Online Supporting Material

Supplemental Table 1

Bisulfite-converted gene-specific primers (BS), methylation-specific PCR (MSP) primers, and their associated parameters for the first study

Primer Set	Sense Primer (5' to 3')	Anti-sense Primer (5' to 3')	Size[1] (bp)	Anneal temp., ℃	Genomic position[2]
CYP27A1 BS	TTTTTTAGGGATTAGATGATTGG	ATTCCCCACCCAAAACRACCCT	146	59	-69
CYP27A1 MSP Methylated	TTTTTCGTTTCGAATTGATTTCG	AAAAAAAACGAAAACCGACCG	91	60	-44
CYP27A1 MSP Unmethylated	TTTTTTGTTTTGAATTGATTTTG	AAAAAAAACAAAAACCAACCA	91	57	-44
CYP2R1 BS	GGTTTTAGGGTTGTTGTGGAGT	CTTCAACAACTAACRAACCCCTA	139	61	-45
CYP2R1 MSP Methylated	CGTATTTTTAGTTCGGGTCGAT	AAAAACAACAAAAAAAACGCG	88	61	-22
CYP2R1 MSP Unmethylated	TGTATTTTTAGTTTGGGTTGAT	AAAAACAACAAAAAAAACACA	88	59	-22
CYP27B1 BS	TTTTAAGTAYGTTTTTAGAGTGTTTTAT	AAACRTCCCTTCCTACCTACAACT	200	59	163
CYP27B1 MSP Methylated	TTATCGCGTTCGTTGGGCGTTCGA	CTACCTACAACTCGTATAACCTCGA	164	70	191
CYP27B1 MSP Unmethylated	TTATTGTGTTTGTTGGGTGTTTGA	CTACCTACAACTCATATAACCTCAA	164	60	191
CYP24A1 BS	GTAGTTTTTTTGGGGTTYGTATT	AAATAACCCCCAAAAAATCAT	178	61	-430
CYP24A1 MSP Methylated	ATCGCGATTGTGTAAGCGT	ATACTTCGAACGCGCCTC	91	64	-387
CYP24A1 MSP Unmethylated	ATTGTGATTGTGTAAGTGT	ATACTTCAAACACACCTC	91	57	-387

[1]Size: the size of a PCR product

[2]Genomic position is the location of the 5' nucleotide of the sense primer in relation to the major transcriptional start site defined in the genome assembly named NCBI Build 36.1 (released in Mar. 2006).

Supplemental Table 2

Pyrosequencing primers and their associated parameters for the validation study

Primer Set	Sense Primer (5' to 3')	Anti-Sense Primer (5' to 3')	Pyrosequencing Primer (5' to 3')	Size[1] (bp)	Anneal temp., ºC	Genomic position[2]
CYP2R1	TGTTTGGGTTTTAGGG TTGTTG	CCTCTACTTCAACAA CTAA	GGTTTTAGGGTTGTTGTG GAGT	151	58	-45
CYP24A1	GGTATYGYGATTGTGT AAG	AAAATAACCCCCAAA AAATCATA	GGTATYGYGATTGTGTAA G	139	54	-390

[1]Size: the size of a PCR product.

[2]Genomic position is the location of the 5' nucleotide of the pyrosequencing primer in relation to the major transcriptional start site defined in the genome assembly named NCBI Build 36.1 (released in Mar. 2006).