

# **NRF1 and ZSCAN10 bind to the promoter region of the SIX1 gene and their effects body measurements in Qinchuan cattle**

**Da-Wei Wei<sup>1,4†</sup>, Lin-Sheng Gui<sup>1,4†</sup>, Song-Zhang<sup>1,4</sup>, Rajwali Khan<sup>1</sup>, Sayed Haidar Abbas Raza<sup>1</sup>, Li-Wang<sup>1,4</sup>,  
Hong-Fang Guo<sup>1,4</sup> and Lin-Sen Zan<sup>1,2,3</sup>**

<sup>1</sup>College of Animal Science and Technology, Northwest A&F University, Yangling 712100 Shaanxi, People's Republic of China. <sup>2</sup>National Beef Cattle Improvement Center, Northwest A&F University, Yangling 712100 Shaanxi, People's Republic of China. <sup>3</sup>Modern Cattle Biotechnology and Application of National-Local Engineering Research Center, Yangling 712100 Shaanxi, People's Republic of China. <sup>4</sup>Shaanxi Beef Cattle Engineering Research Center, Yangling 712100 Shaanxi, People's Republic of China. Correspondence and requests for materials should be addressed to L.-S.Z. (email: zanlinsen@163.com).

† These authors contributed equally to this study.

**Table S1. Primers used in the expression and functional analysis of the SIX1 promoter experiments**

Reaction	Name	Function	Primer Sequence (5' to 3')	Tm (°C)	Product Length(bp)	Amplified Region
qRT-PCR	GAPDH	Reference	F: CCAACGTGTCTGTTGTGGAT R: CTGCTTCACCACCTTCTTGA	61.0	80	778-857
	SIX 1	RT-PCR	F: GCCAAGGAAAGGGAGAACA R: GACTCTGGGGAGGTGAGAACT	61.0	127	866-992
5' RACE	R1		GGCAGGGTCTACCAAGCACCGTTTCC	66.5	555	
	R2		CACTCCGCTCTTGACCACCGAGA	60.0	475	
Promoter cloning	Primer A	5'flanking region	F: ATCAGCATTGCTTCCAAGT R: TTGAGAGATGTCTTGAATTTTC	62.5	728	-1824/-1097
	Primer B	5'flanking region	F: ACTAAGCATTCTCCACACATAC R: TGTGGAATGAATAAGTAATG	59.0	750	-1164/-415
	Primer C	5'flanking region	F: GTCTACGCTTGTATTATTTATCG R: AAGGAACGGCCGGCTCACTC	61.5	602	-483/+118
	-1802	5'flanking region	F: <i>CGGGGTACCTAATTGCCTGGGATACTGAA</i>	63.5	1947	-1802/+144
	-1488	5'flanking region	F: <i>CGGGGTACCGAAGCAAATTTAAAGAACCTA</i>	62.0	1633	-1488/+144
	-1044	5'flanking region	F: <i>CGGGGTACCCACACACATGCACACACACAC</i>	65.0	1189	-1044/+144
	-708	5'flanking region	F: <i>CGGGGTACCAATCTCTATTTGCTCAGCAAC</i>	60.5	853	-708/+144
	-483	5'flanking region	F: <i>CGGGGTACCGTCTACGCTTGTATTATTTATCG</i>	64.0	628	-483/+144
	-216	5'flanking region	F: <i>CGGGGTACCTCACGTTGCAAGGTCCTGAC</i>	63.5	361	-216/+144
	-28 +144	5'flanking region Exon 1	F: <i>CGGGGTACCAATCAGCGCGCCGAGAACGC</i> R: <i>GGAAGATCTGAGGAGGAGTAGAGCGCGCGC</i>	60.0	173	-28/+144
EMSA	NRF1 forward		CCGGCCCGGCGCGC <i>CGCA</i> GGCTGGCCAGCCCCGG			-80/-46
	NRF1 reverse		CCGGGGCTGGCCAGCC <i>TGCG</i> CGCCCGGGCCCG			
	mNRF1 forward		CCGGCCCGGCGCGC <i>TTTT</i> GGCTGGCCAGCCCCGG			-80/-46
	mNRF1 reverse		CCGGGGCTGGCCAGCC <i>AAAA</i> CGCCCGGGCCCG			
	ZF15 forward		CGGCCCGGCGCGC <i>CGCA</i> GGCTGGCCAGCCCCGGA			-79/-45
	ZF15 reverse		TCCGGGGCTGGCCAGCC <i>TGCG</i> CGCCCGGGCCCG			
	mZF15forward mZF15 reverse		CGGCCCGGCGCGC <i>TTTT</i> GGCTGGCCAGCCCCGGA TCCGGGGCTGGCCAGCC <i>AAAA</i> CGCCCGGGCCCG			-79/-45
ChIP	ChIP-NRF1/ ZSCAN10		F: CCCACTTTCGCTTCTCACCACCTC R: CCGGCTCACTCCGAGCTTTT	60.0	261	-152/+109
	ChIP- control	Exon 2	F: TCCTAAGAAGGGAGGGACTGG R: AGGCTGGTAACCTGGATGTTT	60.0	283	3180/3463

The italicized letters of the forward (F) and reverse (R) primers were enzyme cutting sites of *KpnI* and *BglII*, the underlined bases are core putative transcription factor-binding sites, respectively.

**Table S2. The different potential *cis*-acting elements between wild types and SNPs sequence in the proximal minimal promoter of bovine SIX1.**

Item	Transcription factors	Optimized	<i>Cis</i> -acting elements (Recognition sequence)	Target strand	SNPs
WT1	Regulatory factor X 3 (RFX 3)	0.84	cgggCTG <i>C</i> gaggccgccc	(-)	
SNP1	NONE	0.9	cgggCTG <i>A</i> gaggccgccc	(-)	g. -85 G > T
WT2	ZF5 POZ domain zinc finger (ZF5F)	0.83	cagcc <i>C</i> GCGcgcgc	(-)	
	Cell cycle-dependent element (CDEF)	0.87	ggcgCGC <i>G</i> ggctg	(+)	
SNP2	Nuclear respiratory factor 1 (NRF1)	0.92	cggcgCGC <i>A</i> ggctggcc	(+)	g. -63 G > A
	Zinc finger and SCAN domain containing 10 (ZSCAN10)	0.86	tggccagcc <i>T</i> GCGcgc	(-)	g. -63 G > A

WT stands for wild type. SNP loci are underlined in the table; capital letters are the core sequence of the transcription factors, and the value > 80 denotes a sequence combination with the *Cis*-acting elements.