

Supplementary Materials: Cell-free DNA Methylation of Selected Genes Allows for Early Detection of the Major Cancers in Women

Sandra P. Nunes, Catarina Moreira-Barbosa, Sofia Salta, Susana Palma de Sousa, Inês Pousa, Júlio Oliveira, Marta Soares, Licínio Rego, Teresa Dias, Jéssica Rodrigues, Luís Antunes, Rui Henrique and Carmen Jerónimo

Table S1. Promoters' methylation levels cut-off values used to categorize BrC, CRC and LC samples vs. AC samples used for validity estimates calculation in Figure 1 and Tables S2, S3 and S4.

Genes	BrC vs. AC	CRC vs. AC	LC vs. AC
<i>APC</i>	6.710	4.711	4.115
<i>FOXA1</i>	68.01	142.4	30.12
<i>RARβ2</i>	24.88	3.949	3.056
<i>RASSF1A</i>	58.27	39.47	26.57
<i>SCGB3A1</i>	4.130×10^{-3}	6.291×10^{-5}	6.140×10^{-3}
<i>SEPT9</i>	49.92	8.973	0
<i>SOX17</i>	184.7	0.6633	28.16

Table S2. Biomarker performance of each promoter's gene methylation for BrC detection in ccfDNA.

Genes	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
<i>APC</i>	32·41	94·17	85·37	57·06	62·56
<i>FOXA1</i>	38·89	79·61	66·67	55·41	58·77
<i>RASSF1A</i>	19·44	100·0	100·0	54·21	58·77
<i>SCGB3A1</i>	21·30	92·23	74·19	52·78	55·92

Abbreviations: PPV – Positive Predictive Value; NPV – Negative Predictive Value.

Table S3. Biomarker performance of each promoter's gene methylation for CRC detection in ccfDNA.

Genes	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy %
<i>APC</i>	20·83	94·17	71·43	62·99	64·00
<i>FOXA1</i>	50·00	88·35	75·00	71·65	72·57
<i>RARβ2</i>	16·67	95·15	70·59	62·03	62·86
<i>RASSF1A</i>	13·89	99·03	90·91	62·20	64·00
<i>SCGB3A1</i>	26·39	90·29	65·52	63·70	64·00
<i>SEPT9</i>	11·11	100·0	100·0	61·68	63·43
<i>SOX17</i>	23·61	90·29	62·96	62·84	62·86

Abbreviations: PPV – Positive Predictive Value; NPV – Negative Predictive Value.

Table S4. Biomarker performance of each promoter's gene methylation for LC detection in ccfDNA liquid biopsies.

Genes	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy %
<i>APC</i>	35·62	94·17	81·25	67·36	69·89
<i>FOXA1</i>	72·60	73·79	66·25	79·17	73·30
<i>RARβ2</i>	24·66	95·15	78·26	64·05	65·91
<i>RASSF1A</i>	21·92	98·06	88·89	63·92	66·48
<i>SOX17</i>	38·36	95·15	84·85	68·53	71·59

Abbreviations: PPV – Positive Predictive Value; NPV – Negative Predictive Value.

Table S5. Additional clinicopathological features of breast cancer (BrC) patients' plasma samples used in this study.

Clinicopathological features	BrC
Estrogen Receptor Status ^a	
Positive	91
Negative	15
Progesterone Receptor Status ^b	
Positive	81
Negative	24
ERBB2 Receptor Status ^c	
Positive	13
Negative	84
Molecular subtype ^d	
Luminal	90
ERBB2 overexpression (HR-, ERBB2+)	4
TNBC	10

^a Not amenable to obtain information for 2 cases; ^b No information available in 3 cases; ^c Not possible to determine in 11 cases; ^d No information available in 4 cases. Abbreviations: AC—Asymptomatic Control; ERBB2—Erb-B2 receptor tyrosine kinase; HR—Hormone Receptor; TNBC—Triple Negative Breast Cancer.

Table S6. Primers and probes sequences with respective fluorochrome and quencher.

Gene	Sequences
<i>ACTβ</i>	Primers F – 5' TGG TGA TGG AGG AGG TTT AGT AAG T 3' R – 5' ACC AAT AAA ACC TAC TCC TCC CTT AA 3'
	Probe 5' Cy5 – ACC ACC ACC CAA CAC ACA ATA ACA AAC ACA – QSY 3'
<i>APC</i>	Primers F – 5' TGT GTT TTA TTG CGG AGT GC 3' R – 5' CAC ATA TCG ATC ACG TAC GC 3'
	Probe 5' VIC – CAATCGACGAACTCCCGAC – MGB 3'
<i>FOXA1</i>	Primers F – 5' CGA CGT TAA GAC GTT TAA GC 3' R – 5' CGC TCA ACG TAA ACA TCT TAC 3'
	Probe 5' FAM-ATA TAC GAA TAA AAC GAC TTA ACG – MGB 3'
<i>MGMT</i>	Primers F – 5' TTT CGA CGT TCG TAG GTT TTC GC 3' R – 5' GCA CTC TTC CGA AAA CGA AAC G 3'
	Probe 5' VIC – TGC GTA TCG TTT GCG – MGB 3'
<i>RARβ2</i>	Primers F – 5' TCG AGA ACG CGA GCG ATT 3' R – 5' GAC CAA TCC AAC CGA AAC 3'
	Probe 5' HEX – CTT ACA AAA AAC CTT CCG AAT ACG TTC CGA – Iowa Black RQ-Sp 3'
<i>RASSF1A</i>	Primers F – 5' AGC GAA GTA CGG GTT TAA TC 3' R – 5' ACA CGC TCC AACC GA ATA 3'
	Probe 5' NED – CGG GAG TTG GTA TTC GTT GGG CG – QSY3'
<i>SCGB3A1</i>	Primers F – 5' GTA CGG TCG TGA GCG GAG C 3' R – 5' GAA ACT TCT TAT ACC CGA TCC TC 3'
	Probe 5' FAM – GCC GAC CTC GCC CGC GCT CCT AAA – Iowa Black RQ-Sp 3'
<i>SEPT9</i>	Primers F – 5' TTA GTT AGC GCG TAG GGT TC 3' R – 5' ACC TTC GAA ATC CGA AAT AA 3'
	Probe 5' NED – GCG TTA ACC GCG AAA TCC GAC ATA ATA ACT – QSY 3'
<i>SHOX2</i>	Primers F – 5' ATT CGT ATT TGG TCG CGT AC 3' R – 5' CTA CTA CGA CCG CCA CTA CC 3'
	Probe 5' FAM – CAA CGT AAC GAA CG – MGB 3'
<i>SOX17</i>	Primers F – 5' GAT CGG TTC GTT TTC GTC G 3' R – 5' GCC CGT ATT CTA ACC TAT CG 3'
	Probe 5' Cy5 – ACC GAC CTA ATA ACA CTA CGA ACG C – Iowa Black RQ-Sp 3'

