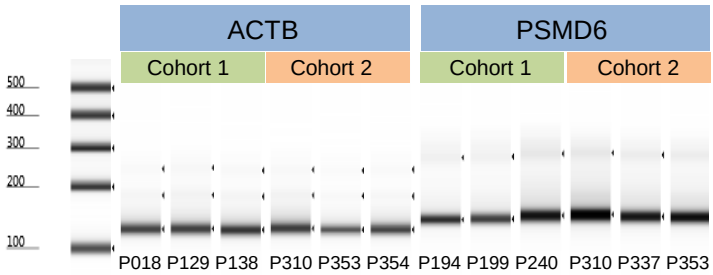
A



B

Primers used for methylation-specific PCR

Gene[a]	Primer sequences, 5'-3'	Amplicon (bp)[b]	Tm (°C)[c]
ACTB_F	TGGTGATGGAGGAGGTTTAGTAAGT	133	60
ACTB_R	AACCAATAAAACCTACTCCTCCCTTAA		
PSMD6_F	GTGTAAATTTTTCGATTGGTAGATAGAC	154	60
PSMD6_R	CGACCTTTACCATCATTACATCG		

[a]F: Forward primer, R: Reverse primer
[b]bp: Base pair
[c]Tm: Melting temperature

Supplementary Figure S2. Methylation-specific PCR (MSP) of bisulfite converted DNA isolated by different methods. (A) MSP products for selected tumors from cohort 1 and cohort 2 using primers for ACTB (β-actin) and PSMD6. DNA from tumors in cohort 1 was isolated using proteinase K, phenol, chloroform, and isoamylalcohol, whereas DNA from tumors in cohort 2 was isolated using PureLink™ Genomic DNA Mini Kit. (B) Primer sequences, amplicon length, and melting temperature for ACTB and PSMD6.