

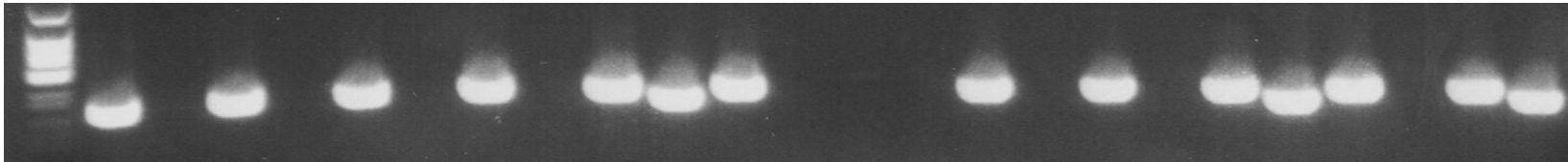
Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Brock MV, Hooker CM, Ota-Machida E, et al. DNA methylation markers and early recurrence in stage I lung cancer. *N Engl J Med* 2008;358:1118-28.

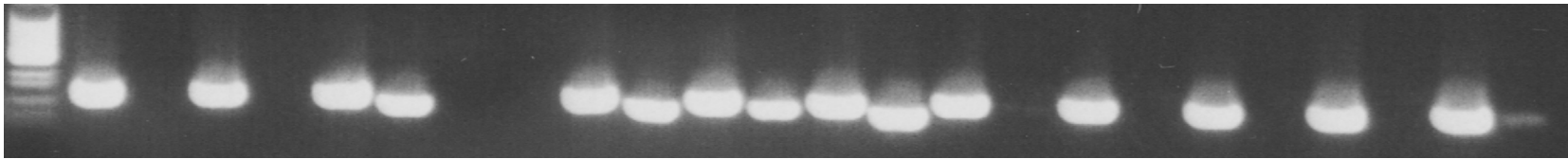
1 2 3 4 5 6 7 8 9 10 11 12

* U M U M U M U M U M U M U M U M U M U M U M U M



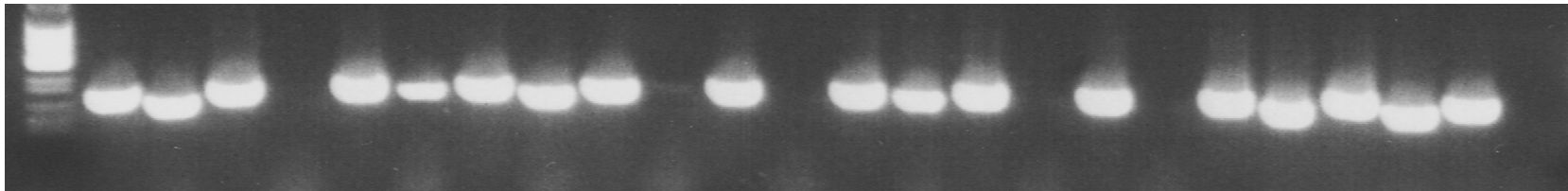
13 14 15 16 17 18 19 20 21 22 23 24

* U M U M U M U M U M U M U M U M U M U M U M U M



25 26 27 28 29 30 31 32 33 34 35 36

* U M U M U M U M U M U M U M U M U M U M U M U M



Supplementary Figure 1:

Methylation Specific PCR for the *H-cadherin* gene in 36 samples of tumors and lymph nodes, numbered at top of each panel. * represents the molecular weight marker. For each sample, the presence of a visible PCR product in Lanes U indicates the presence of an unmethylated promoter region amplified and serves as a control for sample preparation; the presence of product in Lanes M indicates a methylated gene promoter and was scored as positive for methylation. Samples 7 and 16 did not amplify for this gene. Samples 1, 2, 3, 4, 8, 9, 11, 13, 14, 20, 21, 22, 23, 26, 30, 32, 33, 36 are scored as unmethylated. Samples 5, 10, 12, 15, 17, 18, 19, 25, 27, 28, 31, 34, and 35 are methylated and samples 24 and 29 had very minimal M amplification that was scored as negative.

Supplemental Table 1: METHYLATED SPECIFIC PCR CONDITIONS USED

FLANK PCR for APC, MGMT, ASC, P16, DAPK, H-CADHERIN, RASSF1A:
Using 1:500 diluted Flank PCR product as the PCR templates:

95°C 5 Min 1 cycle
95°C 30 Sec
55°C 30 Sec
72°C 40 Sec 36 cycles
72°C 5 Min 1 cycle

INSIDE PCR for APC, MGMT, DAPK, RASSF1A:

95°C 5Min 1 cycle
95°C 30 Sec
60°C 30 Sec
72°C 30 Sec 25 cycles
72°C 5Min 1 cycle

INSIDE for P16:

95°C 5Min 1 cycle
95°C 30 Sec
64°C 30 Sec
72°C 30 Sec 20 cycles
72°C 5 Min 1 cycle

INSIDE for ASC:

95°C 5Min 1 cycle
95°C 30 Sec
66°C 30 Sec
72°C 30 Sec 20 cycles
72°C 5 Min 1 cycle

INSIDE for H-CADHERIN:

95°C 5Min 1 cycle
95°C 30 Sec
64°C 30 Sec
72°C 30 Sec 30 cycles
72°C 5 Min 1 cycle

Supplemental Table 2. Crude and Adjusted Odds Ratios (ORs) and 95% Confidence Limits (95% CLs) for Risk of Recurrence for Selected Demographic Characteristics.

Characteristic	Crude OR	95% CL	p value	Adjusted OR*	95% CL	p value
Stage						
1A	1.00	---		1.00	---	
1B	1.76	0.90 - 3.43	0.098	1.71	0.86 - 3.41	0.127
Age, continuous	0.98	0.94 - 1.01	0.228	0.97	0.94 - 1.01	0.212
Sex						
Female	1.00	---		1.00	---	
Male	1.02	0.53 - 1.97	0.952	0.98	0.49 - 1.96	0.960
Race						
Caucasian	1.00	---		1.00	---	
African American	0.89	0.36 - 2.19	0.804	0.84	0.33 - 2.12	0.712
Histology						
Adenocarcinoma	1.00	---		1.00	---	
Squamous Cell	0.79	0.37 - 1.68	0.417	0.81	0.37 - 1.79	0.610
Other**	1.07	0.36 - 3.18	0.952	0.89	0.28 - 2.76	0.835
Smoking status						
Never	1.00	---		1.00	---	
Ever	0.63	0.24 - 1.66	0.351	0.72	0.26 - 1.99	0.529
* Multivariable logistic regression model adjusted for stage (1A/1B), age (continuous), sex, race (C/AA), histology (adenocarcinoma, squamous cell, other), smoking status (ever/never).						
** Other histology- large cell (16), basoloid (1), mucoepidermoid (1).						

Supplemental Table 3. Univariate Odds Ratios (OR) and 95% Confidence Limits (95% CLs) for Estimation of Risk of Recurrence by Methylation Status for Selected Gene Markers.

	Gene Marker	Validation (n=20)		
		OR*	95% CL*	p value
Single Genes	Unmethylated	1.00	referent	
	APC			
	Tumor**	Undefined	Undefined	
	N1	0.53	(0.08 – 3.31)	0.500
	N2	0.35	(0.03 – 4.65)	0.426
	Tumor and N2	0.70	(0.04 – 13.18)	0.812
	RASSF1A			
	Tumor	1.75	(0.27 – 11.15)	0.554
	N1	1.50	(0.19 – 11.93)	0.702
	N2	3.00	(0.25 – 35.33)	0.383
	Tumor and N2	1.56	(0.12 – 20.85)	0.739
	P16			
	Tumor	3.60	(0.49 – 26.40)	0.208
	N1	8.17	(1.03 – 64.94)	0.047
	N2	6.13	(0.83 – 45.02)	0.075
	Tumor and N2 [†]	Infinity	(0.811 – infinity)	0.079
	HCAD			
	Tumor	2.67	(0.39 – 18.17)	0.316
	N1	8.00	(1.00 – 63.96)	0.050
	N2	5.33	(0.78 – 36.33)	0.087
Tumor and N2	8.40	(0.76 – 93.43)	0.083	
Gene Combinations	APC & RASSF1A			
	Tumor	1.75	(0.27 – 11.15)	0.554
	N1	3.43	(0.29 – 40.95)	0.330
	N2 [‡]	0.00	(0.00 – 31.91)	0.900
	Tumor and N2 [‡]	0.00	(0.00 – 28.36)	0.842
	APC & P16			
	Tumor	3.60	(0.49 – 26.40)	0.208
	N1	1.50	(0.19 – 11.93)	0.702
	N2	0.80	(0.04 – 14.89)	0.881
	Tumor and N2 [†]	Infinity	(0.04 – infinity)	0.579
	APC & HCAD			
	Tumor	2.67	(0.39 – 18.17)	0.316
	N1	5.33	(0.47 – 60.80)	0.178
	N2	0.80	(0.04 – 14.89)	0.881
	Tumor and N2 [†]	Infinity	(0.04 – infinity)	0.579
	RASSF1A & P16			
	Tumor	1.71	(0.23 – 12.89)	0.601
	N1 [†]	Infinity	(0.17 – infinity)	0.526
	N2 [†]	Infinity	(0.15 – infinity)	0.579
	Tumor and N2 ^{††}	Undefined	Undefined	
	RASSF1A & HCAD			
	Tumor	1.39	(0.22 – 8.92)	0.729
	N1	3.43	(0.29 – 40.95)	0.330
	N2 [†]	Infinity	(0.15 – infinity)	0.579
	Tumor and N2 [†]	Infinity	(0.04 – infinity)	0.579
	P16 & HCAD			
	Tumor	2.50	(0.34 – 18.33)	0.367
	N1	18.67	(1.56 – 222.93)	0.021
N2	6.13	(0.83 – 45.02)	0.075	
Tumor and N2 [†]	Infinity	(0.53 – infinity)	0.170	

* Univariate odds ratios and 95% confidence limits

** All subjects positive for methylation.

† No methylation in controls.

‡ No methylation in cases.