

Supplementary Information

***In Vitro* Diagnosis of DNA Methylation Biomarkers with Digital PCR in Breast Tumors**

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Table S1. Gene, primer and probe sequences (5' to 3') used in the real-time quantitative PCR (qPCR) and chip-based dPCR (dPCR) assays.

Gene (Accession)	CpG ID (Sequences)	Methy. Status ^a	Methy. Freq. ^b	Primer sequences	Methy Probe sequences	Tm (°C)
GCK ^c (NM_033507)	cg17650622 (CGGAGAGATGGCG ATGGATGTCACAAG GAGCCAGGCCAG ACAGCCTTGA)	Hyper	80%	Forward: GGTAGGGGGTAGAGT ATTTGAGTAGT Reverse: TCCCAATACAAAATC CCTAACTTTA	FAM- TAGTTTTTCGGAGAG GAGAT-MGBNFQ ^d	46 (qPCR) 50 (dPCR)
PDX1 ^e (NM_000209)	cg01771019 (GGCCGCCAGGCCT AGCGGATCATGCCC AGGCAGGGGGTTG GACGTCACCG)	Hyper	100%	Forward: GGGTTATTTTTTTTGT ATTTATTGA Reverse: TAATTCTTTATAATTC AAACTCTCTTAC	FAM- TTGGTGGCGGTGACG T-MGBNFQ	43 (qPCR) 39 (dPCR)

a: Methylation status. *b*: Methylated frequency;^{1,2} *c*: Glucokinase. *d*: minor groove binder non-fluorescent quencher. *e*: pancreatic-duodenal homeobox 1.

Table S2. PCR primers and probes for Gene *RARβ2*, *BRCA1*, *GSTP1* and *RASSF1A* methylation assays.

Gene (Accession)	Primer sequences	Methy. Status ^a	Probe sequence	T _m (°C)
<i>RARβ2</i> (NM_001290217)	Forward: TGTCGAGAACGCGA GCGATTC Reverse: CGACCAATCCAACCG AAACGA	Hyper	FAM- TTGGGTATCGTCGGGGTA- BHQ	60(qPCR) 56(dPCR)
<i>BRCA1</i> (NM_007294)	Forward: GAGAGGTTGTTGTTT AGCGGTAGTT Reverse: CGCGCAATCGCAATT TTAAT	Hyper	FAM- CCGCGCTTTTCCGTTACCAC GA-BHQ	60(qPCR) 62(dPCR)
<i>GSTP1</i> (NM_000852)	Forward: GTCGGCGTCGTGATT TAGTATTG Reverse: AAACCTACGACGACG AAACTCAA	Hyper	FAM- AAACCTCGCGACCTCCGAA CCTTATAAAA-BHQ	60(qPCR) 62(dPCR)
<i>RASSF1A</i> (NM_001206957)	Forward: ATGAGTTGCGGGAG TTGGT Reverse: ACACGCTCCAACCGA ATACG	Hyper	FAM- CCCTTCCAACGCGCCCA- BHQ	60(qPCR) 62(dPCR)

^a: Methylation status.³

References

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