

Gene symbol	<i>Lep</i> ^{m1Kyo}
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primer	Sequence(5'-3')	Size	target gene	accession
Lep_F3	GCACCTGTTGTTCTCTTCC	583bp	Leptin precursor	NW_003807990
Lep_R3	TGGAATCGTGCGATAACTT			
comment	Lep mutation (Q92X)is determined by sequencing.			

PCR condition							
Taq polymerase		BIOTAQ™ HS DNA Polymerase (Bioline Reagents Ltd., UK)					
Thermal cycler		PC-808(ASTEC)					
PCR buffer		Ampdirect Plus(Shimadzu.Co)					
		first denature	denature	anneal	extension	cycle	final extension
PCR	°C	94	94	60	72	30	72
	min	3	0.5	1	0.75		3
comment		①PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm FTA disc was removed from the bloody–stained region on FTA®card. Untreated sample discs were placed directly in 15 μ L PCR mixture containig 1 × Ampdirect®Plus, 0.2 μ M each primer and 0.4 units of BIOTAQ™ HS DNA Polymerase.					

Sequenece: ABI3130xl	
Mutant Allele Wild Allele Heterozygote	
Strain harboring mutation	F344- <i>Lep</i> ^{m1Kyo}