Supplementary materials

Comparative Expression Profiling and Sequence Characterisation of ATP1A1 Gene Associated to Heat Tolerance in Tropically Adapted Cattle

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1. Additional materials and methods -q-RT-PCR

For loading samples, the plate was placed into the Eco sample-loading dock, the notch was aligned with the matching indentation on the adapter. The dock light was turned on and the dock was inclined to a comfortable angle for pipetting. The samples and q-RT PCR reagents were pipetted into the plate and the plate was sealed with an Eco optical seal, which held the plate in place on the Eco sample loading dock, before the squeegee was dragged firmly across the surface to ensure the seal was secure. The plate adapter with sealed plate was placed into a plate centrifuge along with a second plate adapter for balance. The plate was centrifuged at 250 x g for 30 sec. The Eco lid was opened and the plate was placed on the block, and the notch aligned against the top left corner. The lid was closed and the reaction was started.

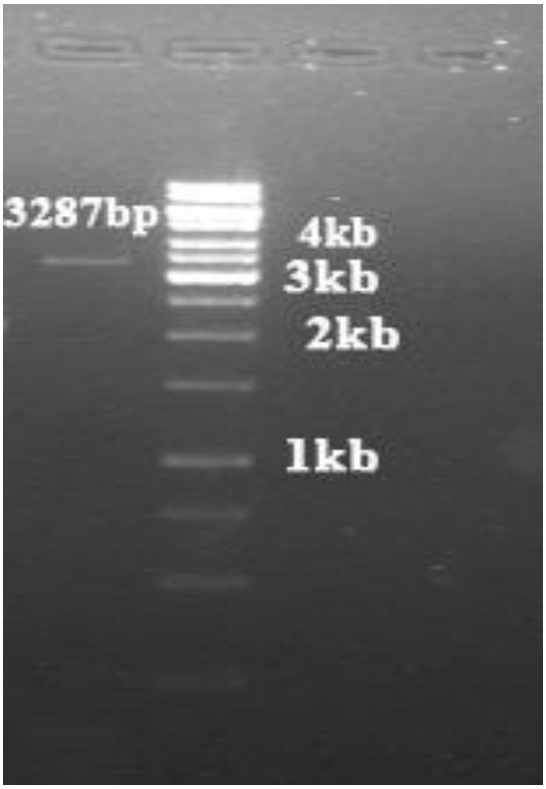

Figure S1. Gel demonstrating the 3287 bp PCR amplified product of ATP1A1 gene of Vechur cattle in 1% agarose gel (M- 1 Kb DNA Marker, A-ATP1A1 gene-3287bp)

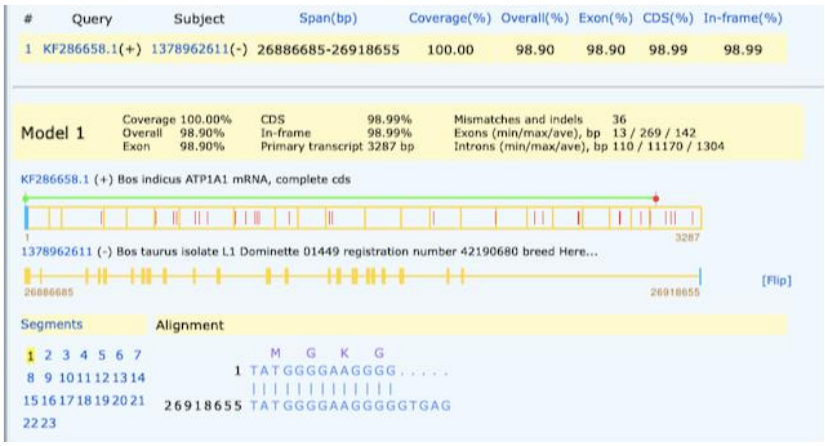

Figure S2. Results of mRNA (cDNA) to genomic alignment created by Splign. Graphical view the alignment between Vechur ATP1A1 sequence KF 286658.1 and Bos taurus reference genome (1378962611).

#	Query	Subject	Span(bp)	Coverage(%)	Overall(%)	Exon(%)	CDS(%)	In-frame(%)
1	KF286658.1(+)	1378962611(-)	26886685-26918655	100.00	98.90	98.90	98.99	98.99

#	Query	Subject	Idty	Len	Q.Start	Q.Fin	S.Start	S.Fin	Type	Details
+1	KF286658.1	1378962611	1	13	1	13	26918655	26918643	GC<exon>GT	M13
+1	KF286658.1	1378962611	1	105	14	118	26907473	26907369	AG<exon>GT	M105
+1	KF286658.1	1378962611	1	60	119	178	26906748	26906689	AG<exon>GT	M60
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+1	KF286658.1	1378962611	0.993	135	497	631	26903240	26903106	AG<exon>GT	M134R
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+1	KF286658.1	1378962611	1	137	1832	1968	26894778	26894642	AG<exon>GT	M137
+1	KF286658.1	1378962611	0.987	151	1969	2119	26893393	26893243	AG<exon>GT	M21R2M
+1	KF286658.1	1378962611	1	169	2120	2288	26892646	26892478	AG<exon>GT	M169
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+1	KF286658.1	1378962611	0.984	124	2444	2567	26891837	26891714	AG<exon>GT	M37RM
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+1	KF286658.1	1378962611	0.972	249	3039	3287	26886933	26886685	AG<exon>	M4R2M

Figure S3. Tabular format for the alignment between Vechur ATP1A1 sequence KF 286658.1 and Bos taurus reference genome (1378962611).

```
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>lcl|Seg6   gb|KF286658.1| [497,631] x gi|1378962611
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TGGAAGTGAAAGGAGGGGATCGAATTCCTGCCGATCTCAGGATCATTTCTGCAAATGGCTGCAAC
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CCCTGGAGACACGGAACATTGCCTTATTTTCAACTAAGTGTGTTGAAG
>lcl|Seg8   gb|KF286658.1| [750,1018] x gi|1378962611
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GCGGTGTTCCTGGGCGTGTCGTTCTTCATCCTTTCTCTGATCCTTGAGTACACCTGGCTGGAGGCTGTCA
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>lcl|Seg9   gb|KF286658.1| [1019,1217] x gi|1378962611
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GGCCCACATGTGGTTCGACAACCAAATCCACGAGGCGGACACGACAGAGAATCAGAGCG
>lcl|Seg10   gb|KF286658.1| [1218,1327] x gi|1378962611
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CGTGTTTCAGGCTAACCAGGATAACCTGCCTATCCTGAAG
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AGGAGATGAGAGAGAGATACACCAAGATCGTCGAGATCCCATTCAACTCCACCAACAAGTACCAG
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>lcl|Seg12   gb|KF286658.1| [1463,1655] x gi|1378962611
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CGCTTTTCAGAATGCCTACCTGGAGCTGGGTGGCCTCGGAGAGCGTGTGCTGG
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TTGAAGTACCACACAGAGATTGTGTTCGCCAGGACCTCTCCGCAGCAGAAGCTCATCATTGTGGAAGGCT
GCCAGAGACAG
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>lcl|Seg18   gb|KF286658.1| [2444,2567] x gi|1378962611
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CAGTGG
>lcl|Seg20   gb|KF286658.1| [2714,2844] x gi|1378962611
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>lcl|Seg21   gb|KF286658.1| [2845,2946] x gi|1378962611
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>lcl|Seg23   gb|KF286658.1| [3039,3287] x gi|1378962611
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ACCGAAGCATGTGGGGAAGGGAGACGTCCCGGAATGAAGCATGTAGCTCTATGGGGGGGGTGGGGGTGGG
AAGAGGGGTGCCCGAGAAACATCCGTCTGTGGAAACGAC
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Figure S4. Segments and boundaries of the aligned regions of 23 exons between Vechur ATP1A1 sequence (KF 286658.1) and Bos taurus reference genome (1378962611).

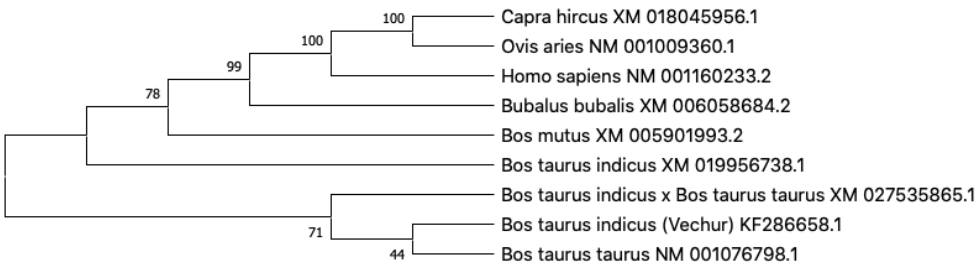

Figure S5. The evolutionary history of ATP1A1 gene in Vechur cattle inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.12680380 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.