Table W1. Treatment Received for each CRC Patient before the Blood Collection.

Sample	Treatment before Blood Collection
CRC1	CT neoadjuvant 1 [oxaliplatin–5-fluorouracil (5-FU)]
CRC2	CT palliative 1 (oxaliplatin–egafur uracil–bevacizumab), CT 2 palliative (UFT-irinotecan-bevacizumab)
CRC3	Untreated
CRC4	Untreated
CRC5	Untreated
CRC6	Untreated
CRC7	Untreated
CRC8	Untreated
CRC9	Untreated
CRC10	Untreated
CRC11	Untreated
CRC12	Untreated
CRC13	Untreated
CRC14	Untreated
CRC15	Untreated
CRC16	Untreated
CRC17	CT 1 palliative (5-FU oxaliplatin)
CRC18	CT 1 palliative (5-FU irinotecan)
CRC19	CT 1 palliative (5-FU), CT 2 palliative (5-FU avastin oxaliplatin), CT 3 (5-FU avastin)
CRC20	CT 1 palliative (5-FU irinotecan), CT 2 palliative (5-FU irinotecan avastin), CT 3 palliative (5-FU avastin oxaliplatin), CT 4 palliative (5-FU avastin)
CRC21	CT 1 palliative (5-FU irinotecan avastin)
CRC22	CT 1 palliative (5-FU irinotecan), CT 2 palliative (5-FU oxaliplatin), CT 3 palliative (5-FU irinotecan avastin), CT 4 palliative (5-FU avastin)
CRC23	CT 1 palliative (irinotecan 5-FU avastin), RT 1 neoadjuvant, CT 2 palliative (5-FU avastin)
CRC24	Untreated
CRC25	Untreated
CRC26	RT 1 neoadjuvant, CT 1 neoadjuvant (5-FU oxaliplatin), CT 2 neoadjuvant (5-FU irinotecan avastin), CT 3 palliative (irinotecan avastin)
CRC27	CT 1 neoadjuvant (5-FU oxaliplatin irinotecan), RT 1 neoadjuvant
CRC28	CT 1 (Camptothecin-Erbitux)
CRC29	RT 1 neoadjuvant, CT 1 neoadjuvant
CRC30	CT 1 palliative
CRC31	CT 1 palliative
CRC32	CT 1 palliative
CRC33	Untreated
CRC34	Untreated
CRC35	Untreated
CRC36	Untreated
CRC37	Untreated
CRC38	Untreated

RT, radiotherapy; CT, chemotherapy; the number indicates the sequence of treatment.

Table W2. Characteristics of the Selected Primers and of the Amplicons Obtained.

Species	Gene	Primer Name	Direction	Sequence 5′-3′	Tm (°C)	Amplicon Size (bp)
Human	*KRAS*	KRAS Hf 2	Sense	AATCCGTGTGGGTCAGAGAG	59.4	189
		KRAS Hr 2	Antisense	GAAACAATAGCCACCCTCCTT	57.9	–
Mouse	*KRAS*	KRAS Mf 3	Sense	GGCCAGGAGTGCATTAAGAC	59.4	214
		KRAS Mr 3	Antisense	GCACGTCAGATAGTCTCCAAA	57.9	–
Human	*KRAS*	KRAS G12V f	Sense	ACTTGTGGTAGTTGGAGCTGT	59.3	142
		KRAS G12V r	Antisense	GAATGGTCCTGCACCAGTAA	58.6	–
Human	*BRAF*	BRAF V600E f	Sense	GATTTTGGTCTAGCTACAGA	49.7	145
		BRAF V600E r	Antisense	TAGTAACTCAGCAGCATCTCAGG	58.8	–
Human	*KRAS*	Kras 46 Hr	Antisense	GCTGTATCGTCAAGGCACTC	59.4	46
		Kras 82 Hr	Antisense	TTGGATCATATTCGTCCACAA	54.0	82
		Kras 138 Hr	Antisense	CAAAGAATGGTCCTGCACC	56.7	138
		Kras 200 Hr	Antisense	TGAAAATGGTCAGAGAAACCTT	54.7	200
		Kras 250 Hr	Antisense	TGAAACCCAAGGTACATTTCAG	56.5	250
		Kras 300 Hr	Antisense	GAACATCATGGACCCTGACA	57.3	300
		Kras 350 Hr	Antisense	TTCTACCCTCTCACGAAACTCTG	60.6	355
		Kras 400 Hr	Antisense	AAAGATTGTCTTTTAGGTCCAGATAGG	60.4	390
		KrasNonMutated Hf	Sense	GTAGTTGGAGCTGGTGGC	58.2	–
		Kras G13D Hf	Sense	GTAGTTGGAGCTGGTGA	52.8	–
		Kras G12V Hf	Sense	TTGTGGTAGTTGGAGCTGT	54.5	–
		Kras G12D Hf	Sense	TGTGGTAGTTGGAGCTGA	53.7	–
		Kras G12S Hf	Sense	ACTTGTGGTAGTTGGAGCTA	55.3	–
		Kras G12A Hf	Sense	TGTGGTAGTTGGAGCTGC	56.0	–
Intplex primers						
Human	*BRAF*	Braf A1 conv k	Sense	TTATTGACTCTAAGAGGAAAGATGAA	56.9	105
		Braf A2 conv k	Antisense	GAGCAAGCATTATGAAGAGTTTAGG	59.7	–
		Braf V600E conv k	Sense	GATTTTGGTCTAGCTACAGA	53.2	97
		Braf B2 conv k	Antisense	TAGCCTCAATTCTTACCATCCACA	59.3	–
		Braf blocker	Sense	GCTACAGTGAAATCTCGATGG-PHO		
Human	*KRAS*	Kras A1 inv k	Sense	GCCTGCTGAAAATGACTGA	54.5	61
		Kras G12D Inv k	Antisense	CTCTTGCCTACGCCAT	51.7	–
		Kras G12A Inv k	Antisense	CTCTTGCCTACGCCAG	54.3	–
		Kras G12S Inv k	Antisense	TCTTGCCTACGCCACT	51.7	60
		Kras G12C Inv k	Antisense	TCTTGCCTACGCCACA	51.7	–
		Kras G13D Inv k	Antisense	GCACTCTTGCCTACGT	51.7	64
		Kras G12V Inv k	Antisense	CTCTTGCCTACGCCAA	51.7	61
		Kras B1 inv k	Sense	CCTTGGGTTTCAAGTTATATG	54.0	67
		Kras B2 inv k	Antisense	CCCTGACATACTCCCAAGGA	59.4	–
		Kras blocker	Antisense	GCCTACGCCACCAGCTC-PHO		

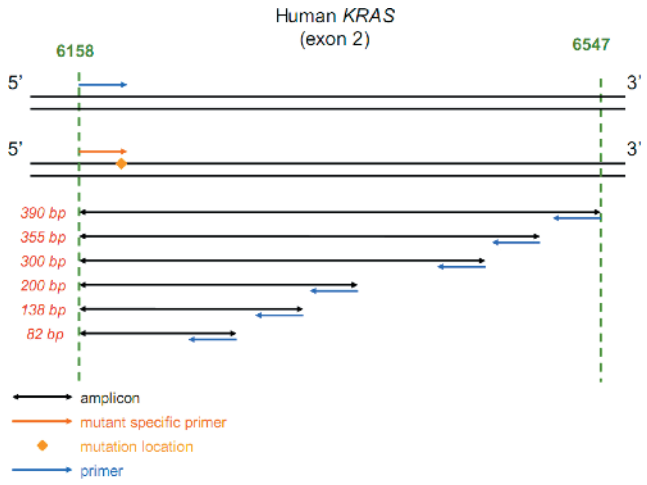

Figure W1. Design of the primers used for the ccfDNA size distribution study.

Table W3. Numerical Values of ccfDNA Quantification Obtained in the Xenografted Mice Experiments.

Mouse No.	mWT	hWT	hKRASm	hBRAFm	Tumor Weight (mg)
1	6.336	ND	ND	ND	0
2	6.627	ND	ND	ND	0
3	13.079	0.056	ND	ND	0
4	4.551	ND	0.066	ND	72.4
5	11.802	1.381	4.068	ND	89.8
6	8.29	3.644	4.304	0.201	174.8
7	3.746	2.516	1.729	ND	311.1
8	9.224	2.608	2.409	ND	358
9	6.776	1.586	6.102	0.131	465
10	5.826	8.456	7.493	0.155	543
11	9.047	9.047	12.531	0.235	1200

Data are expressed as ng/ml plasma.
ND, not determined.

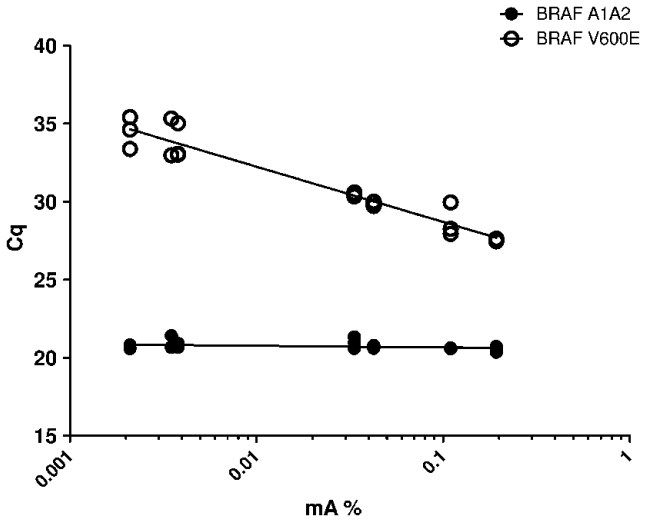

Figure W2. Analysis of the sensitivity of the method for detecting BRAF V600E point mutation. mA% represents the estimated mutation load and Cq represents the quantification cycle when the amplified amplicon is detected during the Q-PCR experiment. Each point corresponds to a specific amplification of the targeted sequence as determined by melting analysis. DNA from HT29 cells harboring a specific mutation was serially diluted six times into high concentrated WT genomic DNA from human placenta up to dilution of 0.2 mutated copies in 20,000 copies (1/100,000 ratio). Assay is carried in triplicate determination.