

The functions and mechanisms of sequence differences of DGAT1 gene on milk fat synthesis between dairy cow and buffalo

Dinesh Bhattarai, Rahim Dad^a, TesfayWorku, SutongXu, Farman Ullah, Min Zhang, XianweiLiang, Tingxian Den, Mingxia Fan and Shujun Zhang

SUPPLEMENTARY FILE

Table S1

List of Primers for SNP study and for Plasmid construction

| primer | Forward* | Reverse* | Length | Tm |
|--------|------------------------------|----------------------------|--------|------|
| BV1 | CGGGTACCCGCCAGCGGACTACAAAG | CCAGATCTATCCCGCACCTCCTCTTC | 193 | 58.0 |
| BV2 | CGGGTACCAAAAGTGGGTGCCTACGGT | CCAGATCTATCCCGCACCTCCTCTTC | 699 | 58.0 |
| BV3 | CGGGTACCTCTTGTGTAAACCAGGCCTA | CCAGATCTATCCCGCACCTCCTCTTC | 1219 | 56.0 |
| CV1 | CGGGTACCCGCCAGCGGACTACAAAG | CCAGATCTATCCCGCACCTCCTCTTC | 363 | 58.0 |
| CV2 | CGGGTACCAAAAGTGGGTGCCTACGGT | CCAGATCTATCCCGCACCTCCTCTTC | 666 | 58.0 |
| CV3 | CGGGTACCAACCTCTGGGTCCCTGCGA | CCAGATCTATCCCGCACCTCCTCTTC | 945 | 56.0 |
| CV4 | CGGGTACCTCTTGTGTAAACCAGGCCTA | CCAGATCTATCCCGCACCTCCTCTTC | 1148 | 56.0 |

*5'-3' direction

Table S2

Comparison of mRNA sequence between cow and buffalo DGAT1 gene

| S.N. | Region | Mutation | mRNA position | AA position | AA codon | AA change |
|------|--------|---------------|---------------|-------------|----------------|------------|
| 1 | 5'UTR | A>G | 8 | | | |
| 2 | Exon 1 | G>T | 34 | 7 | gcG/gcT | A/A |
| 3 | | C>T | 102 | 30 | gCg/gTg | A/V |
| 4 | | T>C | 123 | 37 | gTg/gCg | V/A |
| 5 | | Exon2 | T>C | 262 | 83 | cgT/cgC |
| 6 | Exon4 | G>A | 361 | 116 | caG/caA | Q/Q |
| 7 | Exon6 | T>C | 478 | 155 | gcT/gcC | A/A |
| 8 | | T>C | 568 | 185 | tcT/tcC | S/S |
| 9 | Exon8 | G>A | 707 | 232 | Gcg/Acg | A/T |
| 10 | | C>A | 708 | 232 | gCg/gAa | A/E |
| 11 | Exon9 | C>G | 793 | 260 | ccC/ccG | P/P |
| 12 | Exon11 | T>C | 1066 | 351 | gcT/gcC | A/A |
| 13 | Exon13 | G>A | 1237 | 408 | gtG/gtA | V/V |
| 14 | Exon14 | C>T | 1300 | 429 | ctC/ctT | L/L |
| 15 | | C>G | 1312 | 433 | acC/acG | T/T |
| 16 | Exon15 | G>A | 1318 | 435 | gcG/gcA | A/A |
| 17 | | C>T | 1405 | 464 | atC/atT | I/I |
| 18 | | C>T | 1464 | 484 | gCg/gTg | A/V |
| 19 | 3 UTR | G>C | 1486 | | | |
| 20 | | G>T | 1528 | | | |
| 21 | | G>A | 1530 | | | |
| 22 | | T>C | 1554 | | | |
| 23 | | A>G | 1580 | | | |
| 24 | | T>C | 1611 | | | |
| 25 | | >C | 1620-21 | | | |
| 26 | | >T | 1620-21 | | | |
| 27 | | A>G | 1631 | | | |
| 28 | | A>G | 1664 | | | |
| 29 | | T>C | 1670 | | | |
| 30 | | A>G | 1708 | | | |
| 31 | | T>A | 1728 | | | |

Table S3

Distribution of K232A allele in cow and buffalo population

| mRNA position | AA Change | Amino acid | Frequency (n= 84) | |
|---------------|-----------|------------|-------------------|---------------|
| | | | Cow(n=43) | Buffalo(n=41) |
| G707A | Lys 232 | K | 0.25 | 1.00 |
| G708A | ala | A | 0.75 | 0.00 |

Figure S1

Schematic representation of *DGATI* gene structure cow (left) and buffalo (right). The genomic, mRNA and protein components in details, 5'-UTR: 5'- untranslated region, 3'- UTR: 3'- untranslated region, ORF: open reading frame, and Putative protein domain (MBOAT)

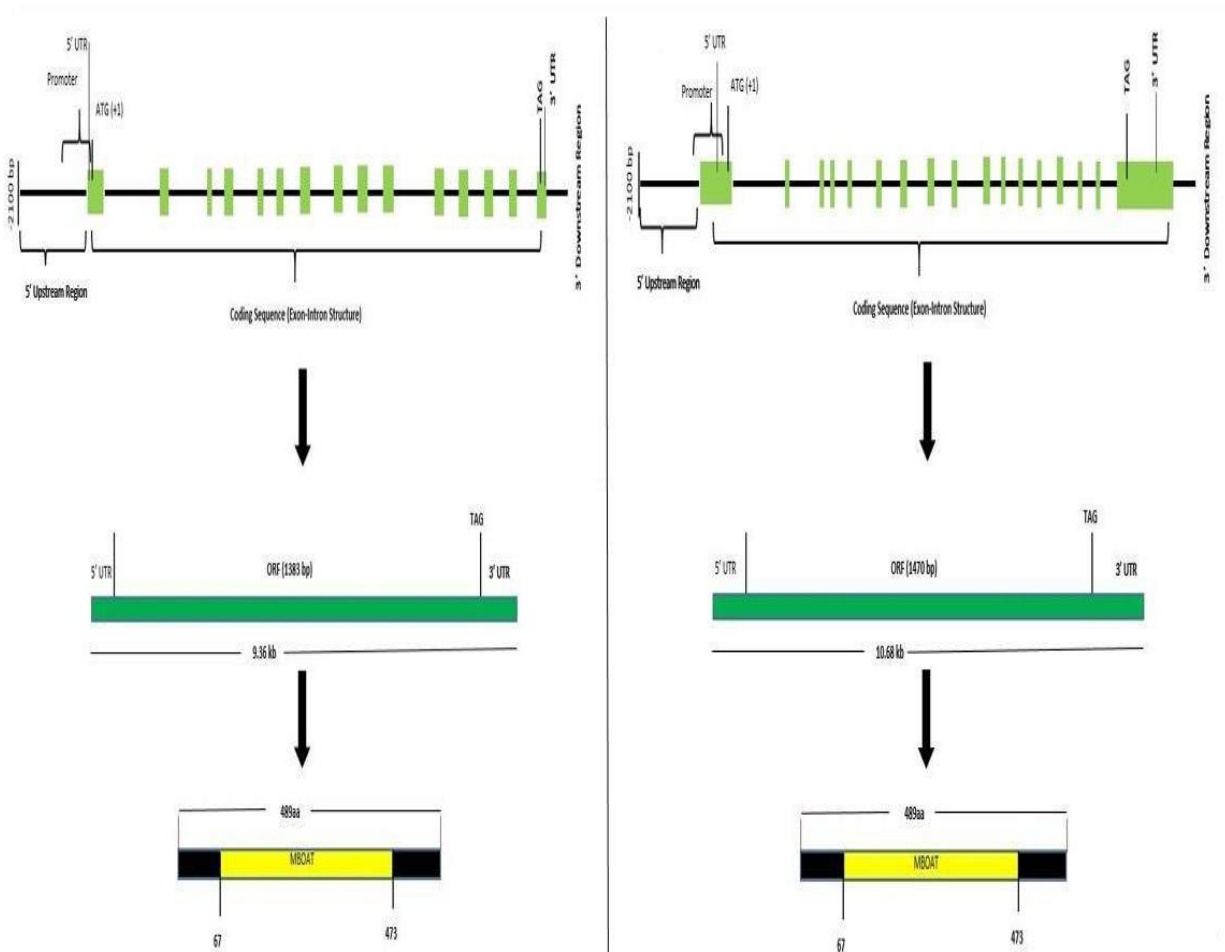
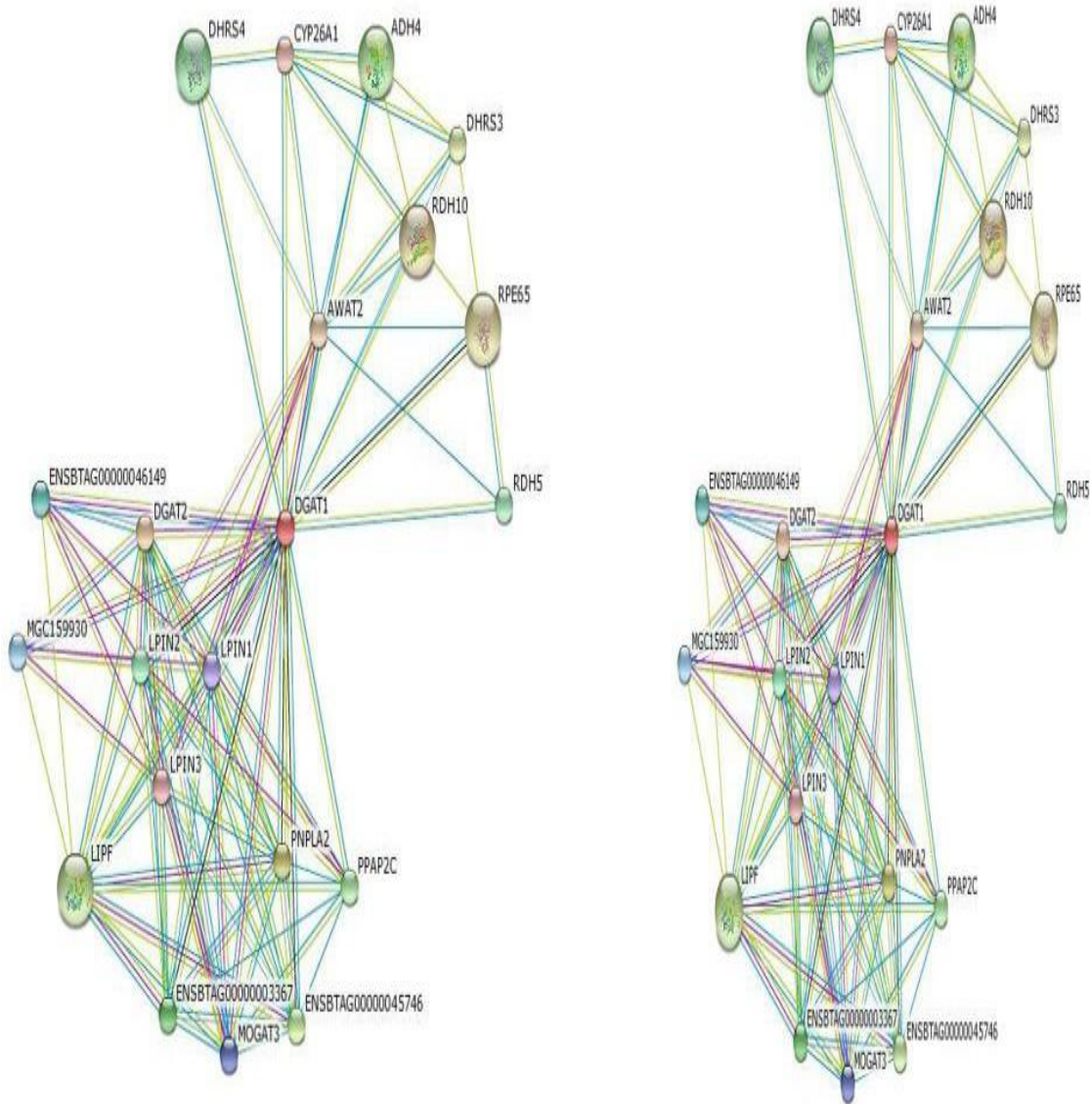


Figure S2

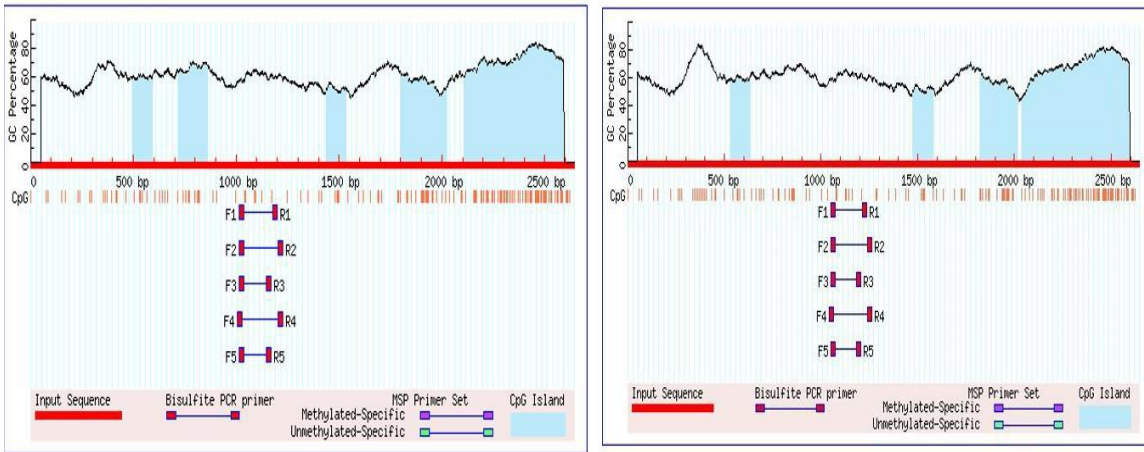
Protein interaction of *DGAT1* gene cow (left) and buffalo (right). The length and color indicates the specificity of nearness and farness of the other protein. In both cow and buffalo, there is similar interaction of other protein with *DGAT1* protein.



14 **Figure S3**

15 CpG island prediction in Cow *DGAT1* (left) and Buffalo *DGAT1* (right). The software
16 <http://www.urogene.org/methprimer/> was used for the prediction. The results showed 5
17 different prediction of CpG Island in cow *DGAT1*; 5' upstream sequence while 4 CpG
18 Island are predicted in Buffalo *DGAT1* 5' upstream sequence.

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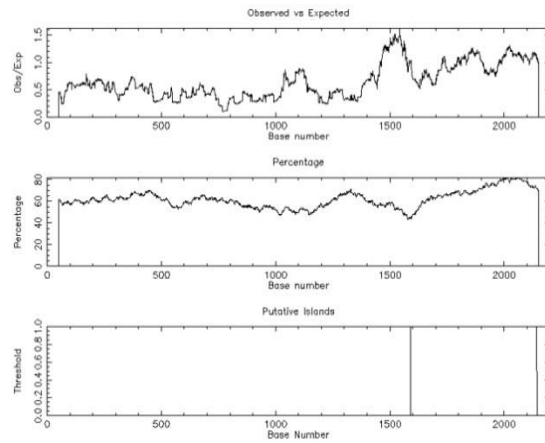
Figure S4

DGAT1 gene 5' untranslated region (UTR) and transcription regulatory region. The 2000 bp 5' UTR sequence of *DGAT1* gene (cow and buffalo). Predicted promoter region of *DGAT1* gene are shaded in gray. The TATA and CAAT box regions are marked with red and blue boxes. ATG is the start of coding sequence (+1).

-2000

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GCCCGCCCTTGG.....CTGAGCGGTCAATTTCTCCTCTGCCAATCAGCGCGCCAGCCAGGCTGCCCTC
TA.....CCATCTCCCTTGGGAGCTATA-TGAGTCTTAGCGCTG.....TAA
GGATGTCAGGCTGGCCCTCAGCCGCAAAATGTCACAGT.....TAGGGGTGTTTCTTGTGTAAGC
CAGGCCATAAATCCCGGTGAACCTCGCATCTGGAGATCCAGGATACTCACACTCCATGCTCTTCCAAATGTTG
TGAACAAGTAAGATGGCCCTTGCCTGGCAAGCCGCTCACTGTGCAAGTTGTTTGGTGATTGGTGTCTTCAATC
AAGGACTGGATGACTGCCGACTGTGCAATGAACAGAA.....CCTACGGTGGTAATTAGTG
GGTTCACAGACGACCCCTGCCGCGGATGTAGCTTGGTAGACGCGTGGGTGTGAGCCTGACGTTAAGCAGCTA
GGCATTTCATAAATACTACAACCCCAATTTCTGCGCTGAGCTGAGAAATGACGAATCCTGTGTATAAGCGG
GACAAAGGGCAGGCGGCTCAGCAGAGGCTTGTTCAG.....GCCTGGGTGCTGCCAGCCGCGGGC
TAGCACTTGGCCGCGGGGTGCGAACTAAGGCGATGGGGACCGCGCGGGCGGGCTCCCGCGCGGAGG
ACGGGGTCCGCGSCTTCGATCCAGGGCGGCGAGTGGGCGCGCGGACGGAAGAGAGGTGGGGATGGGCGCGG
AGGGGACGCGCGSCTCCGGGACACAGACAAGGACGAGACTAGACSTGGCGAGCGCCACTGGGACCTGAG +191
  
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CGCCTTGGTACA.....CCTGAGCGGTCAATTTCTCCTCTGCAATAGCGCGCCAGCCAGGCGCCCTCTC
AGTCAC.....GGGAGCTATACTGAGTCC.....AAGACAGGGCCAGGTAGCT
CCAGCCTGCCAGGTAAGGATGTGAGCTGGCCCTCAGCCCAAAATGTCACAGT.....AACGGATGGGTTT
TGTGGCTAGGGGTGTTCTTGTGTAAACAGGCGCTAAACTCCGCGTGAACCTCGCATCTGGAGATCCAGGATGCTC
ACACTCCATGCTCTTGGCAAAATGTTTGTGAAA.....TGGATGACTGCTACTGTGCAATGAACAGAAACCT
CTGGATCCACAGCCGTCGCG.....CGGGATGCAGTTCGTTAGCAGCGTGGGTGTGAGCCTGACGTTAAG
CACTAGGCATTTGTAATATCTACAACCCACACTCTGCGCTGCTGAGAAATGACGAATCCTGTGTATATAG
CGGGACAAGGGCCAGGCGGCTCAGGAGAGGCTTGTGTCAGCTGCCAGGAAGCCCGCGGTGCTCTTTCAGTCC
GGGATTGCATTTGCCAGGACTACAACCTCCAGGCTGCTGCGCGCGCGCGGCGGACCACTCCAGGCTGAC
.....GCGGTGGCGCAGTTAGCCAAGGTCGGAGGCGGGGCGGCGGCGGCGGCTCGGCGCAGC
CGCGGGCTACGACTTGGCCGCGGGGTGCGGGCTGAGGCCATGGGCGACCGCGCGGCGCTGGCGGCTC +29
  
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