

Supplementary table S5. Primers used in the study for Sanger sequencing and qPCR.

Gene, primer	5' to 3' primers sequence	Reference sequence	Bases in sequence	Bases in GRCh38 assembly	Amplicon (gDNA)	PCR method
NRASex1-F	GCTCGCCAATTAACCCTGAT	NG_007572.1	682-701	Chr1:114716194-114716213	213 bp	58,BE
NRASex1-R	CGACAAGTGAGAGACAGGATCA	NG_007572.1	873-894	Chr1:114716022-114716001		
NRASex2-F	GGGCTTGAATAGTTAGATGCT	NG_007572.1	2802-2822	Chr1:114714093-114714073	543 bp	58,BE
NRASex2-R	GGTTCCAAGTCATTCCCAGTA	NG_007572.1	3324-3344	Chr1:114713571-114713551		
NRASex3-F	GCTGAGATTGCAGGCATGA	NG_007572.1	7087-7105	Chr1:114709790-114709808	285 bp	58,BE
NRASex3-R	CAACTGATGCAAACTCTGCACA	NG_007572.1	7349-7371	Chr1:114709524-114709546		
KRASex1-F	GTCACATTTTCATTATTTTATTATAA	NC_000012.12	5509-5535	Chr12:25245397-25245423	155 bp	60,NA
KRASex1-R	TTTACCTCTATTGTTGGATCATATTC	NC_000012.12	5638-5663	Chr12:25245269-25245294		
KRASex2-F	GGTGCACTGTAATAATCCAGACTGT	NC_000012.12	23479-23503	Chr12:25227429-25227453	301 bp	60,NA
KRASex2-R	GCATGGCATTAGCAAAGACTCA	NC_000012.12	23758-23779	Chr12:25227153-25227174		
KRASex3-F	GATATTTGTGTTACTAATGACTGTGCTATA	NC_000012.12	25110-25139	Chr12:25225793-25225822	211 bp	60,NA
KRASex3-R	ACCTGTCTTGCTTTGCTGAT	NC_000012.12	25300-25320	Chr12:25225612-25225632		
RRASex1-F	GCGAAGGCAGCAGCAGCGGT	NC_000019.10	20-39	Chr19:49640105-49640124	201 bp	58,BE
RRASex1-R	CTCCCGGGTGAGGGCCCACTA	NC_000019.10	200-220	Chr19:49639924-49639944		
RRASex2-F	GTCTGCCGGGCACTGCAGTA	NC_000019.10	2969-2989	Chr19:49637155-49637175	173 bp	58,BE
RRASex2-R	GACACCACCCCATCCATCAT	NC_000019.10	3121-3141	Chr19:49637003-49637023		
RRASex3-F	GGAGCCTGGTCCCCACGAT	NC_000019.10	3176-3194	Chr19:49636950-49636968	201bp	58,BE
RRASex3-R	GAGCCAGGTCCTCCCACA	NC_000019.10	3357-3376	Chr19:49636768-49636787		

RRASex4-F	CCTCATGCCTCCGGCTTCACT	NC_000019.10	3391-3411	Chr19:49636733-49636753	189 bp	58, BE
RRASex4-R	GGAAGGAGCTCCAGACTGAGAT	NC_000019.10	3556-3579	Chr19:49636565-49636588		
RRASex5_6-F	CCTGAGTGCAGGACCTGACT	NC_000019.10	4247-4267	Chr19:49635877-49635897	374 bp	58, BE
RRASex5_6-R	CGAAGGCAGCTAGTCCCGAGAGCT	NC_000019.10	4597-4620	Chr19:49635524-49635547		
PTENex1-F	GCAGCTTCTGCCATCTCTCT	NG_007466.2	980-999	Chr10:87864418-87864437	200 bp	63, BE
PTENex1-R	CATCCGTCTACTCCACGTT	NG_007466.2	1160-1179	Chr10:87864598-87864617		
PTENex3-F	GTGGGGAAAACCTTCTTTTCA	NG_007466.2	30482-30502	Chr10:87893920-87893940	282 bp	63, BE
PTENex3-R	TCTTTTCTGTGGCTTAGAAATCTT	NG_007466.2	30739-30763	Chr10:87894177-87894201		
PTENex4-F	AATCTGTCTTTTGGTTTTTCTTGA	NG_007466.2	61977-62000	Chr10:87925415-87925438	233 bp	62, BE
PTENex4-R	ACTCTACCTCACTAACAAGCAGA	NG_007466.2	62185-62209	Chr10:87925623-87925647		
PTENex5-F	AAAGATTCAGGCAATGTTTGTT	NG_007466.2	67516-67537	Chr10:87930954-87930975	235 bp	62, BE
PTENex5-R	TCTCACTCGATAATCTGGATGAC	NG_007466.2	67728-67750	Chr10:87931166-87931188		
PTENex6i-F	GGAATCCAGTGTCTTTTAAATACC	NG_007466.2	69452-69477	Chr10:87932890-87932915	261 bp	62, BE
PTENex6i-R	TTCGTCCCTTCCAGCTTTA	NG_007466.2	69693-69712	Chr10:87933131-87933150		
PTENex6ii-F	TGAAGATCTTGACCAATGGCT	NG_007466.2	69636-69656	Chr10:87933074-87933094	222 bp	63, BE
PTENex6ii-R	CAGATCCAGGAAGAGGAAAGG	NG_007466.2	69837-69857	Chr10:87933275-87933295		
PTENex10-F	GGCTACGACCCAGTTACCAT	NG_007466.2	88588-88607	Chr10:87952026-87952045	271 bp	62, BE
PTENex10-R	TCCAATACATGGAAGGATGAGA	NG_007466.2	88837-88858	Chr10:87952275-87952296		
PTENex11-F	CAGTAAAGGCATTTCTGTG	NG_007466.2	94363-94383	Chr10:87957801-87957821	262 bp	62, BE
PTENex11-R	TCCTTATTTTGGATATTTCTCCC	NG_007466.2	94602-94624	Chr10:87958040-87958062		

PTENex12-F	GGACAAAATGTTTCACTTTTGGGTA	NG_007466.2	97455-97479	Chr10:87960893-87960917	260 bp	62,BE
PTENex12-R	CAACAACCCCAAAAAATGT	NG_007466.2	97695-97714	Chr10:87961133-87961152		
PTENex13-F	GATGAGTCATATTTGTGGGTTTTTC	NG_007466.2	101803-101826	Chr10:87965241-87965264	272 bp	62,BE
PTENex13-R	TTATTTTCATGGTGTTTTATCCC	NG_007466.2	102052-102074	Chr10:87965490-87965512		
CDKN2Aex1a-F	GAAGAAAGAGGAGGGGCTG	NC_000009.11	19561-19579	Chr9:21974913-21974931	340 bp	60,BE
CDKN2Aex1a-R	GCGCTACCTGATTCCAATTC	NC_000009.11	19881-19900	Chr9:21974592-21974611		
CDKN2Aex1b-F	GCGCTGAGGGTGGGAAGAT	NC_000009.12	21994706-21994724	Chr9:21994706-21994724	678 bp	56,PCRx
CDKN2Aex1b-R	GCCTGGGCTAGAGACGAATTATCTG	NC_000009.12	21994071-21994047	Chr9:21994071-21994047		
CDKN2Aex2-F	GGAAATTGGAACTGGAAGC	NC_000009.11	23134-23153	Chr9:21971339-21971358	574 bp	59,BE
CDKN2Aex2-R	GTGAGCTGAGCAAGACC	NC_000009.11	23690-23707	Chr9:21970785-21970802		
CDKN2Aex3-F	CAGGCGGGCAGTGGACTAGC	NC_000009.11	26051-26070	Chr9:21968422-21968441	319 bp	60,DM
CDKN2Aex3-R	CTACGAAAGCGGGTGGTTGTG	NC_000009.11	26347-26369	Chr9:21968123-21968145		
CDK4-F	GGTGATAGGAGTCTGTGATTGTA	NG_007484.2	688-710	Chr12:57751738-57751760	213 bp	62,NA
CDK4-R	ACCTCACGAACTGTGCTGAT	NG_007484.2	881-900	Chr12:57751548-57751567		
MC1R-F	GGTCCAGAGGTGTCGAAAT	NG_012026.1	1044-1062	Chr16:89918922-89918940	1324 bp	58,BE
MC1R-R	TGCCAGCACACTTAAAGC	NG_012026.1	2349-2367	Chr16:89920227-89920245		
MC1R-S1, seq primer	CCATCTCTGACGGGCTCT	NG_012026.1	1496-1513	Chr16:89919374-89919391	NA	58,BE
MC1R-S3, seq primer	TCCAGCCTCTGCTTCCTG	NG_012026.1	1769-1785	Chr16:89919646-89919663	NA	
RAC1ex1F	CCGCCGCTTCTATCTCA	NG_029431.1	184-201	Chr7:6374678-6374695	227 bp	58,BE
RAC1ex1R	CCGCGACACAGGCCCA	NG_029431.1	395-410	Chr7:6374904-6374889		
RAC1ex2F	GTGAAAGCTAAGATTACAT	NG_029431.1	12660-12679	Chr7:6387154-6387172	231 bp	58,BE
RAC1ex2R	GTTTCATATGAGGATTAAGATGGCT	NG_029431.1	12866-12890	Chr7:6387360-6387384		

RAC1ex3F	CTAGGATGGCTGGGACAGT	NG_029431.1	17363-17381	Chr7: 6391857-6391875	271 bp	58, BE
RAC1ex3R	GCAAGTCCATAGGTAACATGCACT	NG_029431.1	17610-17633	Chr7: 6392104-6392127		
RAC1ex4F	GTTGTCCTCAGTCTGACTAACTCA	NG_029431.1	24118-24143	Chr7: 6398612-6398637	209 bp	58, BE
RAC1ex4R	CACTCAAGGAGCTTGAGGCT	NG_029431.1	24307-24326	Chr7: 6398801-6398820		
RAC1ex5F	GTAGAAAGCAAAGTGCATGCTTCA	NG_029431.1	25562-25585	Chr7: 6400056-6400079	183 bp	58, BE
RAC1ex5R	CTGAGAGGATCATTCTATAACTTACAT	NG_029431.1	25718-25744	Chr7: 6400238-6400212		
RAC1ex6F	GTGATTTAGGTGAAGGACATCTGTA	NG_029431.1	27309-27333	Chr7: 6401803-6401827	291 bp	58, BE
RAC1ex6R	GGACTCCAGTCTGTCTACTACAAT	NG_029431.1	27575-27599	Chr7: 6402093-6402069		
RAC1ex7F	GGTGGTGTGATCAGAAGAGAGT	NG_029431.1	27759-27780	Chr7: 6402253-6402274	226 bp	58, BE
RAC1ex7R	GGGACAGGACCAAGAACGA	NG_029431.1	27966-27984	Chr7: 6402478-6402460		
METex1F	CTGAACTGCTCTCGCCTTGA	NG_008996.1	26633-26652	Chr7: 116699037-116699056	703 bp	58, BE
METex1intr	GCCTTCTCACTGATATCGAATGCA	NG_008996.1	27312-27335	Chr7: 116699739-116699716		
METex1intF	CCGGTTCATCAACTCTTTTGTGA	NG_008996.1	27250-27271	Chr7: 116699654-116699675	713 bp	58, BE
METex1R	GCCCTGGATATTCTTTTTGAGACT	NG_008996.1	27939-27962	Chr7: 116700366-116700343		
METex2F	CAGGCTCTGAAAATACACACTGA	NG_008996.1	59176-59198	Chr7: 116731580-116731602	411 bp	58, BE
METex2R	CAGTCTGTGTTCCAGTTTCTACTATGAT	NG_008996.1	59559-59586	Chr7: 116731990-116731963		
METex3F	ACTGAGCTTGTGGAATAAGGAT	NG_008996.1	67500-67522	Chr7: 116739904-116739926	251 bp	58, BE
METex3R	GTGACACTGGTTGTAATATGCAT	NG_008996.1	67727-67750	Chr7: 116740154-116740131		
METex16F	CACTGTTCCATAATGAAGTTAATGTCT	NG_008996.1	106326-106352	Chr7: 116778730-116778756	336 bp	58, BE
METex16R	TGTGCATCTTTGGCTACTAGCT	NG_008996.1	106640-106661	Chr7: 116779065-116779044		
METex17F	GGTCAAATTAGAACAGTAGATGCTTA	NG_008996.1	109525-109551	Chr7: 116781929-116781955	229 bp	58, BE

METex17R	GGAAACAGATTCTCTTGTCACT	NG_008996.1	109730-109753	Chr7: 116782157-116782134		
METex18F	GGATTTCAAATACTGAAGCCACT	NG_008996.1	110798-110820	Chr7: 116783202-116783224	418 bp	58, BE
METex18R	GTACGTATCAGACAAGTCCATTTTACAT	NG_008996.1	111187-111215	Chr7: 116783619-116783591		
METex19_20F	ATGTATGGTCACATCTCTCACCTCA	NG_008996.1	123201-123225	Chr7: 116795605-116795629	564 bp	58, BE
METex19_20R	CCATTGGACAAAGTGTGGACTGT	NG_008996.1	123742-123764	Chr7: 11679614-116796168		
Expression primers used in the study						
Primer	Sequence 5' to 3'	Ref sequence	Bases	Coding transcripts	Amplicon size	PCR (A _{temp} , mix)
EPHA2cDNA-F	CCGCAAGAAGGGGAGACTCCA	NM_004431.3	c.1419-1438	NM_004431	164 bp	60, Max
EPHA2cDNA-R	ACAGCGTCTGGAATTCGTGC	NM_004431.3	c.1563-1582			
FLIcDNA-F	GGATCCAGGGAGTCTCCGGT	NM_002017.4	c.223-242	NM_002017, NM_001271010, NM_001167681	192 bp	60, Max
FLIcDNA-R	CACATGCTCCTGTGCCACAGT	NM_002017.4	c.393-414			
EFNA1cDNA-F	CCACACCGTCTTCTGGAACA	NM_004428.2	c.59-79	NM_004428, NM_182685	213 bp	60, Max
EFNA1cDNA-R	GCCAGCGGACTTGGTCCT	NM_004428.2	c.254-271			
ANPEPcDNA-F	CCAAAATCAGACCAGATTGGC	NM_001150.2	c. 1015-1035	NM_001150	209 bp	60, Max
ANPEPcDNA-R	CACAGGTCATTCCACCACTCTA	NM_001150.2	c. 1202-1223			
CAV1cDNA-F	GTGGTCAAGATTGACTTTGAAGAT	NM_001753.4	c. 187-210	NM_001753, NM_001172896, NM_001172897, NM_001172895	249 bp	60, Max
CAV1cDNA-R	GCTGATGCACTGAATCTCAATCA	NM_001753.4	c.413-435			
METcDNA-F	CTGGAAGAAGATCACGAAGAT	NM_001127500.1	c. 1517-1538	NM_001127500, NM_000245	128 bp	60, Max
METcDNA-R	CGATCGCACACATTTGTCGT	NM_001127500.1	c. 1625-1644			
FH1_CCND1 ^A	GCCTTAAGATGAAGGAGAC	NM_053056.2	c.328-347	NM_053056	94 bp	60, Max
RH1_CCND1 ^A	CCATTTGCAGCAGCTC	NM_053056.2	c.406-421			
FH3_EGFR ^A	TCTTAAAGACCATCCAGGAG	NM_005228.3	c.233-252	NM_005228.3, NM_201282.1, NM_201283.1, NM_201284.1	85 bp	60, Max
RH_EGFR ^A	ATCTGCAGGTTTTCAAAG	NM_005228.3	c.299-317			
KRAScDNA	GATCCAACAATAGAGGATTCTACAG	NM_004985.3	c.97-122	NM_004985, NM_033360	82 bp	60, Max
KRAScDNA-R	CTGCTGTGTCGAGAATATCCAAG	NM_004985.3	c.156-178			

MITFcDNA-F	CCCAAGTCAAATGATCCAGAC	NM_198159.2, NM_000248.3	c.997-1017 c.694-714	NM_198159, NM_198177, NM_006722, NM_000248, NM_198158, NM_198178, NM_001184967	129 bp	60, Max
MITFcDNA-R	CAGTTTCTTCTGTCGGTTTTCAAGT	NM_198159.2, NM_000248.3	c.1104- 1125 c.801-822			
RRAScDNA-F	CGCTGACCATCCAGTTCAT	NM_006270.4	c.131-149	NM_006270	124 bp	60, Max
RRAScDNA-R	GCGGTGTCCAGGATGTCCA	NM_006270.4	c. 236-254			
SOX10cDNA-F	GCTCTGGAGGCTGCTGA	NM_006941.3	c.420-436	NM_006941	207 bp	60, Max
SOX10cDNA-R	CTCTGTAGTGGCCTGGAT	NM_006941.3	c.607-626			
MAP3K8cDNA-F	CTGGAATTTTATTAACATGGTCATCAC T	NM_005204.3	c.317-345	NM_005204, NM_001244134	181 bp	60, Max
MAP3K8cDNA-R	CACGCCATTCTTTCTTCGTCT	NM_005204.3	c.476-497			
HPRT1cDNA-F	GACTGCGCAAACAATGCAGAC	NM_000194.2	c.412-434	NM_000194	93 bp	60, Max
HPRT1cDNA-R	GGTCCTTTTACCAGCAAGCT	NM_000194.2	c.484-504			
HBMScDNA-F	ACAACCGGGTGGGGCAGA	NM_000190.3	c.596-613	NM_000190, NM_001024382	111 bp	60, Max
HBMScDNA-R	CCACCAGATCCAAGATGTCCTG	NM_000190.3	c.685-706			

A Commercial expression primer sequences from SigmaAldrich.

PCR method is given as annealing temperature, additives BE=betaine 1 M final concentration, NA=no additives, PCRx= PCRx Enhancer system, ThermoFisher Scientific), DM=DMSO 5% final concentration, Max=Maxima SYBR Green qPCR Master Mix, ThermoFisher Scientific.

BRAF mutational analyses on genomic DNA were performed using primers published in Davies H. et al. (2002) Nature 417(6892):949-954.