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Chinese Yellow Cattle *PPARA* Gene: Analyses of Expression, Polymorphism and Trait Association

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Supplementary Online Material (SOM)

Table S1. Information of primer sequences for the bovine *PPARA* gene

Name	Primer sequence (5'-3')	Size (bp)	Tm (°C)	Used for
PPARA-P1	F: ATACTGCCTTGGACTTCGC R: TAGTCTGTAGGGCAATGGAAGTA	443	57	partial intron 1 and 2; exon 2
PPARA-P2	F: CAAGCGGAAGGAGAATGGC R: AAGGCAGAAAGACGCAACC	494	60	partial intron 2 and 3; exon 3
PPARA-P3	F: TGAACAAGAAGCGTGAATAAAGG R: CGTTCCAAGCCCACAAGG	285	60	partial intron 4 and 5; exon 5
PPARA-P4	F: TCTGACTTCCTGCTGATGTTCG R: CTGGGAGCGTTGAGGTGG	561	60	partial intron 5 and 6; exon 6
PPARA-P5	F: GATGACGCTGACTATCTTTCTGA R: TACCGCTGCTGGGTTCTC	732	60	partial intron 6 and 7; exon 7
PPARA-P6	F: TGAAGACTGTTCCTTGGCGT R: AAAACAGCAAACTGAACCGAAAC	947	60	partial intron 7 and 3'utr
PPARA-P7	F: TTACAGCAGACAATCACGGGT R: GCTCCTCCTTTGGGAACGA	573	60.5	partial promoter region
PPARA-P8	F: GCTCGCCGCCACAAATAGA R: GGGAACGCTGGTTGGGAG	469	63	partial promoter region
PPARA-P9	F: TCTTGCTGCTCACCATTG R: CCACCTCCTGTCCCACTG	692	57	partial promoter region
PPARA-qRT	F: GCTCCGTTATTACAGACACCC R: AACCCTTGCAGCCCTCAC	180	54	mRNA expression
ACTB -qRT	F: CTGGGCGTAATGGTGGGC R: CTGATGCCGTGCTCAATGG	107	54	mRNA expression
TUBA1A-qRT	F: GGAGGTTCGCACTGGCAC R: CGCCTTGCCAATGGTGTAG	112	54	mRNA expression

Table S2. Primers for identifying mutations by PCR-RFLP and ACRS-PCR within the *PPARA* gene

No.	Locus*	rsID #	Primers (5'-3')	Size (bp)	Tm (°C)	Enzyme	Cleavage sites	Genotype (bp)
1	g.117148558 A>T	rs471506343	F: TTTCAGTTGGGATGTCCCATAC R: TTACTTTCTTAGGCTCTCGTGTTAC	338	57.5	*Hap*II	AGCGC^T	*TT*: 338 *TA*: 338, 318, 20 *AA*: 318
2	g.117195033 A>G	rs134580633	F: CAAGCGGAAGGAGAATGGC R: AAGGCAGAAAGACGCAACC	494	60	*Hinf* I	G^ANTC	*AA*: 494 *AG*: 494, 388, 106 *GG*: 388, 106
3	g.117195348 A>G	rs135735531	F: CAAGCGGAAGGAGAATGGC R: AAGGCAGAAAGACGCAACC	330	60	*Hind*III	A^AGCTT	*AA*: 330, 164 *GA*: 330, 164, 89, 75 *GG*: 330, 89, 75
4	g.117204210 G>A	rs477982176	F: AATCCACAGGGTTTCTTTCG R: AATAGGCAGACGGAGGCAT	201	55	*Asu*II	TT^CGAA	*GG*: 201 *GA*: 201, 183, 18 *AA*: 183, 18
5	g.117204336 T>C	rs137668765	F: GTCTTCCCTTTTTACCGCTT R: ACCAAAGTCTTCCAAATAAATATTG	168	53	*Hha*I	C^CGG	*TT*: 168 *TC*: 168, 146 *CC*: 146
6	g.117228031 T>C	rs110745628	F: TCCGTGGAGACCGGCAC R: TAGGCTACCAACATCCCATCTTTAT	164	57.7	*HgiC* I	G^GYRCC	*TT*: 164 *TC*: 164, 150, 15 *CC*: 150, 15
7	g.117228160 T>C	rs446377435	F: TGCTGTCTTCTGTGATGAATAAAGC R: AATGATAGCAGCCACAAAGAGG	191	55	*Hpa* II	C^CGG	*TT*: 191 *TC*: 191, 166, 25 *CC*: 166, 25
8	g. 117232845 T>C	novel	F: CACTACAGAGAGACAGGAGCAC R: ACAGTCAAAAAGCGGTAAAAAGGG	333	60	*Pst*I	CTGCA^G	*TT*: 309 *TC*: 333, 309 *CC*: 333
9	g.117233248 A>G	rs432147085	F: CTTCCTTCGCCCTTATTCAA R: TTGCACATGCTATATAGCGC	147	55	*Hae*II	GCG^C	*AA*: 147 *AG*: 147, 122

PCR-RFLP = PCR-restricted fragment length polymorphisms, ACRS-PCR = artificially created restriction site-PCR, Tm = annealing temperature

rsID in database of dbSNP in NCBI; *identification of polymorphisms in *PPARA* gene among Chinese cattle according the reference sequence (GenBank No. NC_007303.6)

Primers with N for identifying mutations byACRS-PCR, N represents mismatched bases. Primers without N for identifying mutations by PCR-RFL

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Table S3. Genotype and allelic frequencies, value of χ^2 test, and diversity parameters of the *PPARA* gene analysed in the study

SNPs	Breeds	Sample	Genotype frequencies			Minor allele frequency	HWE *P*-value*	PIC	H_e	N_e
			AA	*TA*	*TT*					
	JX	141	0.000	0.496	0.504	0.248	**13.970**	0.304	0.373	1.596
g.117148558 A>T	LX	114	0.026	0.518	0.456	0.285	**7.273**	0.325	0.408	1.688
	NY	139	0.129	0.403	0.468	0.331	1.132	0.345	0.443	1.795
	QC	30	0.000	0.400	0.600	0.200	0.810	0.269	0.320	1.471
			AA	*AG*	*GG*					
	JX	141	0.121	0.539	0.340	0.390	2.486	0.363	0.476	1.908
g.117195033 A>G	LX	114	0.105	0.763	0.132	0.487	**31.706**	0.375	0.500	1.999
	NY	139	0.187	0.597	0.216	0.486	5.298	0.375	0.500	1.998
	QC	30	0.033	0.900	0.067	0.483	**16.970**	0.375	0.499	1.998
			AA	*GA*	*GG*					
	JX	141	0.326	0.489	0.184	0.429	0.0154	0.370	0.490	1.961
g.117195348 A>G	LX	114	0.044	0.763	0.193	0.425	**35.882**	0.369	0.489	1.957
	NY	139	0.108	0.561	0.331	0.389	4.556	0.362	0.475	1.905
	QC	30	0.033	0.933	0.033	0.500	**20.017**	0.375	0.500	2.000
			GG	*GA*	*AA*					
	JX	141	0.639	0.308	0.053	0.207	0.483	0.274	0.328	1.488
g.117204210 G>A	LX	114	0.708	0.236	0.057	0.175	3.491	0.247	0.288	1.405
	NY	139	0.682	0.296	0.023	0.171	0.067	0.243	0.283	1.394
	QC	30	0.793	0.138	0.069	0.138	2.504	0.210	0.238	1.312
			TT	*TC*	*CC*					
	JX	141	0.319	0.553	0.128	0.404	3.109	0.366	0.482	1.929
g.117204336 T>C	LX	114	0.491	0.430	0.079	0.294	0.145	0.329	0.415	1.709
	NY	139	0.425	0.482	0.093	0.334	0.948	0.346	0.445	1.803
	QC	30	0.300	0.500	0.200	0.450	0.003	0.373	0.495	1.980
			TT	*TC*	*CC*					
	JX	141	–	0.066	0.934	0.033	0.845	0.062	0.064	1.068
g.117228031 T>C	LX	114	–	0.055	0.946	0.027	2.158	0.052	0.053	1.056
	NY	139	–	0.061	0.939	0.030	1.193	0.057	0.059	1.062
	QC	30	–	0.069	0.931	0.035	**6.389**	0.064	0.067	1.071
			TT	*TC*	*CC*					
	JX	141	0.698	0.198	0.103	0.202	**18.718**	0.271	0.323	1.477
g.117228160 T>C	LX	114	0.709	0.209	0.081	0.186	**11.616**	0.257	0.302	1.433
	NY	139	0.640	0.270	0.090	0.225	5.099	0.288	0.349	1.536
	QC	30	0.778	0.185	0.037	0.129	0.063	0.200	0.228	1.291
			TT	*TC*	*CC*					
	JX	141	0.858	0.121	0.021	0.082	3.244	0.139	0.150	1.176
g. 117232845 T>C	LX	114	0.684	0.281	0.035	0.175	0.007	0.248	0.289	1.407
	NY	139	0.734	0.216	0.050	0.158	5.017	0.231	0.266	1.363
	QC	30	0.833	0.167	0.000	0.083	0.413	0.141	0.153	1.180
			AA	*AG*	*GG*					
	JX	141	0.078	0.922	–	0.461	**100.569**	0.374	0.497	1.988
g.117233248 A>G	LX	114	0.167	0.833	–	0.417	**55.962**	0.368	0.486	1.946
	NY	139	0.252	0.748	–	0.374	**47.724**	0.359	0.468	1.881
	QC	30	0.433	0.567	–	0.283	3.278	0.324	0.406	1.684

SNP =single nucleotide polymorphism, HWE = Hardy–Weinberg equilibrium, PIC = polymorphism information content, H_e = heterozygosity, N_e = effective allele numbers, JX = Jiaxian, LX = Luxi, NY = Nanyang, QC = Qinchuan

*values in bold indicate that the genotype distribution was not in agreement with the HWE ($P < 0.05$)