

Functional impact of c.V15M variation in LHβ gene on silent estrus behavior in river buffalo of Pakistan

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SUPPLEMENTARY FILE

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B4_LH2      ACTGACCTTGTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGGCATGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
B5_LH2      ACTGGCCCTTGTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
K1_LH2      -CTGGCCCTTGTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
B2_LH2      -CTGGCCCTTGTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAAGCCCACTGGCGGCTGAGAA
Mediterranean ACTGGCCCTTGTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
Swamp      -ATGGAGATGTTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
River      GATGGAGATGTTCCAGGGAAGTCTGCTGTGGCTGCTGCTGGCGGTGGCCGCGGTGTGGCTTCCAGGGGGCCATTGCGGCCGCTGTGCCAGCCATCAACGCCAACCTGGCGGCTGAGAA
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Figure S1 LHβ Gene Sequence Comparison in various buffalo breeds (B4, B5, B2: Buffalo sequences of Nili Ravi Breed for primer-2 of LHβ Gene, K1: Cattle sequences of Sahiwal breed for primer-2 of LHβ Gene)

Table S1. Primer Sequence for LHβ Gene in Nili-Ravi Buffalo

Primers	Sequence	Length	Tm
LHB F1	CCTGACCTTGTCTGCCTCTC	20	60°C
LHB R1	CACTTCCTCCCAGCTGTTTC	20	60°C
LHB F2	GGTCCCTGAACGTGTGCAT	19	62°C
LHB R2	AATCTGCCTGGAGCTGAGTTG	21	61°C
LHB F3	CCTGAGTCTGGGATCTGTGG	20	60°C
LHB R3	GAGGAGTGTCTGCTGGCTTC	20	60°C

Table S2: Heat score of Nili Ravi Buffalo breed of Pakistan

Sample ID for Nili-Ravi Buffalo	Heat Score	Symptoms
69	155	Standing heat, Mounted but not standing, Mounting head side of other cows
85	120	Standing heat, Mounted but not standing, Sniffing
29	95	Mounted but not standing, Mounting, Restless, Mounted but not standing
23	100	Standing heat
49	95	Mounted but not standing, Mounting, Restless, Mounted but not standing
44	105	Standing heat, Restless
41	125	Standing heat, Mounted but not standing, Sniffing, Mucus discharge
57	83	Mounted but not standing, Mounting, Mucus discharge
31	45	Mounting head side of other cows
34	26	Sniffing, chin resting
30	-	-
33	11	Mucus discharge, Restless, Cajoling
38	05	Restless
46	26	Sniffing, chin resting
50	20	Restless, chin resting
58	20	Restless, chin resting
27	10	Restless
20	11	Mucus discharge, Restless, Cajoling
22	35	Sniffing, chin resting, Mounted not standing
25	36	Sniffing, chin resting, Mounted not standing
21	45	Mounting head side of other cows
23	15	Restless, vaginal discharge
24	20	Restless, chin resting
74	145	Standing heat, Mounted but not standing, Mounting head side of other cows
48	6	Restless, vaginal discharge
60	15	Mucus discharge, Restless, Cajoling
52	6	Mucous discharge, Cajoling
57	11	Mucus discharge, Restless, Cajoling
45	18	Mucus discharge, Restless, Cajoling, Sniffing
58	20	Restless, chin resting
13	11	Mucus discharge, Restless, Cajoling
7	10	Mounted not standing
56	10	Sniffing