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Genetic variation, association analysis, and expression pattern of *SMAD3* gene in Chinese cattle

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Supplementary Online Material (SOM)

Table S1. Primers in bovine *SMAD3* gene for mutations discovery and genotyping

Name[a]	Primer sequences (5'→3')	Position of amplicon in NC_007308.5[b]	Size (bp)	T_a (°C)	Location within gene
P1	F: CTGCAACTCTAGAAGGCAGC R: ACTCGCAAGAAGTGGAGCAA	nt-2291 to -1446	846	67.3	Promoter
P2	F: CGTCCGTTCGTGCGAGTCTAA R: AGGCAGTAGCGAAGCGGGAT	nt -951 to -243	708	63.1	Promoter, 5'UTR
P3	F: CAAAGTTTGGCTGGGGGTTG R: GAGGTGAAAAGCCAATGCGG	nt- 409 to 750	847	67.3	5'UTR, Exon 1, partial Intron 1
P4	F: TCACTGTCCGTTTCTGCTCC R: GCCTTGCTATTTCCGCTTC	nt 100225 to101019	795	61.4	Exon 2, 3 and partial Intron 1, 2, 3
P5	F: CCTTTCGGAGCCATCTAC R: TCTTCCATCAGGGCATACA	nt 101364 to 102434	1071	57.0	Exon 4 and partial Intron 3, 4
P6/SNP3	F: TGCGGGCTTGATTAGACG R: GCCCACTTGTCAGCCGTA	nt 105182 to 106585	1404	62.5	Exon 5 and partial Intron 4, 5
P7	F: CTTGAAGATGGCTCTGACCT R: AAGGGTCTTCCTCTACTGCT	nt 114439 to 115116	696	61.4	Exon 6 and partial Intron 5, 6
P8	F: GCTGCTAGAGACCAGTCACC R: TGAGACAGCAAGGCACAGAG	nt 117001 to 1177415	415	61.4	Exon 7 and partial Intron 6, 7
P9	F: CCTCCCTATTGAAGTGGCG R: ACTGCTGGCATCTCCTCC	nt 119469 to 120312	861	61.4	Exon 8 and partial Intron 7, 8
P10	F: ACCTCCTTTCCCTTCCTG R: CTCTGGGTTTGCTTTCGT	nt 122029 to 122531	503	57.0	Exon 9 and partial Intron 8, 9, 3'UTR
SNP1	F: CCCTTCTCTTTTTCCATGAGA T C T A R: AAGGAGAGAAAGAGCTCTGGC	nt -2042 to -1821	221	55.9	Promoter
SNP2	F: TTGAGACGTCCCCTCAAGAGAC R: CCACCTGAATCGCTCTGAAC	nt 101627 to 101884	258	58.7	Intron 3
SNP4	F: GGGCAGAGATCAGCAAGGGT R: GGCAGAACCCTTAGGTTTTCG TC GA	nt 114293 to 114548	256	60	Intron 5

[a]P1, P2, P3, P4, P5, P6/SNP3, P7, P8, P9, P10 were primer pairs for sequencing, covering the promoter region (–2000 to –1) and all of exons of *SMAD3* gene

[b]location of SNPs in promoter was counted from the translation initiation site; of other SNPs was counted from transcription start site

SNP1, SNP2, P6/SNP3, and SNP4 were primers for genotyping. T and TC showed mismatch to create restriction site at SNP1 and SNP4. At SNP2, a total of two *Msp*I restriction sites "CCGG" were in primary PCR product, and A represented mismatch allele to destroy the extra one and the target site was recognized by *Msp*I in the 258-bp product

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Table S2. Primers for quantitative real-time PCR

Name	Primer sequences (5'→3')	Size (bp)	T_a (°C)
GAPDH	F: AATGAAAGGGCCATCACCATC R: GTGGTTCACGCCCATCACA	204	60
SMAD3	F: CAAGTGACCACCAGATGAA R: GTGAAGCCATCCACCGTCAT	211	60

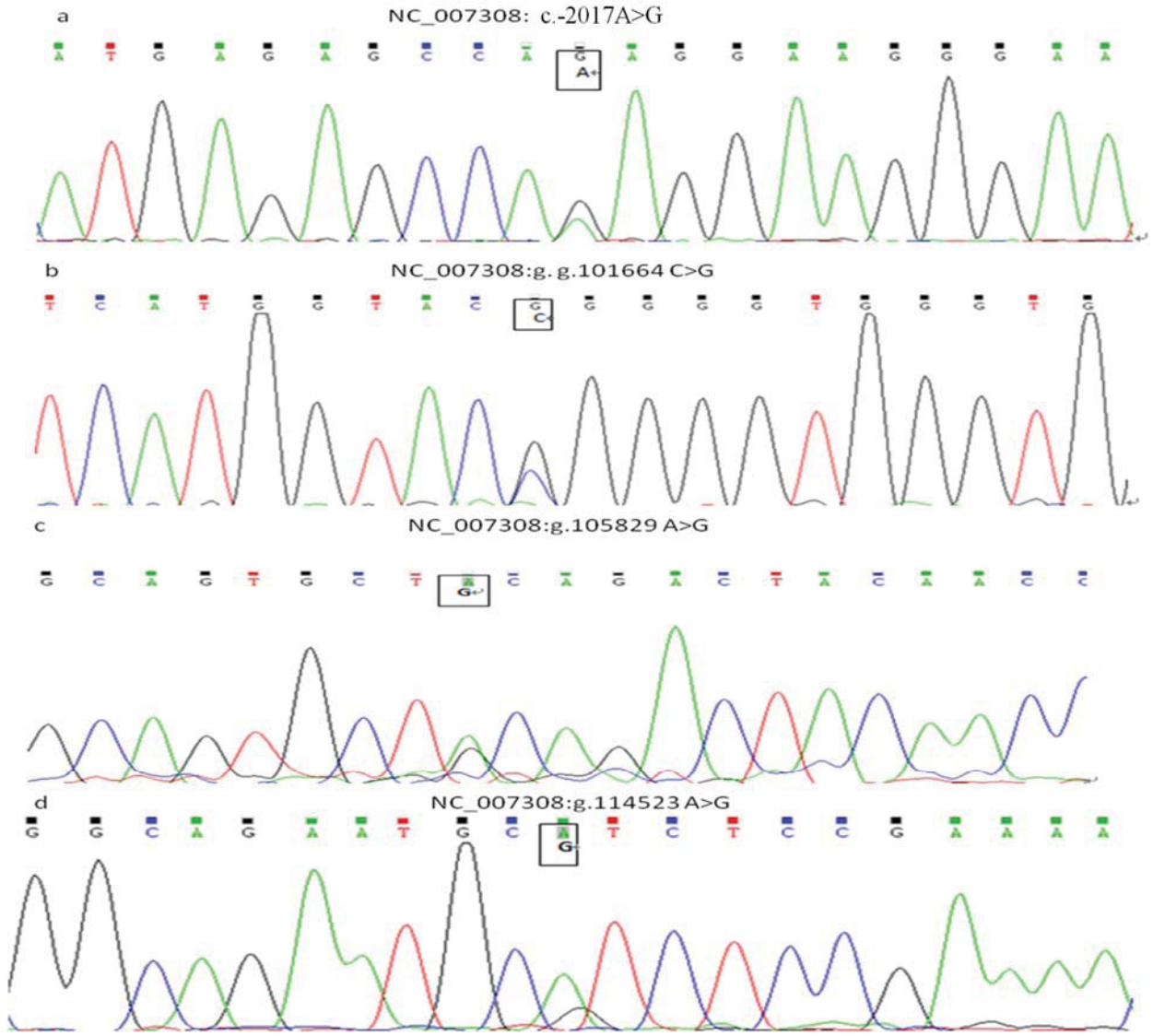

Figure S1. Sequencing maps with DNA pool for four SNPs in *SMAD3* gene; location of SNPs was referred to the reference sequence (GenBank Acc. No. NC_007308.5)

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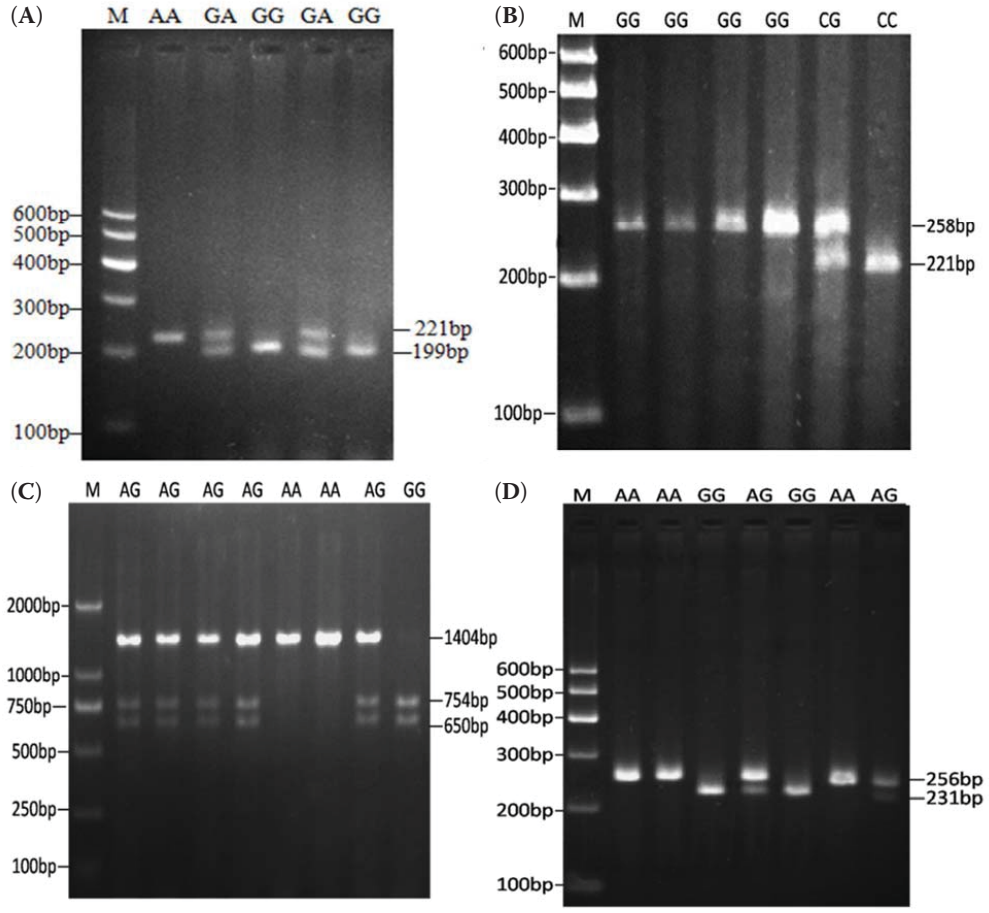

Figure S2. Agarose gel electrophoresis patterns of the four SNPs within *SMAD3* gene by PCR-RFLP

(**A**) PCR products' digestion with *Xba*I showed three geneotypes at SNP1 (*AA*, *AG*, *GG*) detected by 3% agarose gel electrophoresis, (**B**) PCR products' digestion with *Msp*I showed three geneotypes at SNP2 (*CC*, *CG*, *GG*) detected by 3% agarose gel electrophoresis, (**C**) PCR products' digestion with *Pst*1 showed three geneotypes at SNP3 (*AA*, *AG*, *GG*) detected by 1.5% agarose gel electrophoresis, (**D**) PCR products' digestion with *Sal*I showed three geneotypes at SNP4 (*AA*, *AG*, *GG*) detected by 3% agarose gel electrophoresis