

Table W1. Primer Sequences and dHPLC Conditions.

Exon	Primer FW	Primer RV	T_m PCR (°C)	Ampl. Size (bp)	tdHPLC	% B
1	5'-CTCCACAGACCCTCTCCTTG-3'	5'-GGACAGAGTAGGTGAGGGGA-3'	58	242	64–68	57–54
2	5'-AAACGTCATGATTTTCAATGGC-3'	5'-GGGGAATTTGCTTTCTTTTCTT-3'	58	281	55.5	56.6
3	5'-TTTCACTTTTCAGATGTGTGTG-3'	5'-CTTTGTGAATTTGATCTTGAG-3'	58	210	55.5	54.8
4a	5'-GTTTGAATAATTTTCATAATAGAAA-3'	5'-CTCACAGCAGCTTTGACCTCC-3'	58	417	51–57	61–57
4b	5'-CAAGTGGTCTCCTCCCTT-3'	5'-GTCAAAAAGTAGTATCATGAATG-3'	58	283	55	55
4c	5'-TTTCTAGCAGACAACATCGA-3'	5'-ATTTGTGTTGTGTAGCATCCT-3'	62	308	54.5	57.5
5	5'-GAAGGAAGTTAGAAAGTTGTGACA-3'	5'-ATGGCTGGTAAGGATACGATTG-3'	62	172	54	51
6	5'-CATGTTTATCTTTTAAAAGTTTG-3'	5'-ATGTGAAGCAGTTTTATTTTACTCAA-3'	62	332	54.5–56	58.6–56.6
7	5'-ATTTGCTATAATATAGTACATCTGG-3'	5'-GTTGATAAGTTTCATAGAGCTGCTTT-3'	62	385	53–56.5	58–54.3
8	5'-GGATTTTACTGCATTTGTGTG-3'	5'-TATCTAACTATATTTACTGATGCTGTTA-3'	58	276	56	56
9	5'-GCTGTCTTTTGGCTTC-3'	5'-CCAAAAGGTATGCTAAATAC-3'	58	183	54.5–56	52.2–49.7
9br	5'-GCTTAAAATTTGTATACATAA-3'	5'-CCTGGAGTGGTGCTTCATGCAT-3'	58	193	55–60	52–48
10a	5'-CTACAGTGATAAACAAGACAT-3'	5'-ATTCCTGCTGCTTTGGTT-3'	62	292	55–58	58–55
10a2	5'-CATTTTTTGGTGTTTATGATAGCAAG-3'	5'-GTGTATAGTTACCATTATAGTCACATC-3'	62	252	54–57–60.5	57–54–50
10b	5'-ATTATCCTGAGTCTTATGTC-3'	5'-TCTCAAAATATCACACTAAGTTA-3'	58	229	54–57	56.2–51.2
10c	5'-ACCTTTAGCAGTCACTGTC-3'	5'-CTGTGAGTAACAGGTAGATG-3'	58	307	54–59	59–54.4
11	5'-GAAAGAGCTCAATTTCTAGC-3'	5'-CACTTCAAAGGTTTATGGT-3'	58	307	52–55	58–55
12a	5'-TGTAATCAATATGGGAGAATGCC-3'	5'-TGGAAGAATATTTGGAATGGTAAT-3'	58	269	54–56	56–54
12b	5'-GAGGTTTTTAGGAGAGTCTC-3'	5'-ATGTGCTCTGTTGTTTCTG-3'	58	315	54–57.5	58–53.4
13	5'-CACAGTTTATGCACTTGTAG-3'	5'-CTGCCTCAAAGCACATGCC-3'	62	380	57–61	59–54
14	5'-GCTCTTCCCTACTCTTTTGG-3'	5'-TATGCCCTTAGCAACAAA-3'	58	191	60	54
15	5'-ACTTGGCTGTAGCTGATTGA-3'	5'-TCAAGAGTCCGCTCAGTAAAGT-3'	62	247	57	57
16	5'-CATTTTTTGTACTTTTGTGATGG-3'	5'-CTCTATTTTTTACCTTTCTC-3'	58	579	55–58	63–60
17	5'-ATTTGGCTCTATGCCTGTGG-3'	5'-ACTGCACACAACTAGGGTG-3'	58	385	55.5	58.8
18	5'-AGAAAGTTGTGTAGCTCTTTTCT-3'	5'-GCGGTTATTTGGTGAAGAGG-3'	58	367	53–56	57–54.4
19a	5'-TCATGTCACTTAGGTTATCTGG-3'	5'-CCTTCAAGTATTAGTGGGTTTTA-3'	58	242	55–57.5	58–55.7
19b	5'-TGAGGGGAAGTGAAGAAGT-3'	5'-GCAAAAAGCAAATAAAGCC-3'	58	236	53.5–57.5	56.5–52.5
20	5'-CCACCCTGGCTGATTTATCG-3'	5'-GCATGTAAGAGAAGCAAAAATTA-3'	62	402	57–59	59–57
21	5'-AGCAAAAATTAAGTCCAGCAA-3'	5'-TCAGAGCCAGAGAAGATG-3'	58	393	57–59	59–57
22	5'-TGCTACTCTTTAGCTTCTAC-3'	5'-GGCTGATTGTCTTCTTTAAGG-3'	58	331	56.5–58	58.6–57
23.1	5'-TTTGTATCACTTATTTGTGTGA-3'	5'-CTTTTACATAGAACCCGCTGTTTTT-3'	58	283	56–57	58.2–57.2
23.2	5'-GGCTTAAATGCTGTATA-3'	5'-GAGATTACCATTATTAATCTAAAGT-3'	58	270	53–59	57–51.3
23a	5'-AGCCAGAATATGAGACATGATGGG-3'	5'-TCTACTAATTTCTGGCACAATAAG-3'	62	446	54.5	60.3
24	5'-TTGAAGTCTTTGTTCATGCTTT-3'	5'-GATAATCTAGCTATCTTAAATTCC-3'	58	266	53–58	57–52.1
25	5'-AATTTATAGATGAGGAATG-3'	5'-GTACTCTTTTACATGAAGTTCCT-3'	54	335	52–54–57	58–56–53.7
26	5'-GCTTTGCTAATGTCAAGTCA-3'	5'-GATAGTGAACACTCTCCGTTTAA-3'	62	342	56–58	58–54
27a	5'-ATGGTCTGTAGGTTTGTG-3'	5'-GCCACCAGGCCACTGTGTAG-3'	62	361	57	59
27b	5'-TTGCTTTTAAAATATTTTTTCATTTTAG-3'	5'-CCCAGTTGACTTAACAGGAATT-3'	58	330	55	55
28	5'-AAAATAAAATTTGATTTAGTGGCATCTG-3'	5'-AAATGTCACGTAAAGGCTGTGCG-3'	62	636	55–58	62–60
29	5'-TCTGGAGCCTTTTAGAATTTTATGT-3'	5'-TCAGTTTGTATTTGGGGTTTGTTC-3'	62	460	58–60.5	60–55.5
30	5'-GAAAAAATTTTGGAACTATAAGG-3'	5'-TAACAATTAATCTAAGACAATAAG-3'	58	322	51–56.5	58–54
31	5'-TTTTTCCCGAATTTCTTTATG-3'	5'-CTTCAGAAAGCATGTAGACTCAC-3'	58	425	55–57	61–59
32	5'-ATCTAGTATTTTGTAGGCCTCAG-3'	5'-CCTTCTGTACTATAGCATATCTG-3'	58	312	53–56	58–55
33	5'-TGCTAAAACCTTTGAGTCCCATG-3'	5'-GTGCTCTAACCCAAGTTGC-3'	64	448	56–59	59–53.8
34	5'-TTCTAAAATTCAAAATGAAAATGG-3'	5'-AAAAACACTTGCAGTGTAG-3'	58	432	51.5–57	60–55
35	5'-GCATGGACTGTGTATTGGTA-3'	5'-TCTGTGGATCTTTAATTGCA-3'	58	319	53.5	56.8
36	5'-GCTGGACCAGTGGACAGAAC-3'	5'-GACGTTTAAATTTGAGGTCAATGA-3'	62	389	53–58	57.8–54.3
37	5'-TCCTGAATTCATCCGAGATT-3'	5'-TCATTTTGGGTATCAGTGTGAA-3'	58	237	54–56	55.5–53.5
38	5'-AACTGCAATGTGTTTGAAGAG-3'	5'-GAGGTTCCAGATTACTCAAATTTAG-3'	62	257	57–60	56–53
39	5'-TTGAACACAAAATTAAGTGAAGCC-3'	5'-GAAGTAAAGTTAGCCCTTATGTCTTAC-3'	62	318	56	55
40	5'-ATTCACATTCACATATGCATGTTTACCTTC-3'	5'-CTTTGGTTCAAGACACTACAG-3'	62	547	55–56	61.6–61.1
41	5'-GTGCACATTAACAGTACTAT-3'	5'-ATCTAGAGATGGCCTAGGAAG-3'	62	373	55.5	58
42	5'-CTTGGAAAGGCAAACGATGGTTG-3'	5'-CCATGTCAGTGAAGCAAGTTTGTG-3'	58	356	55–60	56–52.2
43	5'-AGTGTATTTCCCATTTATAGACTG-3'	5'-CATTTAAAATAAGGTGGGAGA-3'	58	234	55–57.5	56–52.4
44	5'-GAAGTAACATTGAAATAGTTAGG-3'	5'-TCCAGTCTACTTTTAGGAGGCC-3'	58	271	58.5	55
45	5'-CATGAATAGGATACAGTCTTCTAC-3'	5'-GTTAAATGCTTACCAGTAATGTG-3'	62	269	57	56
46	5'-CTCATCTCCCTTTAATTTTGGC-3'	5'-TCTGGAGAAGGATGGTTGATG-3'	58	295	54–56.5	57–55.1
47	5'-CTGTTACAATTTAAAGATACCTTG-3'	5'-GTATGCCCTGCTTTAAGAACACACA-3'	62	185	55.5	51.4
48	5'-AAGGAAGAAAAATAGTAAATTAAGTCC-3'	5'-GTTTATAGCAAATTTTGCTCCTT-3'	58	423	53–58	61–56.9
48a	5'-ATTCAATAATTTAAACCAGATCC-3'	5'-CTTTAGGAACCTTGTAAAGCCACC-3'	58	327	54	58
49	5'-AGAATGTGTCCCGTTGTGTTAA-3'	5'-TAATGAACCCATCCGGTTTGTG-3'	58	369	58.5	58.4
KRAS ex2	5'-ACTGGTGGAGTATTTGATA-3'	5'-GTATCAAAGAATGGTCCCT-3'	50	—	—	—
KRAS ex3	5'-ATAATAGCCAATCCTAA-3'	5'-ATGGCATTAGCAAAG-3'	53	—	—	—
BRAF ex11	5'-TCATAATGCTTGTCTGATAGGA-3'	5'-GGCCAAAATTTAATCAGTGGGA-3'	60	—	—	—
BRAF ex15	5'-TCCCTCTCAGGCATAAGGTAA-3'	5'-CGAACAGTGAATATTTCTTTGTAT-3'	58	—	—	—

T_m PCR, indicates PCR melting temperature (°C); Ampl. Size, amplicon size; tdHPLC, range in temperature used with high-performance liquid chromatography; % B, starting concentration for buffer B used in dHPLC.

Table W2. Detailed Somatic Events of Four Components in the MAPK Pathway.

Tumor ID	MSI Status	<i>KRAS</i> ^{mut}	<i>BRAF</i> ^{mut}	<i>NF1</i> ^{mut}	MLPA	Real-time	<i>RASSF1A</i>
848	MSI	WT	WT	NP	NP		U
854	MSI	c.184-189delGAG	WT	NP	NP		U
884	MSI	WT	V600E	D1302Y/V2577G	WT		U
894	MSI	c.49insTTG	WT	NP	NP		U
910	MSI	WT	WT	c.(3114-50)delTG	WT		U
912	MSI	G13D	WT	NP	NP		U
955	MSI	WT	V600E	NP	NP		U
965	MSI	WT	V600E	NP	NP		U
980	MSI	WT	V600E	WT	WT		U
984	MSI	WT	V600E	WT	WT		U
988	MSI	WT	WT	NP	NP		ND
1022	MSI	WT	WT	NP	NP		U
1044	MSI	WT	V600E	c.480-57C>T	WT		U
1047	MSI	G12A/V14I	WT	WT	WT		M
1066	MSI	WT	WT	WT	WT		M
1117	MSI	WT	WT	NP	NP		U
1132	MSI	G12V	WT	NP	NP		U
1141	MSI	WT	WT	NP	NP		U
1190	MSI	WT	V600E	NP	NP		M
1193	MSI	WT	V600E	c.7395-7C>T	WT		U
1268	MSI	WT	V600E	Ex3+24G>A	WT		U
1273	MSI	WT	V600E	c.(1392+46_+53)delTT	WT		U
1314	MSI	WT	WT	NP	NP		U
1326	MSI	G13D	WT	c.(1392+46_+53)delT	WT		U
1341	MSI	WT	V600E	c.(61-4_-12)delT	WT		M
1349	MSI	WT	WT	NP	NP		M
1363	MSI	G13D	WT	WT	Gain of IVS27b-Ex49	1.66	U
1388A	MSI	WT	WT	NP	NP		M
1388C	MSI	G13D	WT	NP	NP		M
868	MSS	WT	WT	NP	NP		U
886	MSS	G12D	WT	NP	NP		U
887	MSS	G12C	WT	NP	NP		M
896	MSS	WT	WT	WT	Gain of whole gene	1.59	M
904	MSS	WT	WT	NP	NP		U
922	MSS	G12V	WT	NP	NP		U
923	MSS	G13D	WT	NP	NP		U
927	MSS	G12V	WT	NP	NP		M
946	MSS	WT	WT	NP	NP		U
948	MSS	G12R	WT	NP	NP		U
953	MSS	WT	WT	WT	WT		U
966	MSS	Q61L	WT	NP	NP		U
974	MSS	G12A	WT	NP	NP		U
976	MSS	G12D	WT	NP	NP		M
1013	MSS	WT	D594G	NP	NP		U
1024	MSS	G12C	WT	NP	NP		U
1027	MSS	G13D	WT	NP	NP		U
1029	MSS	G12D	WT	NP	NP		U
1046	MSS	WT	WT	NP	NP		U
1060	MSS	WT	WT	NP	NP		M
1069	MSS	WT	WT	NP	NP		U
1103	MSS	WT	WT	WT	Gain of whole gene	1.25	U
1111	MSS	WT	WT	NP	NP		M
1121	MSS	G12A	WT	WT	WT		M
1124	MSS	G12D/G13D	WT	WT	WT		ND
1166	MSS	G13D	WT	WT	WT		M
1167	MSS	WT	WT	WT	Gain of whole gene	1.67	M
1194	MSS	WT	WT	NP	NP		M
1197	MSS	WT	WT	NP	NP		ND
1287	MSS	WT	WT	c.2252-31A>G	WT		ND
1294	MSS	WT	G469R	NP	NP		U
1296	MSS	G13D	WT	WT	WT		U
1340	MSS	WT	WT	WT	WT		ND
1364	MSS	WT	L597Q	NP	NP		M
1369	MSS	G12D	ND	NP	NP		ND
1391	MSS	G12D	WT	c.2252-31A>T	WT		U