

**An association analysis between *PRL* genotype and milk production trait in Italian Mediterranean river buffalo**

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**SUPPLEMENTARY FILE**

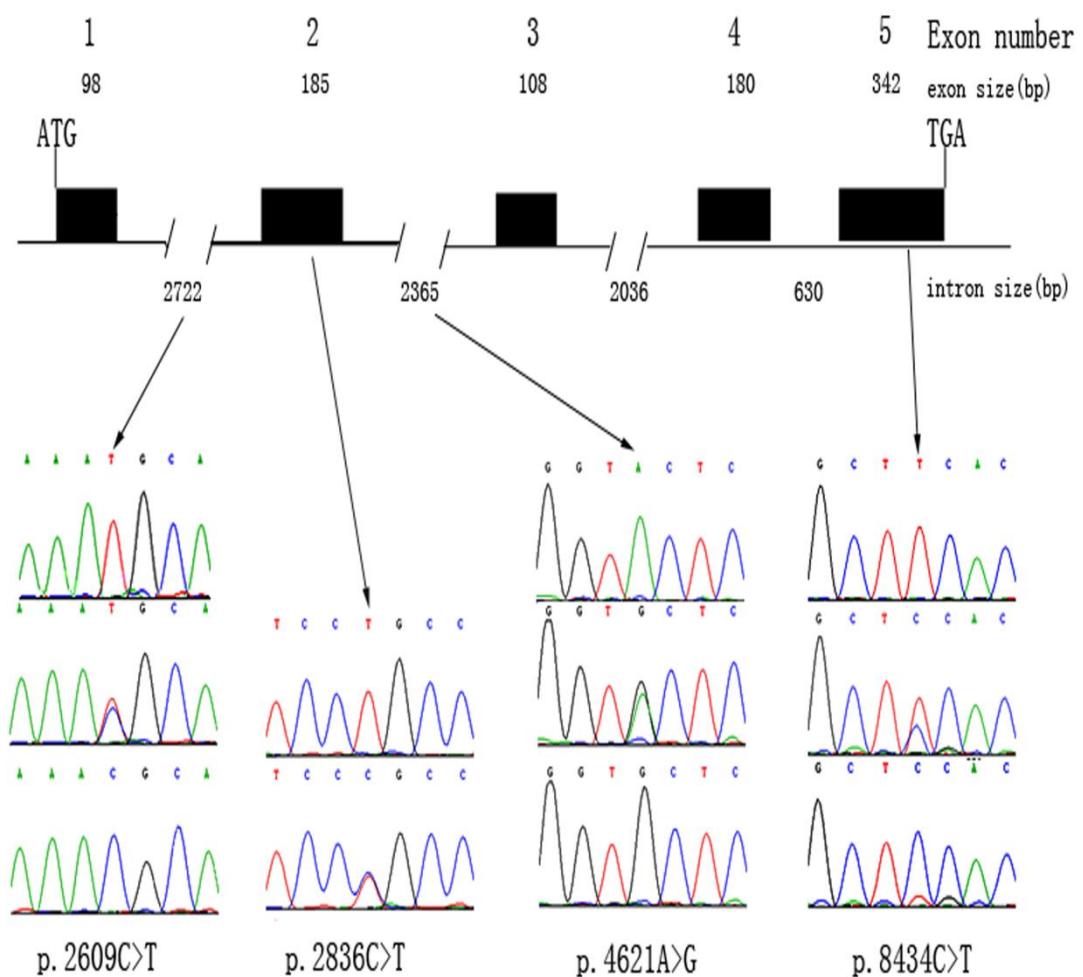
**Supplementary Table S1** Primers used for HRM analysis of the *PRL* gene

No	Primer sequence (5'-3')	Target region	Size (bp)	Temp. (°C)
1	FWD: AACTGTGCTGTTCTGCCATT  REV: AGAAGCAACACCTTCCACTGT	Intron1	294	56
2	FWD: TCTCAGAGCGGCTCATGTTTT  REV: AACAGGTCTCGAAGGGACAC	Exon2	150	57
3	FWD: GGAGCTCATAGTAGAGCACACA  REV: TGGACTGAGAAGGAGAGTCG	Intron2	227	56
4	FWD: TACCCTGTGTGGTCAGGACT  REV: TGAAACCCATTAGAGCCAAGCA	Exon5	272	60

**Supplementary Table S2** Genotypes and allele frequency within the SNPs in the *PRL* gene

Location	Position	SNP	Genotype frequency			Allele frequency		Amino acid change and position
			AA	AB	BB	A	B	
Intron1	2609	C/T	0.829(435)	0.145(76)	0.027(14)	0.901	0.099	
Exon2	2836	C/T	0.840(441)	0.160(84)	0	0.920	0.080	R12C
Intron2	4621	G/A	0.851(447)	0.131(69)	0.017(9)	0.917	0.083	
Exon5	8434	C/T	0.825(433)	0.150(79)	0.025(13)	0.900	0.100	L202L

**Supplementary Figure S1** Sequencing results of 4 SNPs in PRL gene



**Supplementary Figure S2** The HRM analysis showed a difference between the temperatures of melting ( $T_m$ ) for the heterozygote, the homozygous mutation and the wild type as different genotypes due to different bonds

