Supplemental Table 1A: $MgCl_2$ Concentrations Used in the Methylation-Specific PCR Assays

$MgCl_2$ (mM)	Gene Target
1.5	p14(M), p16(M), CDKN2B(M), DAPK1(M), GSTP1(M), MGMT(M), CDH1(M), PTEN(U&M)
2	RASSF1A(U&M)
2.5	p14(U), p16(U), CDKN2B(U), DAPK1(U), GSTP1(U), MGMT(U), CDH1(U)

U:unmethylated target; M:methylated target

Supplemental Table 1B: Thermal Profiles for Methylation-Specific PCR Assays

Reaction Profile			Thermal profile
Initial denaturation	10 min		95°C
Denaturation	40 sec*	x 40	95°C
Annealing	45 sec cycles	x 40	**56°C** for CDH1(U&M) **60°C** for RASSF1A(U&M), p14(U&M), p16(U&M), CDKN2B(U&M), DAPK1(U&M), GSTP1(U&M) & MGMT(U&M) **64°C** for PTEN(U&M)
Extension	45 sec	x 40	72°C
Final extension	7 min		72°C

U:unmethylated target; M:methylated target; * 30 sec for PTEN(U&M)

Supplemental Table 1C: Assay Conditions and Thermal Profiles for Bisulfite PCR Assays

Assay Conditions		Reaction Profile			Thermal profile
10X Buffer II	1X	Initial denaturation	10 min		95°C
$MgCl_2$	3.0mM*	Denaturation	40 sec		95°C
dNTP	200μM	Annealing	45 sec**	x 40 cycles	**57°C** for HsPromoter, HsExon1, MmExon1, and PTENexon1 **60°C** for RhPromoter, RhExon1, and MmPromoter
Forward primer	200nM				
Reverse primer	200nM				
AmpliTaq Gold	1U				
Bisulfite converted DNA	80ng	Extension	45 sec**		72°C
Total reaction volume	*25ml*	Final extension	7 min		72°C

* 2.0mM for PTENexon1; ** 40 sec for PTENexon1

Supplemental Table 1D: Assay Conditions and Thermal Profiles for Colony PCR Assays

Assay Conditions		Reaction Profile			Thermal profile
10X Buffer II	1X	Initial denaturation	10 min		95°C
$MgCl_2$	4.0mM	Denaturation	1 min		95°C
dNTP	200μM	Annealing	1.5 min	x 10 cycles	**55°C – 0.5°C**/cycle
Forward primer	100nM	Extension	1 min		72°C
Reverse primer	100nM	Denaturation	1 min		95°C
AmpliTaq Gold	1U	Annealing	1.5 min	x 18 cycles	50°C
Template DNA	colony	Extension	1 min		72°C
Total reaction volume	*25μl*	Final extension	7 min		72°C

Supplemental Table 1E: Assay Conditions and Thermal Profiles for Real-Time Quantitative Methylation-Specific PCR

Assay Conditions		Reaction Profile			Thermal profile
10X Buffer A	1X	Initial denaturation	10 min		95°C
$MgCl_2$	4mM	Denaturation	15 sec		95°C
dNTP	200μM	Annealing	15 sec	x 50cycles	56°C
Forward primer	200nM	Extension	30 sec		72°C
Reverse primer	200nM				
TaqMan probe	400nM				
AmpliTaq Gold	1U				
Bisulfite-converted DNA	80ng				
Total reaction volume	*25μl*				

Supplemental Table 1F: Assay Conditions and Thermal Profiles for Real-Time RT-PCR for RASSF1A mRNA

Assay Conditions		Reaction Profile			Thermal profile
EZ RT-PCR Buffer	1X	Annealing	2 min		50°C
$MnCl_2$	3mM	Reverse transcription	30 min		60°C
dATP, dCTP, dGTP	300μM	Initial denaturation	2 min		95°C
dUTP	600μM	Denaturation	20 sec	x 45 cycles	94°C
Forward primer	400nM	Annealing & Extension	1 min		60°C
Reverse primer	400nM				
TaqMan probe	200nM				
UNG	0.25U				
r*Tth*	2.5U				
Total RNA	450ng				
Total reaction volume	*25μl*				