

Supplementary Material
S1/ Primer sequences and amplification conditions for QMPSE

Gene/Exon	Primer sequence (5' to 3') *		Concentration † μM	Amplicon size ‡ (bp)
	Forward §	Reverse		
Multiplex 1 				
TCF2/exon 3	<u>CGTTAGATAGATTCAACCAGACAGTCCAGA</u>	<u>GATAGGGTTATCTCTTCCTTGCTGGGGTTC</u>	0.4	258
TCF2/exon 4	<u>CGTTAGATAGCTGGGCTCCAACCTTGTC</u>	<u>GATAGGGTTAGACAGCTTGTTGGAGGAGA</u>	0.15	208
TCF2/exon 5	<u>CGTTAGATAGGAGTGCCTACAGCCAG</u>	<u>GATAGGGTTAATCAGGTGAGAGGAGATTG</u>	0.3	174
TCF2/exon 6	<u>CGTTAGATAGAGTCTCAGGAGGAGGTTTGC</u>	<u>GATAGGGTTAATTGCCATGACTCCAGAGAG</u>	0.15	140
TCF2/exon 7	<u>CGTTAGATAGTCAACACCTCCCAAGCACA</u>	<u>GATAGGGTTATGAGTCACAGCTGCCATGA</u>	0.4	197
TCF2/exon 8	<u>CGTTAGATAGGTACGCACACAAGCAGGAAC</u>	<u>GATAGGGTTATTGAAGACATGTTGGTGAGTG</u>	0.15	130
<i>TCF1/exon 3</i>	<u>CGTTAGATAGTGATGAGCTACCAACCAAGA</u>	<u>GATAGGGTTAGAGTCACCTTCCCCTTCC</u>	0.3	235
Multiplex 2 #				
TCF2/exon 1	AACTTCGGGGTGAAGCTG	AGGATGGGAGGTGTGTCATA	0.5	164
TCF2/exon 2	TGTCTAGTGAGGACCCTTGG	GGATCTCTCGTTGCTTTCTG	0.3	201
<i>TCF1/exon 3</i>	<u>CGTTAGATAGTGATGAGCTACCAACCAAGA</u>	<u>GATAGGGTTAGAGTCACCTTCCCCTTCC</u>	0.3	235

* 5' forward and reverse extensions are underlined

† forward and reverse used equimolar

‡ including 5' extension, if any

§ The forward primer was 5' end-labeled with 6-FAM fluorochrome

|| Multiplex 1 PCR was performed in a 30 μl reaction mixture containing 0.2 mM each dNTP, 2 mM MgCl₂, 2.5% DMSO, 2 U TaqGold DNA polymerase (Applied Biosystems), 0.15-0.4 μM each primer and 20 ng of genomic DNA. The amplification conditions consisted of an initial step of denaturation at 94°C for 12 min, followed by 24 cycles of denaturation at 94°C for 20 sec, annealing at 54°C for 20 sec and extension at 72°C for 20 sec. A final extension was performed at 72°C for 10 min.

Multiplex 2 PCR was performed in a 30 μl reaction mixture containing 0.2 mM each dNTP, 2 mM MgCl₂, 5% DMSO, 1M betaine, 2 U TaqGold DNA polymerase (Applied Biosystems), 0.3-0.5 μM of each primer and 20 ng of genomic DNA. The amplification conditions consisted of an initial step of denaturation at 95°C for 12 min, followed by 24 cycles denaturation at 95°C for 45 sec, annealing at 54°C for 45 sec and extension at 72°C for 45 sec. A final extension was performed at 72°C for 10 min.

S2/ Primer sequences and amplification conditions for real-time quantitative PCR based on SYBR-Green I fluorescence

Gene/Exon	Primer sequence (5' to 3')		Amplicon size (bp)
	Forward	Reverse	
TCF2/exon 1	AACTTCGGGGTGAAGCTG	AGGATGGGAGGTGTGTCATA	164
TCF2/exon 2	CTCCCACTAGTACCCTAACC	GAGAGGGCAAAGGTCACCTCAG	293
TCF2/exon 3	AGTGAAGGCTACAGACCCTATC	TTCTGGGTCTGTGTACTTGC	367
TCF2/exon 4	CCAAGACTGCTGTGATTGTG	CAGATAAGATCCGTGGCAAG	383
TCF2/exon 5	GGAGTGCCTACAGCCAG	TCAGGTGAGAGGAGATTGTGG	152
TCF2/exon 6	CTGCTCTTTGTGGTCCAAGTCC	GAGTTTGAAGGAGACCTACAG	290
TCF2/exon 7	ATCCACCTCTCCTTATCCAG	ACTTCCGAGAAAGTTCAGACC	342
TCF2/exon 8	TTGCCTGTGTATGCACCTTG	GCCGAGTCCATGCTTGCCAC	259
TCF2/exon 9	CTTTGCTGGTTGAGTTGGGC	TTCCATGACAGCTGCCCAGAG	210
TRIP3/exon 5	TTATTGCTCAATCCACACCTC	ATGGCTCCACAATTCCTAAAC	139
AATF/exon 3	CTGCTGAGGAACAGGAGTGT	AGCCCTGTCTTCCTCACTCT	224
STS-AC113211	CAGTTCATCTCCCGAAAGACT	GCTGTAGGGGACTTGTAAGAGG	151
MRPL45/exon 8	AATATGAAGAGGCACAAGGAGA	TGATGATGAGACTGCTGAAAAG	188
MEL18/exon 11	GTGTGAGTCAGTCAGCGACA	CTGCAGGCAGTTCAGCTAC	202
<i>HBB/exon 1*</i>	GTGCACCTGACTCCTGAGGAGA	CCTTGATACCAACCTGCCCAG	102

* used as reference gene

Real time quantitative PCR was performed in a 20 µl reaction mixture containing SYBR-green I PCR master mix (applied Biosystems), 0.3 µM of each primer and 20 ng genomic DNA. Thermocycling conditions were performed according to manufacturer's recommendations.