 (Illumina)	HCC2218 (Illumina)
100%	Downloaded	Downloaded	Downloaded	Downloaded
95%	Downloaded	Downloaded	Generated in-house	Generated in-house
80%	Downloaded	Downloaded	Generated in-house	Generated in-house
60%	Downloaded	Downloaded	Generated in-house	Generated in-house
40%	Downloaded	Downloaded	Generated in-house	Generated in-house
20%	Downloaded	Downloaded	Generated in-house	Generated in-house
10%	Generated in-house	Generated in-house	Generated in-house	Generated in-house
5%	Downloaded	Downloaded	Generated in-house	Generated in-house
0%	Downloaded	Downloaded	Generated in-house	Generated in-house



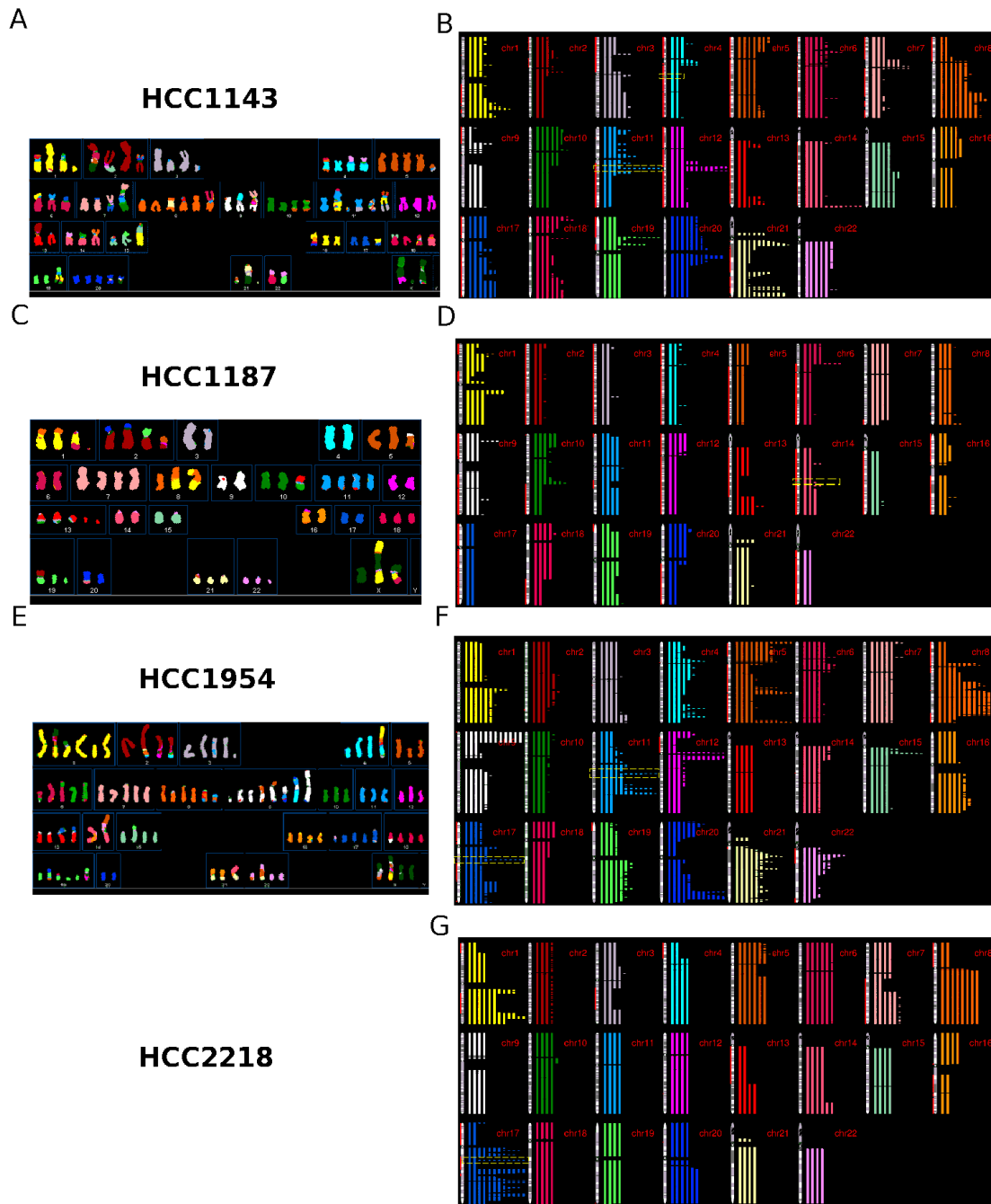
Supplementary Figure S1. Ploidy estimation at different tumor purity for HCC2218. As sCNaphase searches possible average ploidy values, (a) shows the maximum likelihood calculated for the ploidy of 1.9 to 5 for 100% tumor purity sample. There are two peaks corresponding to two possible ploidy estimation roughly at 2.3 or 4.3. In **Supplementary Figure 4**, the dSKY of HCC2218 shows the number of the chromosome arms were mostly estimated to be even numbers, except for a region in chr3p estimated to be 3 copy. Because this region supported for average ploidy of ~ 4.3 , the peak at 4.3 is clearly higher, a preferred ploidy estimation. However, at the 5% tumor purity, the lower tumor ploidy estimation became more likely in (b). As shown in the copy number profile of chr3 HCC2218 in (c), this copy number reduced by a half at 5% tumor purity. Even though the reduction from 3 to 2 copies is unfavorable in region from 0 to 50M, this region cannot produce as strong support for the higher ploidy as for the pure tumor sample. In (c) legend, p stands for pure tumor samples.



Supplementary Figure S2. The segment size distribution merging every 40 SNPs calculated from HCC1143.



Supplementary Figure S3. Inflation of test-statistic for detecting somatic copy number aberration for normal sample under null assumption of $\text{BAF} = 0.5$. Each of the black dots in the Q-Q plots corresponded to a P-value for a pair of allelic depths under different assumptions. P-values were calculated under the assumption that both alleles had equal copy numbers for a normal samples. The red line, $y=x$, is the expected fitness under null hypothesis.



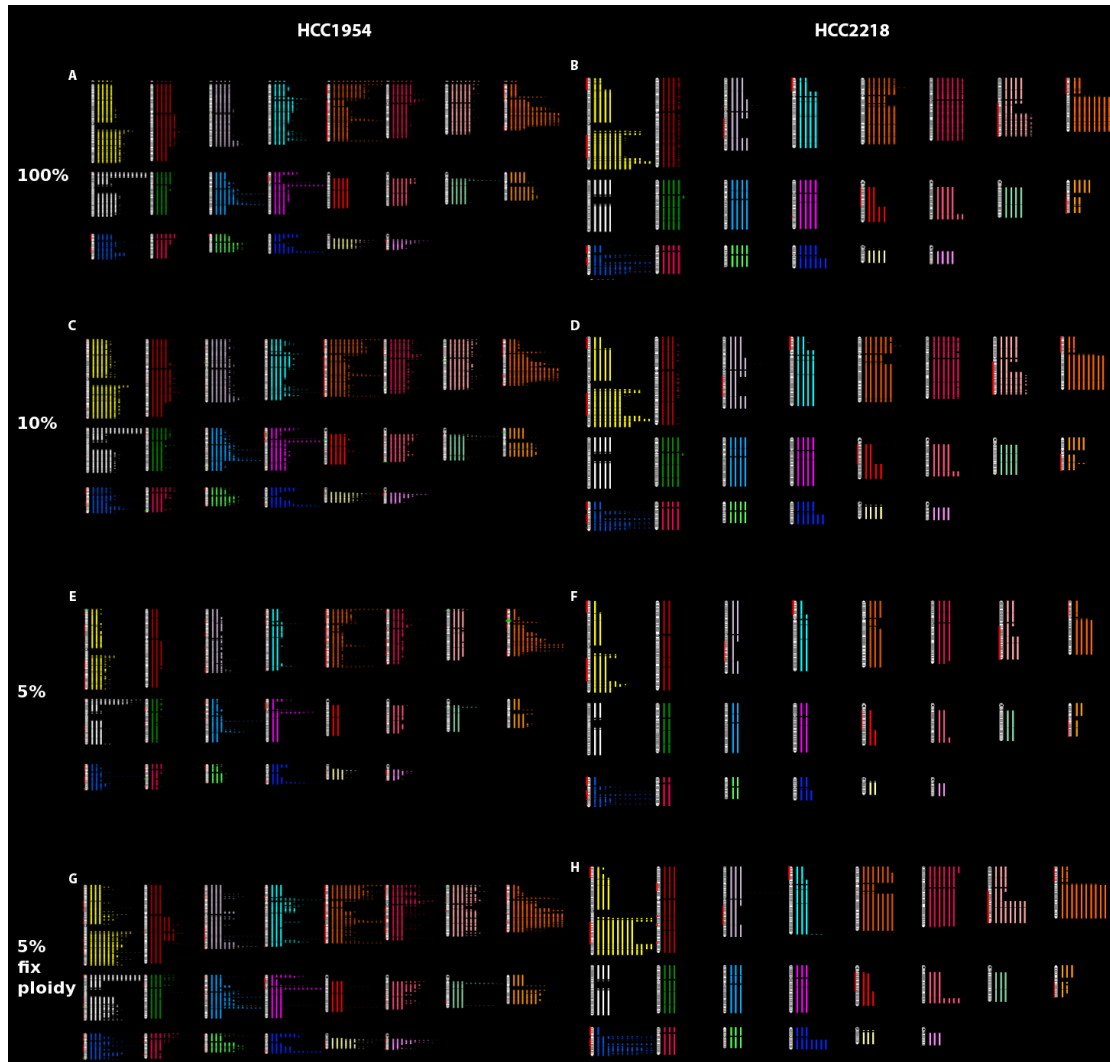
Supplementary Figure S4. dSKY plots capture the same large scale copy number change present in existing SKY experiments. (A), (C) and (E) all show the results from a traditional SKY analysis of the cell-lines characterised in this study. Each chromosome is represented by a specific colour and are grouped together, making it possible to count the number of copies of each chromosome. This work was completed by Mira Grigorova & Paul A.W. Edwards (Department of Pathology, University of Cambridge) - see <http://www.path.cam.ac.uk/~pawefish> - and is reproduced with permission. We were unable to find publicly available results from a SKY analysis of HCC2218. To allow a direct comparison between the result produced by sCNaphase and the SKY experiments, each chromosome was painted in the same colour used in the traditional SKY analysis and are presented in a similar layout. (B),(D),(F) and (G) all show the corresponding dSKY plots. To show additional information, such as the location of each sCNA and the regions that have undergone an LOH event, a diagram of each chromosome is present in the dSKY plot. This diagram is coloured red to indicate an LOH event or green to highlight a homozygous deletion. High resolution dSKY plots can be found from figshare at <https://figshare.com/authors/ /1365237>.

Supplementary Table S3.

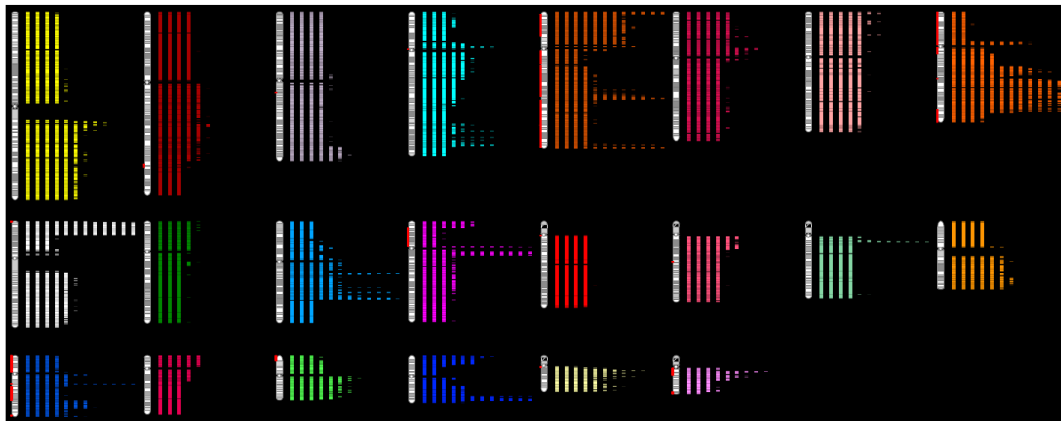
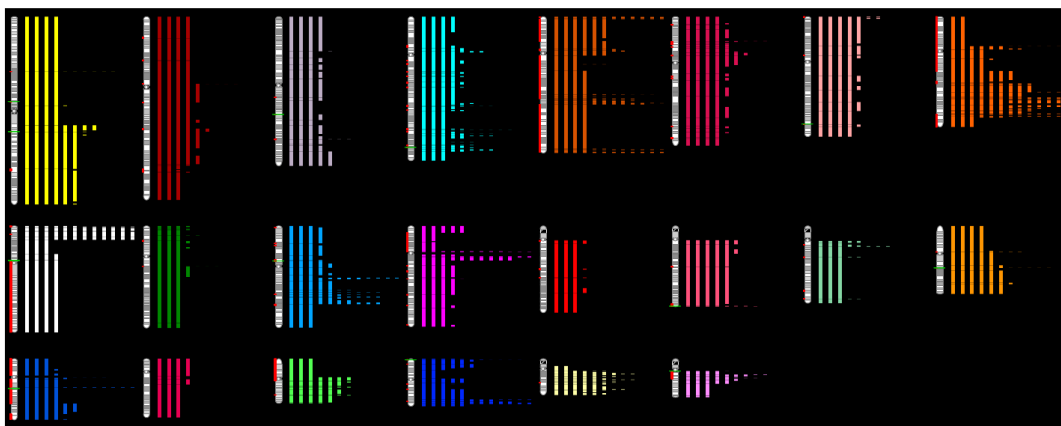
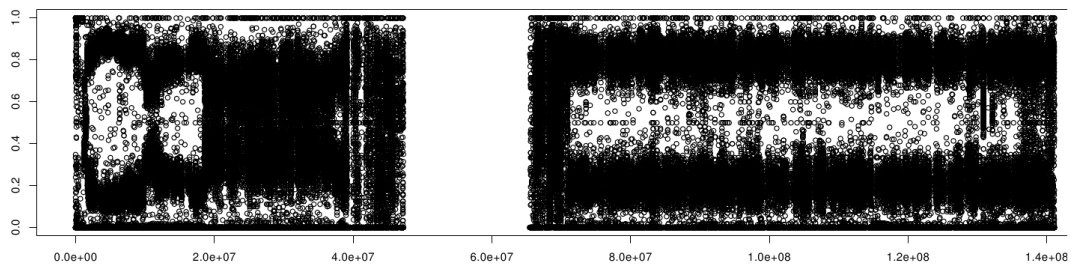
The estimated tumor purity, tumor cellularity, tumor ploidy for tumor-normal mixtures.

TP	HCC1143 (Hypo-tetraploid)										
	sCNaphase			CLImAT				ASCAT			
	tc(%)	tp(%)	pl		tc(%)	tp(%)	Pl		tc(%)	tp(%)	pl
100%	100	100	3.7		98	99	3.77		1.00	99	3.82
95%	91	95	3.7		82	90	3.80		0.93	90	3.80
80%	68	80	3.7		65	78	3.80		0.68	78	3.78
60%	45	60	3.8		43	60	3.91		0.44	60	3.77
40%	27	40	3.8		33	43	3.03		0.27	43	3.76
20%	12	21	3.8		19	27	3.19		0.31	27	1.87
10%	6	11	3.7		48	50	2.19		0.28	50	2.03
5%	4	7	3.7		71	73	2.16		0.29	73	2.00
0%	0.01	0.01	1.8		60	61	2.16		0.27	62	2.00
	HCC1187 (Hypo-triploid)										
100%	100	100	2.8		89	92	2.68		1.00	92	2.72
95%	93	95	2.7		85	88	2.70		0.93	88	2.72
80%	74	80	2.7		71	77	2.69		0.74	77	2.72
60%	52	60	2.7		52	59	2.70		0.51	59	2.73
40%	33	40	2.7		32	39	2.71		0.32	39	2.73
20%	19	23	2.7		18	23	2.75		0.21	23	2.6
10%	8	11	2.7		58	58	2.02		NA	58	NA
5%	4	5	2.8		62	62	2.02		0.22	62	2.00
0%	0.01	0.01	2.5		65	65	2.01		0.25	65	1.99
	HCC1954 (Hyper-tetraploid)										
100%	100	1.00	4.5		49	56	2.67		1.00	56	4.6
95%	86	93	4.7		46	54	2.72		0.97	54	4.2
80%	60	78	4.7		41	48	2.62		0.58	48	4.8
60%	37	58	4.7		36	41	2.50		0.4	41	4.6
40%	22	39	4.7		20	27	2.98		0.24	27	4.5
20%	11	21	4.7		51	52	2.09		0.24	52	2.2
10%	6	12	4.9		50	51	2.05		0.25	51	2.07
5%	4	6	2.8		52	52	1.99		0.26	52	2.02
0%	0.01	0.01	2.3		64	65	2.16		0.26	66	2.00
	HCC2218 (Tetraploid)										
100%	100	100	4.2		48	51	2.3		0.99	51	4.32
95%	89	95	4.2		46	49	2.29		0.9	49	4.36
80%	64	79	4.2		76	77	2.14		0.64	77	4.32
60%	40	59	4.2		54	56	2.16		0.4	56	4.33
40%	23	39	4.4		34	35	2.12		0.4	35	2.08
20%	11	21	4.4		22	23	2.12		0.23	23	2.12
10%	5	11	4.4		41	41	2.02		0.25	41	2.06
5%	5	5	2.2		43	43	2.01		0.23	43	2.01
0%	0.0	0.04	2.4		45	44	1.98		0.24	45	1.99

In the table, *tc* denotes for tumor cellularity, *pl* for ploidy and *tp* for tumor purity. The ploidy for HCC1143, HCC1954 and HCC1187 were derived from the SKY data (<http://old-www.path.cam.ac.uk/~pawefish/BreastCellLineDescriptions/HCC1143.html>). The ploidy of HCC2218 is found from COSMIC (<http://grch37-cancer.sanger.ac.uk/cosmic/sample/overview?id=749716>).



Supplementary Figure S5. Accurate copy number profiling relies on accurate determination of ploidy. Both sCNPhase and the independent SKY analysis of the pure HCC1954 and HCC2218 cell-lines classified them as tetraploids (A),(B), a result maintained across the majority of the mixtures, including those that only contained 20% of cell-line derived reads (C) and (D). However, in the 5% cell-line DNA mixtures, ploidy was miscalculated as a result, sCNPhase failed to produce an accurate copy number profile for these mixtures (E) and (F). When the incorrect ploidy value was manually replaced with the correct one, it was possible to identify the same broad copy number profile in the 5% mixture, that was present in the 100% cell-line mixture (G) and (H).

A**B****C**

Supplementary Figure S6. Discrepancy about LOHs estimation for HCC1954 is presented in dSKY plots in (A) and (B) generated from sCNaphase and COSMIC respectively. The clear differences were found at chr9 q-arm, which is found to be LOHs only by COSMIC. The absolute copy number was predicted to be 4 from COSMIC than 5 from sCNaphase. However the B-allelic frequency at chr9q in (C) shows the allelic copy numbers at chr9 are very imbalance, allelic copy numbers of 4 to 1 from sCNaphase, but clearly the region remains heterozygous. Similarly the extra regions of LOHs from COSMIC at chr8, chr17 and chr19 are also likely to be heterozygous, as supported by the BAFs. These are also coincide with slight lower copy number estimation sCNaphase. COSMIC segmentation also underestimated ploidy for chr7, chr15, which are 5 and 4 copies according to sCNaphase and the SKY plots (**Supplementary Figure S4E**) .

Supplementary Table S4.
The capacity of CLIMAT to detect the focal sCNAs in COSMIC

Tumor purity	HCC1143 15 focal amplification from COSMIC		HCC1954 94 focal amplification from COSMIC		HCC1187 2 focal amplification from COSMIC		HCC2218 18 focal amplification from COSMIC	
	Sen	Spe	Sen	Spe	Sen	Spe	Sen	Spe
100%	33	17	44	55	50	25	6	33
80%	40	17	39	51	50	8	0	0
60%	47	13	14	36	100	13	0	0
40%	20	40	35	39	100	9	22	50
20%	53	31	0	0	100	6	22	25
10%	0	0	0	0	0	0	0	0

Sen for sensitivity; Spe for specificity.

Supplementary Table S5
The detection of ERBB2 and chr11q13 amplification across the mixtures

TP	ERBB2		chr11q13	
	HCC1954	HCC2218	HCC1143	HCC1954
100%	>=12	>=12	>=12	>=12
95%	>=12	>=12	>=12	>=12
80%	>=12	>=12	>=12	>=12
60%	>=12	>=12	>=12	>=12
40%	>=12	>=12	>=12	>=12
20%	>=12	>=12	>=12	>=12
10%	>=12	>=12	>=12	>=12
5%	>=12	>=12	>=12	>=12

Supplementary Table S6. Average 50% overlap specificity and sensitivity across four cell lines as a function of sCNPhase detection threshold.

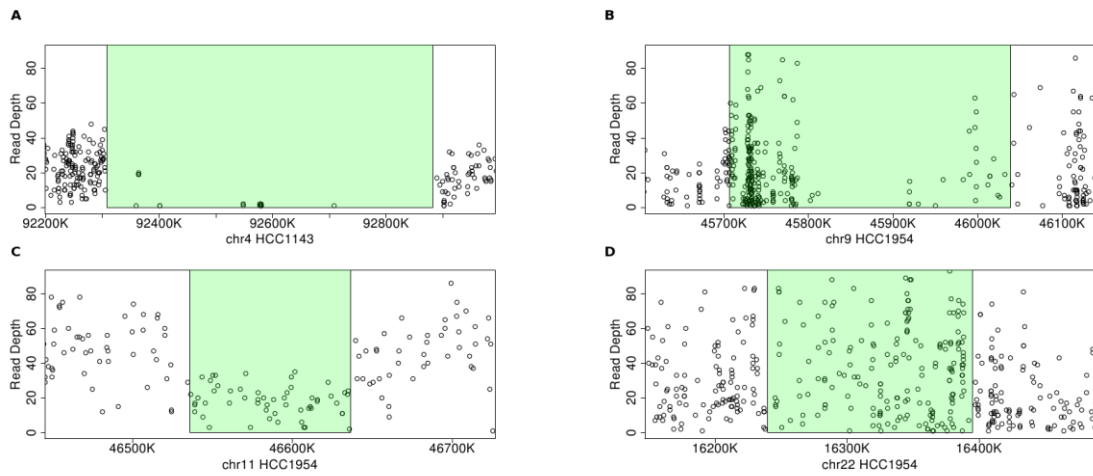
Tumor purity	sCNPhase threshold = 2*ploidy		sCNPhase threshold = 2*ploidy + 1		sCNPhase threshold = 2*ploidy + 2	
	Sen	Spe	Sen	Spe	Sen	Spe
100%	0.67	0.41	0.58	0.59	0.51	0.70
80%	0.62	0.39	0.54	0.58	0.50	0.72
60%	0.67	0.39	0.60	0.58	0.53	0.72
40%	0.63	0.41	0.56	0.59	0.50	0.75

20%	0.64	0.42	0.60	0.57	0.53	0.72
10%	0.67	0.47	0.59	0.64	0.48	0.75
5%	0.41	0.62	0.32	0.69	0.27	0.79

Supplementary Table S7.

A list of the homozygous deletions greater than 100kb in the COSMIC data

Cell-Line	Location	Size	Detected by sCNPhase	Detected by CLImAT
HCC1143	Chr4:92,308,295-92,882,506	574 kb	10% Mixture	Not detected
HCC1187	Chr6:134,329,397-134,537,448	208 kb	Not detected	Not detected
HCC1187	Chr14:63,703,884-64,334,878	630 kb	10% Mixture	Not detected
HCC1187	Chr15:72,194,262-72,443,790	249 kb	10% Mixture	Not detected
HCC2218	Chr16:68,698,153-68,844,967	146 kb	Not detected	Not detected
HCC1954	Chr9:45,706,445-46,038,862	332 kb	Not detected	Not detected
HCC1954	Chr11:46,535,802-46,636,287	100 kb	Not detected	Not detected
HCC1954	Chr22:16,239,488-16,395,163	155 kb	Not detected	Not detected



Supplementary Figure S7. The B-allelic frequency at regions with heterozygous deletions from COSMIC. The plots corresponds to the regions containing the homozygous deletions in HCC1143 and HCC1954 as shown in Supplementary Table 6. The green shading represents the location of the homozygous deletion listed in the COSMIC annotation of these cell-lines, while the black points present the read depth for the individual heterozygous SNPs located within these regions. For HCC1143 (A, C), there is a high level of consistency between deletion in the COSMIC annotation of this cell-line and the read depth of germ-line SNPs in the NGS data. In contrast, the results from HCC1954(B, D) do not show the same level of consistency. In the deletion on chromosome 9(B), the NGS data suggests that the deletion here is significantly smaller than the one listed by COSMIC. In contrast, the deletion on chromosome 22(D), is not consistent with the NGS data.

Supplementary Table S8.

Consensus detection of TP53 LOH at different levels of tumor purity.

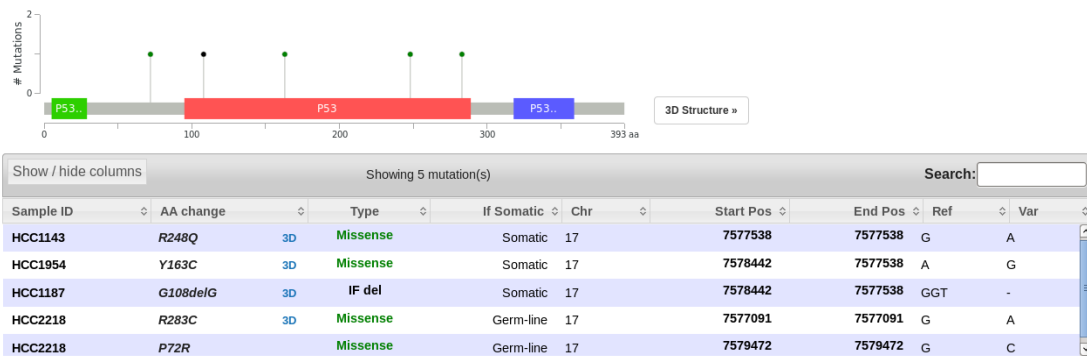
Location of TP3 (chr17:7,571,720-7,590,868)		
Cell-Line	Detected LOHs by sCNaphase	Detected LOHs by CLImAT
HCC1143	100%-20% Mixture (4 copies) 5% mixture (4 copies)	100%-20% Mixture
HCC1187	100%-5% Mixture (2 copies)	100%-20% Mixture
HCC2218	100%-10% Mixture (2 copies) 5% Mixture (1 copy)	80%-20% Mixture
HCC1954	100% Mixture (4 copies) 95% Mixture (4 copies) 80-40% Mixture (4 copies) 20%-10% Mixture (3 copies) 5% Mixture (1 copy)	80%-60% Mixture

Supplementary Table S9.

Germline Heterozygous SNPs present in in the TP53 loci

Position	Ref	Alt	Depth	AF	Phase	Location	h0	h1	h0	Effect
							Normal		Tumor	
chr17:7574775	C	T	31	0.54	01	Non-coding	C	T	C	
chr17:7574779	C	T	30	0.53	01	Non-coding	C	T	C	
chr17:7574936	C	T	41	0.41	01	Non-coding	C	T	C	
chr17:7577091	G	A	29	0.55	--	CDS	A	G	A	R283C*
chr17:7577644	C	G	36	0.47	10	Non-coding	G	C	G	
chr17:7578115	T	C	34	0.5	10	Non-coding	C	T	C	
chr17:7578645	C	T	29	0.55	10	Non-coding	T	C	T	
chr17:7578837	A	G	38	0.28	10	Non-coding	G	A	G	
chr17:7579472	G	C	37	0.59	10	CDS	C	G	C	P72R
chr17:7579801	G	C	31	0.35	10	Non-coding	C	G	C	

Ref and alt are short for reference and alternative allele. AF is for allelic frequency in the normal sample, HCC2218BL. H0 and H1 stand for the two parental chromosomes. The 01 in the phase column means that the reference allele is from H0, the alternative allele is from H1. The 10 means the opposite.



Supplementary Figure S8. TP53 Germ-line and somatic mutations in in each cell line. Mutations annotated by COSMIC, shows the somatic mutations including 1 missense mutation for HCC1143, 1 missense mutation for HCC1954 and 1 inframe deletion for HCC1187. The two germ-line missense mutations for HCC2218 were from aforementioned analysis. The figure is generated from MutationMapper (11). Missense Mutations are showed in green and Inframe Mutations are showed in black. The top colored bars showed the location of the mutations in TP53 as well as the domains of TP53. The table underneath shows the details about the mutation types.

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