Table 8. Summary of the PCR and sequencing primers for DNA methylation analysis

Region	Primer sequences and annealing temperature	Product length, bp	Sequencing primers	CpGs, no.	Sequence length
P16	Forward	175	GGTTGATTGGTTGGTTA	5	21
	GGYGGGGGAGTAGTATGGAGTTTT				
	Reverse (5'-Biotin)				
	ACAAACCCCTCACCCACCTAAA				
	58/56/54/52°C				
RUNX3	Forward	195	GTATTTATTTTGAAGG	3	16
	GGGTATTTTTTATTTTTATTGT				
	Reverse-Universal				
	GGGACACCGCTGATCGTTTAACAACCCCAACTTCCTCTA				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
TIMP3	Forward	296	ATTTTTTATAAGGATTTG AA	6	40
	TTTTGGTTTGGGTTAGAGATAT				
	Reverse-Universal				
	GGGACACCGCTGATCGTTTACCCCCTCAAACCAATAAC				
	Universal (5'-Biotin)				

Region	Primer sequences and annealing temperature	Product length, bp	Sequencing primers	CpGs, no.	Sequence length
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
RASSF1A	Forward	298	Seq1:		21
	GGGGGAGTTTGAGTTTATTGA		GGGTAGTATTAGGTTGGA G	5	
	Reverse (5'-Biotin)		Seq2:		
	CTACCCCTTAACTACCCCTTCC		GATTTTATTTGGGG	3	9
	55°C				
DAPK	Forward		Seq1:		
	GGAGTGTGAGGAGGATAGT	355	TTTTTAGTTGTGTTTT	6	27
	Reverse (5'-Biotin)		Seq2:		
	CCTTAACCTTCCCAATTACT		GGGGGAGTTTTTAGG	4	23
	55°C				
hTERT	Forward	167	TGGGAAGTTTTGGTTT	6	24
	TGTTGYGTAYGTGGGAAGTTT				
	Reverse-Universal				
	GGGACACCGCTGATCGTTTAAACCCCTAAAACCCCAAAC				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
ER	Forward	125	GGATACGGTTTGTATTTT G	3	13

Region	Primer sequences and annealing temperature	Product length, bp	Sequencing primers	CpGs, no.	Sequence length
	TGTGTTTTTTTTTAGGTGG				
	Reverse (5'-Biotin)				
	AACCATCCCAAATACTTTAATA				
	58/56/54/52°C				
MyoD1	Forward	160	GAGGTTTGGAAAGGG	2	10
	AATTAGGGGATAGAGGAGTATTGAAAG				
	Reverse-Univeral		GAAAGTTAGTTTAGAGGTGA	6	27
	GACGGGGACACCGCTGATCGTTTAACAACCCTAAAACRACTACACTTAACTC				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
SFRP1	Forward	218	GTTTGGTTTTAGTAAAT	5	23
	TGTTTTTTAAGGGGTGTTGA				
	Reverse-Universal				
	GGGACACCGCTGATCGTTTACTCCRAAAACTACAAAACTAAAAT				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
N33	GYGYGTGGAGGAGATATTGTTT	195	GGAGGAGATATTGTTTGT	6	33

Region	Primer sequences and annealing temperature	Product length, bp	Sequencing primers	CpGs, no.	Sequence length
	GACGGGGACACCGCTGATCGTTACATTCTACCTCCTTTTTCTTCTAT				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
HPP1	Forward	175	GGGATGTTTAGTAGTT	4	18
	TGTTTTTTYGTYGGGTGTTATTGTTAT				
	Reverse-Universal				
	GACGGGGACACCGCTGATCGTTATCCCACAAACACCATAACTAATTC				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				
	58/56/54/52°C				
BRAF-exon11	Forward				
	GGCTTGACTTGACTTTTTTACTGT	192	TACAGTGGGGACAAAGAAT		TGG/A[TC]ATCTGG/C[AT]ATCATTTG/C[AT]GAACA
	Reverse-Universal				
	GGGACACCGCTGATCGTTTACATTACATACTTACCATGCCACTT				
	Universal (5'-Biotin)				
	GGGACACCGCTGATCGTTTA				

Region	Primer sequences and annealing temperature	Product length, bp	Sequencing primers	CpGs, no.	Sequence length
	55°C				
BRAF-exon15	Forward (5'-Biotin)	156	Seq1:	Analyze1:	
	GCTTGCTCTGATAGGAAAATGA		ATTTTTACTGTGTGAGGTCT T	T/CATGAAGAAATATA TCTG	
	Reverse		Seq2:	Analyze 2:	
	GACAACTGTTCAAACTGATGGG		CCACTCCATGGAGATTT	A/CT/ACTGTAGCTC/A C/GACG/CC/AAAATCA CCT	