

S1 Table. PCR and Single Base Extension (SBE) primer sequences and reaction condition for Multiplex R.

PCR*				
<i>Marker</i>	<i>SNP</i>	<i>PCR Forward Primer (5'-3')</i>	<i>PCR Reverse Primer (5'-3')</i>	<i>PCR fragment size (bp)</i>
M269	T>C	CATGCCTAGCCTCATTCTC	CTGGATGGTCACGATCTCCT	249
L23	G>A	ACACAGTGAAACCCCGTCTC	AAGATTGTGGGGACAAAGGA	229
U106	C>T	TCCTGAATAGCAAATCCCAAAG	AATGGCAGAGGTAGGAGGAAAT	192
U152	G>A	GAAACATTCCACGCTTGAGG	AGCCTCTTTTTGGCTTCCAT	163
S116	C>A	TCAGTCAGGGCAAATCTGAA	GGTGGAGTTGGGGCTAAAGT	241
M529	C>G	GCCCCAAAACAACAGAATA	GGAAGCATTGAGAAGCAGGT	228
M153	T>A	TTCTCAGACACCAATGGTCCTA	TCTGACTTGGAAGGGGAAA	76
M167	C>T	GAGGCTGGGCCAAGTTAAG	CTTCCTCGGAACCACTACCA	131
M207	A>G	CGTTACAACACTATGGGGCAA	TCCTCTCTGAAATGCCGAAT	322
SNaPshot**				
<i>Y-SNP</i>	<i>***</i>	<i>SBE Primer (5'-3')</i>	<i>Concentration in the reaction (μM)</i>	<i>SBE fragment size (bp)</i>
M269	<i>For</i>	GTCTGACAAGGAATGATCAGGGTTTGGTTAAT	0.12	32
L23	<i>For</i>	GCGACAGAGCGAGACTCT	0.16	18
U106	<i>Rev</i>	TCTGACAATAGCAAATCCCAAAGCTCCA	0.12	28
U152	<i>Rev</i>	CAAGGATAAGAAAAATGAGTATTGTGAAAATA	0.08	32
S116	<i>Rev</i>	GAAAGTCTGACAAGAGTTGGGGCTAAAGTAAAAG	0.04	35
M529	<i>For</i>	AATAACAACCGCTCTCTCAGACA	0.04	23
M153	<i>Rev</i>	AAAGCTCAAAGGGTATGTGAACA	0.04	23
M167 (SRY2627)	<i>For</i>	AAGCCCCACAGGGTGC	0.04	16
M207	<i>For</i>	AACAAATGTAAGTCAAGCAAGAAATTTA	0.10	28

*PCR was carried out in 10 μl containing: 5μl Qiagen® multiplex PCR kit, 0.2μM of each primer and 5-10 ng DNA. Thermocycling conditions: denaturation at 95°C for 15 min; 35 cycles at 94°C for 30s, 60°C for 90s and 72°C for 60s; final extension of 72°C, 10 min. Prior to SNaPshot reaction, 1 μl of the PCR product was purified with 0.5 μl of Exo-SAP-it (USB), incubated at 37°C for 15 min, and followed by an enzyme inactivation at 85°C for 15 min. **SNaPshot was carried out in 5 μl reaction containing 1 μl of SNaPshot™ Multiplex Ready Mix (Applied Biosystems), 1.5 μl of purified PCR product and extension primers. The reactions were submitted to 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 30 s. Final products were purified with 1 μl of SAP (USB) at 37°C for 1 h, followed by a denaturing step of 85°C for 15 min. The single-base extension products were run on an ABI 3130 Genetic Analyzer (Applied Biosystems), and the analysis was performed with GeneMapper® Software v4.0. ***Annealing of the SBE primers in forward or reverse sequence direction.