

Supplementary file

Supplementary table 1. Twenty-nine genes displayed a higher level of methylation in sputum of lung cancer patients vs. controls

Genes	Sensitivity	95% confidence interval	Specificity	95% confidence interval
<i>3OST2</i>	0.53	0.46–0.56	0.86	0.83–0.89
<i>APC</i>	0.44	0.34–0.55	0.88	0.71–0.96
<i>CDH1</i>	0.36	0.18–0.57	0.71	0.52–0.85
<i>CDO1</i>	0.79	0.68–0.87	0.68	0.44–0.86
<i>CXCL</i>	0.37	0.27–0.47	0.80	0.72–0.86
<i>CYGB</i>	0.52	0.46–0.58	0.80	0.68–0.87
<i>DAB2</i>	0.04	0.02–0.09	0.98	0.95–0.99
<i>DAL-1</i>	0.31	0.22–0.39	0.87	0.79–0.92
<i>DAPK</i>	0.46	0.41–0.52	0.80	0.65–0.89
<i>DCR2</i>	0.42	0.32–0.51	0.61	0.53–0.69
<i>FAM19A4</i>	0.81	0.73–0.87	0.26	0.22–0.31
<i>FHIT</i>	0.53	0.44–0.62	0.92	0.78–0.99
<i>GATA</i>	0.67	0.32–0.90	0.54	0.33–0.72
<i>H-cadherin</i>	0.51	0.23–0.76	0.58	0.45–0.69
<i>HOXA9</i>	0.80	0.64–0.90	0.51	0.17–0.85
<i>JPH3</i>	0.32	0.22–0.43	0.80	0.72–0.86
<i>KIFLA</i>	0.45	0.36–0.56	0.63	0.55–0.71
<i>MAGE</i>	0.46	0.35–0.57	0.83	0.57–0.93
<i>MGMT</i>	0.43	0.33–0.53	0.92	0.76–0.97
<i>p16</i>	0.49	0.41–0.57	0.91	0.83–0.95
<i>PAX5</i>	0.38	0.28–0.46	0.79	0.71–0.85
<i>PCDH20</i>	0.59	0.49–0.68	0.50	0.40–0.59
<i>PHACTR3</i>	0.61	0.53–0.67	0.69	0.63–0.74
<i>PRDM14</i>	0.63	0.57–0.67	0.77	0.73–0.81
<i>RARβ</i>	0.45	0.29–0.62	0.80	0.59–0.91
<i>RASSF1A</i>	0.29	0.22–0.39	0.96	0.92–0.96
<i>SOX17</i>	0.85	0.76–0.90	0.89	0.69–0.98
<i>SULF2</i>	0.52	0.41–0.62	0.58	0.49–0.66
<i>TAC1</i>	0.87	0.76–0.91	0.76	0.52–0.90
<i>TCF2l</i>	0.30	0.21–0.38	0.72	0.62–0.79
<i>ZFP42</i>	0.88	0.77–0.92	0.64	0.40–0.82

Supplementary table 2. CVs of the four analyses by ddMSP and qMSP on the same specimens

Diluted samples	ddMSP							qMSP							Fold
	Analyse #1	#2	#3	#4	mean	SD	%CV	Analyse #1	#2	#3	#4	mean	SD	%CV	
100.00	101.00	98.00	100.00	98.00	99.00	1.41	1.43	103.00	97.00	103.00	99.00	101.00	3.00	2.99	2.09
25.00	23.00	25.00	26.00	25.00	25.50	0.71	2.77	22.00	26.00	25.00	23.00	24.00	1.83	7.61	2.74
6.25	6.23	6.26	6.25	6.23	6.24	0.01	0.23	6.21	6.22	6.30	6.26	6.24	0.04	0.66	2.90
1.56	1.57	1.54	1.57	1.56	1.57	0.01	0.45	1.60	1.54	1.57	1.53	1.56	0.03	2.03	4.49
0.39	0.38	0.39	0.40	0.39	0.40	0.01	1.79	0.39	0.35	0.40	0.39	0.39	0.02	5.80	3.24
0.10	0.11	0.09	0.10	0.11	0.11	0.01	6.73	0.14	0.11	0.09	0.12	0.12	0.02	18.10	2.69
0.04	0.03	0.02	0.04	0.03	0.04	0.01	20.20								
0.00															

CV, coefficient of variation.

Supplementary table 3. CVs of of repeated measures by ddMSP and qMSP on different times

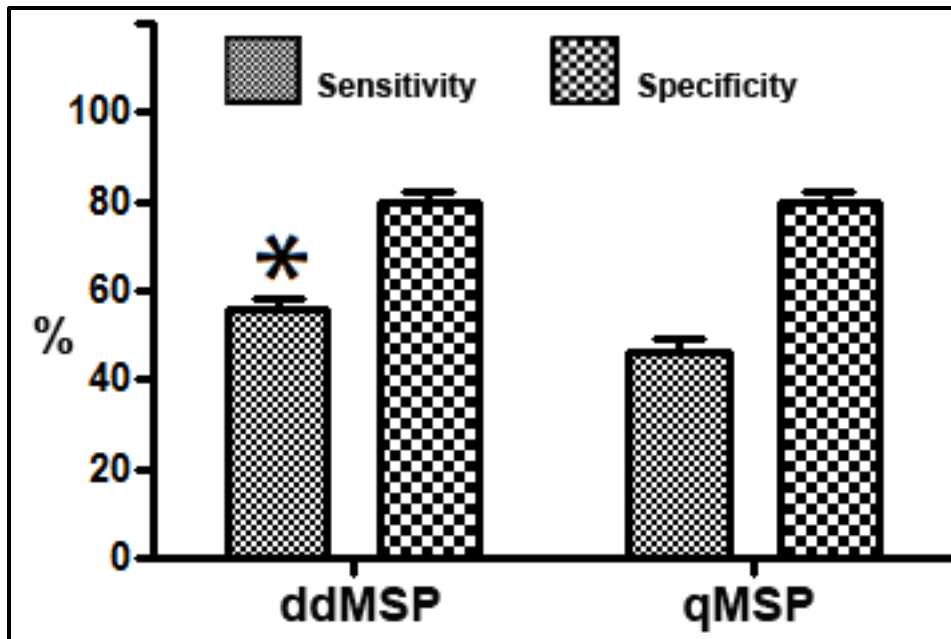
Diluted samples	ddMSP						qMSP						Fold
	Day 1	2	3	mean	SD	%CV	Day 1	2	3	mean	SD	%CV	
5000.00	5001.00	5008.00	4992.00	5001.00	6.56	0.13	5009.00	4998.00	4972.00	4998.00	19.00	0.38	2.90
2500.00	2503.00	2506.00	2490.00	2503.00	7.14	0.29	2512.00	2500.00	2478.00	2500.00	17.24	0.69	2.42
1250.00	1249.00	1235.00	1253.00	1249.00	7.90	0.63	1243.00	1228.00	1264.00	1243.00	18.08	1.45	2.29
625.00	626.00	626.00	622.00	626.00	2.00	0.32	628.00	625.00	612.00	625.00	8.50	1.37	4.28
313.00	312.00	317.00	310.00	312.00	2.99	0.95	310.00	320.00	306.00	310.00	7.21	2.31	2.42
0.00	154.00	149.00	156.00	154.00	2.99	1.95							

CV, coefficient of variation.

Supplementary table 4. CVs of repeated measures by different researches using ddMSP and qMSP

Diluted samples	ddMSP					qMSP					Fold
	Researcher #1	Researcher #2	mean	SD	%CV	Researcher #1	Researcher #2	mean	SD	%CV	
5000.00	5003.00	5008.00	5006	3.54	0.07	5010.00	4993.00	5001.50	12.02	0.24	3.40
2500.00	2505.00	2502.00	2504	2.12	0.06	2499.00	2505.00	2502.00	4.24	0.17	2.83
1250.00	1245.00	1233.00	1239	8.49	0.48	1241.00	1220.00	1230.50	14.85	1.21	2.49
625.00	627.00	623.00	625	2.83	0.32	622.00	630.00	626.00	5.66	0.90	2.82
313.00	311.00	316.00	314	3.54	0.80	312.00	322.00	317.00	7.07	2.23	2.80
156.00	153.00	158.00	156	3.54	1.61						
0.00											

CV, coefficient of variation.



Supplementary Fig. 1. Sensitivities and specificities of measuring DNA methylation of RASSF1A by ddMSP and qMSP for diagnosis of lung cancer in sputum samples of 20 cancer-free controls (normal subjects, N) and 20 patients diagnosed with lung tumor (T). ddMSP analysis of DNA methylation of RASSF1A had a higher sensitivity (55%) than did qMSP (45%) ($p=0.01$) for distinguishing lung cancer cases from controls, while maintaining the same specificity (80%). *, $p=0.01$.