

Additional file 2

Protocols used for Amplifluor and KASP markers and basic PCR cyclers. Protocols 1 and 2 were designed for Amplifluor SNP analysis, while Protocol 3 was used for KASP markers. Protocol 4 was designed as optimal and used in the current study.

Steps	1 [10-11,19-20]			2 [14-15]			3 [25-31]			4 Current study		
	Temp . (°C)	Time	Cyc le	Temp . (°C)	Time (sec)	Cyc le	Temp. (°C)	Time	Cyc le	Temp . (°C)	Time	Cyc le
Initial denaturatio n	95	3-4 min	1	96	4-10 min	1	94-95	10-15 min	1	95	5 min	1
Denaturati on	95	10 sec	35- 45	95	10-15 sec	20	94-95	20 sec	10	95	10 sec	25- 30
Annealing	55-60	20 sec		55-64	5-10 sec		61-65, drop 0.6- 1°C per cycle	25-60 sec		56-60	10 sec	
Extension	72	40 sec		72	10-15 sec					72	20 sec	
Denaturati on	-	-	-	96	10 sec	20- 25	94	10-20 sec	25- 35	95	10 sec	
Annealing				50-56	20-30 sec		55-60	60 sec		51-55	20 sec	
Extension				72	40 sec		-	-		72	40 sec	