

Molecular characterization of the buffalo SCAP gene and its association with milk production traits in water buffaloes

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Supplementary File

Table S1. Primers used to amplify the buffalo SCAP gene, mRNA analysis and their SNP discovery

Primers	Primer sequence (5'~3')	Length (bp)	Tm (°C)	Note
SCAP_F1	ACGAGGTGTCTGCAAATGGT	1639	60	Gene cloning
SCAP_R1	TCGGGCCAGGAAGTAGATGA			
SCAP_F2	CATCCATGCCCCACACCAT	1162	60	Gene cloning
SCAP_R2	GTCTGGCTGGTCCCCAAAG			
SCAP_F3	ACGCCTCCAAGTTACCCG	899	60	Gene cloning
SCAP_R3	CGCTGAAAAGTTGGTGTCAATC			
SCAP_F4	CCTGGGAACGGCTGTCGGA	456	60	Gene cloning
SCAP_R4	TCCGCCCCACCACGATGA			
SCAP_F5	GACAGCGGTGTTGGTAGCG	597	60	Gene cloning
SCAP_R5	CCTTCTGTCCAGGAAAACGAG			
SCAP_F6	ACCTGCTTGATTGACACCAAC	768	60	Gene cloning
SCAP_R6	CTGGTCAATATACACGGTCGTG			
SCAP_F7	ACACCAGAAGCCCATCACAG	706	60	Gene cloning
SCAP_R7	CAGAAGCAGCTTTCCCAAGAC			
QSCAP_F	TTGACATTCGCCGATGGAGC		60	mRNA analysis
QSCAP_R	CGCTTGGGGAGACGCAGGTT			
GAPDH_F	ACTTTGGCATCGTGGAGGGACTT		60	mRNA analysis
GAPDH_R	TTCTCCAGGCGGCAGGTCAG			
SNP_F1	TCCTGATTGGGTCTCCACATCCCCG	1260	58	SNP screening
SNP_R1	TAAACATTAGATGTGCAATGAATCT			
SNP_F2	GATAGCGGTTTGTGGTAGACGATGC	842	58	SNP screening
SNP_R2	ACAACCCAGCTTTTCCAGGACAGT			
SNP_F3	GGCAGGGGTTCTACGTGGAGTG	1315	58	SNP screening
SNP_R3	CCAATGCAGGGGACGCAGTTTCG			
SNP_F4	TTGTGAAATCTTCCGTGTCTCC	1067	58	SNP screening
SNP_R4	GGATGGTCCTGATGATGTCTCG			
SNP_F5	ATCATCAGGACCATCCACCAGCAT	1443	60	SNP screening
SNP_R5	TGGACTTGACCATGTCGATCTTGC			
SNP_F6	TCCAGGGTTCCTCCCACTACTCAC	1662	56	SNP screening
SNP_R6	CGTTCTCCAGCCCGATGACCACC			
SNP_F7	AGTCCTGTGATGTGGGAGTTGCTTG	1278	57	SNP screening

SNP_R7	GGTGAAGTAGCCAATGAGGACGATG			
SNP_F8	AGCAGGGTCCTTAGGGTTGGAGCAT	1932	54	SNP screening
SNP_R8	CCGCTTGGGGAGACGCAGGTTGC			
SNP_F9	CCTGTCCATTGACATTCGC	1505	60	SNP screening
SNP_R9	CCAGTGTGATGTTGTAATAGCTG			
SNP_F10	GGACGCCTCCAAGTTACCCG	1503	62	SNP screening
SNP_R10	GCCGTCCGACAGCCGTTCC			
SNP_F11	CCCCGAGACGGAGATTGTG	619	62	SNP screening
SNP_R11	ACGGCTGAACTCGTATCCTGT			
SNP_F12	CACTGGTGGTTTTTTGACATTTTAG	1319	58	SNP screening
SNP_R12	CCGCAAATGGGTTTAGCAGTAC			
SNP_F13	AGACCCACACTGCCCTCAGCC	1477	55	SNP screening
SNP_R13	CCTGCTGGATGGAGTAGAAGTTGATG			
SNP_F14	GTGTTCCGTCTGGAGGATTC	890	57	SNP screening
SNP_R14	GACAGTCAGAAGCAGCTTTCC			

Table S2. Comparative analysis of SCAP amino acid sequence of different species.

Species	GenBank Accession number	Similarity (%)
<i>Bos taurus</i>	NP_001095359.1	99
<i>Capra hircus</i>	XP_017922557.1	99
<i>Ovis aries</i>	XP_014957938.1	99
<i>Sus scrofa</i>	NP_001185623.2	94
<i>Homo sapiens</i>	NP_036367.2	93
<i>Rattus norvegicus</i>	NP_001094436.1	90
<i>Mus musculus</i>	NP_001096632.1	90
<i>Gallus gallus</i>	XP_001231540.3	75

Table S3. Genetic diversity based on SCAP polymorphisms in the test population of buffalo.

SNPs	Genotype frequencies (%)			Allele frequencies (%)		HWE <i>P</i> value	Location
	AA	AG	GG	A	G		
g.1717600A>G	AA	AG	GG	A	G	0.63	Intron 1
	0.61	0.34	0.04	0.79	0.21		
g.1718168A>G	AA	AG	GG	A	G	0.26	Intron 1
	0.61	0.35	0.04	0.79	0.21		
g.1742850G>A	GG	GA	AA	G	A	0.40	Intron 2
	0.65	0.30	0.05	0.80	0.20		
g.1753880A>G	AA	AG	GG	A	G	0.03	Intron 3
	0.38	0.43	0.20	0.59	0.41		
g.1757922C>T	CC	CT	TT	C	T	1.00	Intron 8
	0.61	0.35	0.05	0.78	0.22		
g.1758953A>G	GG	GA	AA	G	A	0.12	Intron 9
	0.71	0.25	0.04	0.83	0.17		
g.1759116A>G	GG	GA	AA	G	A	0.53	Intron 9
	0.61	0.35	0.04	0.78	0.22		
g.1759142C>T	CC	CT	TT	C	T	0.53	Intron 9

g.1760740G>A	0.61	0.35	0.04	0.79	0.21	0.31	Intron 11
	GG	GA	AA	G	A		
	0.39	0.45	0.16	0.62	0.38		
g.1762368A>G	0.34	0.50	0.15	0.60	0.40	0.51	Intron 14
	AA	AG	GG	A	G		
	0.34	0.50	0.15	0.60	0.40		
g.1766036C>T	0.40	0.44	0.16	0.62	0.38	0.31	Exon 20
	TT	TC	CC	T	C		
	0.40	0.44	0.16	0.62	0.38		

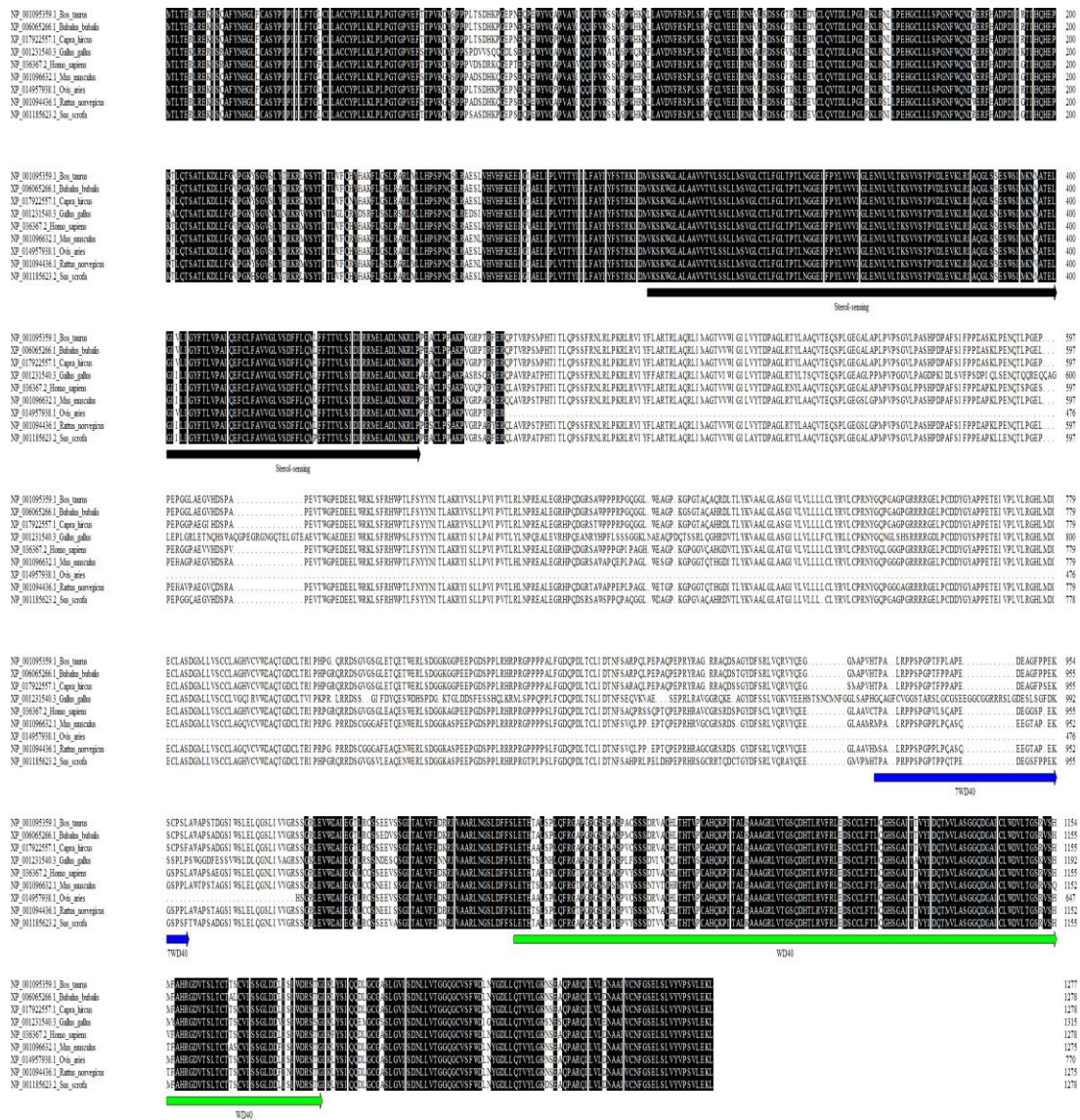


Fig.S1 Multiple alignment of buffalo SCAP with other homologues. Notes. The completely conserved residues across all species are shaded in black. Black arrow represents the sterol-sensing domain. Blue arrow represents the 7WD40 motif. Green arrow represents the WD40 domain.

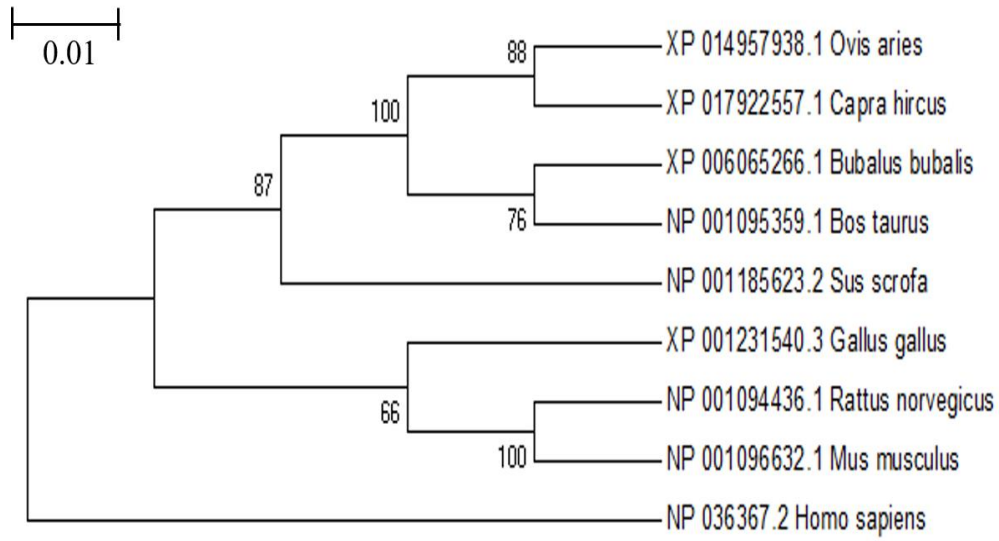


Fig.S2 Phylogenetic tree of *SCAP* family members was constructed with the neighbor-joining method. Node values represent percent bootstrap confidence derived from 1000 replicates.

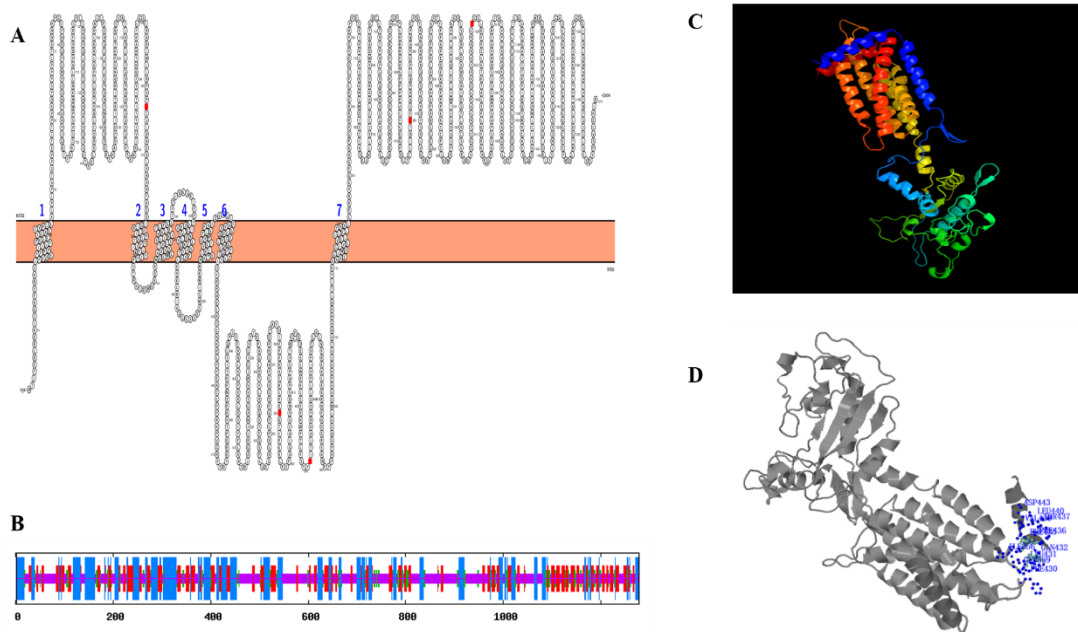


Fig.S3 Secondary structure prediction and 3D structure analysis of buffalo *SCAP*