



Genome wide analysis of growth and development genes in cattle (*Bos taurus*) and Buffalo (*Bubalus bubalis*)

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Abstract

Bos taurus and *Bubalus bubalis* have been major source of meat since about 10000 years. 50 genes associated with growth and development in *Bos taurus* and *Bubalus bubalis* were analyzed integrately by GWAS performing chromosomal mapping, gene structure, conserved motif analysis, gene expression, GO annotation analysis, protein-protein association and phylogenetic analysis, Main bio-informatics tools used were NCBI, Expasy, WebScipio, iTOL, heatmapper, string and MEGA 5. GH, TG and NEB were found as major genes of beef production by visualising expression by heatmapper, motif analysis by MEME and gene structure analysis using webScipio. All other genes were also linked differently with this trait. Each gene had unique structure and discrete role regarding growth. GH affects directly on growth and development. String results revealed that TG associates with other genes in complex networks to stimulate growth. Results of Motif analysis by using MEME and heatmapper showed that NEB has highly conserved domains associated with structural and functional role in skeletal muscle growth and development. Heatmapper analysis showed that CFL1 has role in growth and development of brain, lungs, spleen, colon and testis. ALDH2 showed overexpression in kidney and liver and ACTC1 in heart. Synteny analysis was performed to elucidate syntenic regions between Bt and Bb. Results showed that there is strong similarity and conserved regions. This is due to the strong evolutionary relationship of Bt and Bb. Phylogenetic analysis confirmed strong evolutionary relationship of *Bos taurus* and *Bubalus bubalis*. This comprehensive research could be used by animal scientists to understand growth pattern, association of genes and development of genetic markers to increase beef production to make Pakistan hub of halal food.

Keywords: Gene, GH, growth, development, NCBI

Introduction

Cattle and buffaloes were used as a source of meat and milk production on a global scale about 10000 years ago.

(MacHug *et al.*, 1998). Archeological evidence, historic documents and genetic analysis have recorded the domestication of cattle and buffaloes. Domestication of



cattle and buffaloes occurred autonomously in the Near East and the Indian subcontinent. Human migrations, which advanced the dissemination of domestic cattle over Asia, Africa, Europe and the New World. (Liu *et al.*, 2014) Due to meat production, cattle (*Bos taurus*) and Asian buffaloes (*Bubalus bubalis*) play an essential socioeconomic role. (De Camargo and colleagues, 2015) Meat is a main protein source throughout the world. The meat industry has been rapidly growing to meet the increasing demand for meat, which is one of today's most challenging social issues. (Thornton 2010; Cunningham 1989) The major beef-producing countries in Latin America are Brazil (51.6 %), Argentina (18.5 %) and Mexico (9.4 %). Uruguay, Venezuela, Paraguay, Bolivia, Ecuador, and Chile provide only 1% of regional production. Latin America is a region of the world that boosted supply in response to growing demand for beef. Brazil's beef cattle sector is well-developed, relying primarily on grass-fed cattle such as *Bos taurus*. Beef production in Brazil has grown to become one of the most important sources of job and wealth creation in the last eight years, involving about 200 million head of cattle, 7.5 million jobs, and more than US\$25 billion revenue, with a demand for around 450,000 bulls replacement per year. However, Brazilian beef productivity remains low, and herds require significant improvement (Dunner *et al.*, 2013). There are about 207 million buffaloes in the world, with more than 97 % living in Asia, 2% in Africa, mainly Egypt, 0.7 % in South America, and less than 0.2 % in Australia and Europe. India, Pakistan, China, Egypt, and Nepal are the countries having the most dairy buffaloes. Dairy buffaloes outnumber dairy cows in Pakistan, Egypt, and Nepal. (Vaughn *et al.*, 1999) Pakistan has a diverse range of cattle genetic resources. Buffalo is rightly referred to as Pakistan's "Black Gold" due to their adaptability. The best buffalo animals may be found in the country's

canal-fed districts, where there are enough fodder and crops to consume. With around 250 000 buffalo/cattle, Pakistan's 'Landhi Cattle Colony' has the world's largest concentration (Younas and Yaqoob 2002). In comparison to cattle beef, buffalo meat is slightly dark reddish in colour. As buffalo meat has a reduced cholesterol content, it is of higher quality than cattle beef. Because buffalo meat is devoid of BSE concerns, it is recommended for export to the Gulf countries. (Vaughn *et al.*, 1999; Batzoglou *et al.*, 2000). It is important to recognize that it is our national obligation to change the available genetic resources in a better form for future generations than we received.

Growth and development are economically significant quantitative traits in beef cattle that influence carcass amount. (Kanis *et al.*, 2005; Park *et al.*, 2018) Intramuscular fat deposition, often known as "marbling," contributes greatly to meat production and quality characteristics such as juiciness, flavor, and tenderness. IMF is regulated primarily by genetic variables (breed and sex differences and double-muscling syndrome) as well as age and nutrition. Several number of management elements, including as weaning age, castration, slaughter weight, and environmental conditions also have an impact on IMF. (Wang *et al.*, 2009, Hocquette *et al.*, 2010) Meat production (growth and development) is economically most important trait governed by many genes (Mackay 2001). However, some of these genes may have a major role. These are known as Candidate genes (Mayr 1954). In contrast to natural selection, gene editing and artificial selection favors mutation, which produce "hitch-hiking" effect (Hayes *et al.*, 2009) In subtropical areas, crossbreeding between *Bos taurus* and *Bos indicus* cattle has been used to improve heterosis, growth, and carcass quality. (Davis *et al.*, 2017; Kim *et al.*, 2003).

GWAS (Genome Wide Association Studies) is an authentic method for



connecting traits to its genetics (Korte and Farlow 2013). It has the potential to provide a critical initial look into the genetic architecture and biology of complex characteristics (Belamkar *et al.*, 2011). In beef cattle, GWAS improves the accuracy of genetic predictions for growth, carcasses, reproduction, and health (Kuehn *et al.*, 2011). Dunner *et al.*, (Dunner *et al.*, 2013). The Bovine HapMap Consortium provided the first comprehensive genome-wide investigation of 19 genetically, regionally, and phylogenetically distinct cow breeds in 2009. This was the first study to examine the structure of the bovine genome in different breeds at a high resolution, and it revealed genes involved in production qualities (such as GDF-8 and ABCG2, which are connected to muscle conformation) as well as genes involved in food conversion efficiency (e.g., R3HDM1). Since this initial study, several more have been conducted, all with the same purpose in mind (Silveira *et al.*, 2008; Chung and Kim 2005). CAPN1 (Curi *et al.*, 2009; Tadesse *et al.*, 2006), CAST (Curi *et al.*, 2009, SIRT1) (Tadesse *et al.*, 2006), SIRT2 (GUI *et al.*, 2015), SMO (Zhang *et al.*, 2015), and NEB (Zhang *et al.*, 2015) have all been proposed as functional and positional candidate genes for carcass composition and meat-related production (growth, development) traits (Wyszyska Koko *et al.*, 2006; Davis and Simmen 2000; Herd *et al.*, 1995).

In this study, chromosomal mapping, conserved motif analysis, gene structure, expression analysis, STRING and GO annotation, synteny analysis and phylogenetic analysis were performed to examine 50 genes related with growth and development in *Bos taurus* and *Bubalus bubalis*. The majority of genes for the trait were found in both *Bos Taurus* and *Bubalus bubalis*, but there is difference in chromosome number. Data was collected using various bio-informatics tools e.g. NCBI and Expasy. Web tools used for characterization of genes were iTOL,

WebScipio, heatmapper, string and MEGA 5.

Materials and methods

Sequence Retrieval

Gene bank was searched to identify the growth and development related genes in buffalo and cattle. A query of 50 genes found in various articles was used for this purpose. This was also accomplished with the help of NCBI-BLAsp. Only big open reading frames (ORFs) were employed for further inquiry, such as gene structure determination, phylogenetic analysis, and domain prediction, and only variants of all genes were cross-checked. NCBI was used to obtain genomic sequences, protein length, chromosomal location, and the number of exons in each gene. Using an online tool ([https://web.expasy.org/compute pi/](https://web.expasy.org/compute_pi/)), the isoelectric point and molecular weight of identified proteins were calculated. For further research and characterization, protein sequence data of *Bos taurus* and *Bubalus bubalis* were created.

Chromosomal mapping

File with a map chart NCBI provided an input file with information on growth and development genes. The Map chart programme (<http://www.biometris.wur.nl/UK/Software/MapChart/download>) was used to determine the loci of growth regulating genes (Wheeler *et al.*, 2007; Voorrips 2002).

Gene structure analysis

The coding and genome succession of all predicted genes was downloaded using NCBI. WebScipio was used to get a high-quality gene structure prediction from protein query (<https://www.webscipio.org/>) (Odrionitz *et al.*, 2008).

Conserved motif analysis

In motif analysis, combined information of 50 residues of amino acids



have been subjected to MEME suit server 4.11.2 to compare the orthologous of two species i.e. buffalo (*Bubalus bubalis*) and cattle (*Bos taurus*). The parameters adopted for MEME analysis include number of repetitions, any; protein sequence length 309.5; maximum number of motifs, 10; and optimal breadth of every motif, among 6 and 60 residuals (Cao *et al.*, 2021).

Gene expression analysis

Transcription analysis and putative functions of 50 genes relating to growth and development in different organs of the body of cattle. The expression levels in different organs or tissues based on RNA-seq data (found at <https://www.ebi.ac.uk/gxa/home> in the Expression Atlas database) were downloaded (FPKM values only) (Cook *et al.*, 2019) and analyzed by using Heatmapper at <http://www.heatmapper.ca/> (Babicki *et al.*, 2016). Data comprising RNA sequences from two different experiments showing the expression values in FPKM or TPM for target growth and development related genes in *Bos taurus* and *Bubalus bubalis* (Gonzalez *et al.*, 2018).

GO Analysis

The bioinformatics programme DAVID (Database for Annotation, Visualization, and Integrated Discovery) was used to do the GO annotation study (<https://david.ncifcrf.gov/tools.jsp>) (Huang *et al.*, 2009; Shao *et al.*, 2009; Jiao *et al.*, 2012). growth gene symbol set used as an input source for GO annotation.

Protein-protein association

STRING Ver. 11.0 (<https://string-db.org/cgi/input.pl>) was used to analyze protein-protein association networks (Kim *et al.*, 2021). The STRING programme was used to input the amino acid sequence, and the protein-protein analysis result was downloaded to reflect the gene-gene interaction.

Synteny Analysis

Synteny analysis performed by using TB tool (Chen *et al.*, 2020). Genome file of cattle and buffalo were sequenced aligned with BLAST for synteny analysis (Sun *et al.*, 2019). Identification of tandem and segmental duplication of gene pair based on gene locus.

Phylogenetic Analysis

ClustalW was used to align roughly 50 growth and development-related genes from various families, with gap opening penalty of 10; gap extension penalty of 0.1; gap separation distance of 4; and delay divergence cut-off of 30%. The Neighbor-Joining approach was used to infer evolutionary history (Saitou and Nei 1987). The evolutionary history of taxa studied is represented by the bootstrap consensus tree generated from 1000 replicates (Webb *et al.*, 2008). Branches that correspond to partitions that have been replicated in less than 50% of bootstrap replicates have collapsed. The branch lengths are in the same units as the evolutionary distances used to estimate the phylogenetic tree, and the tree is drawn to scale. MEGA 5 was used to conduct evolutionary analysis. Using a Newick file made with MEGA 5, a phylogenetic tree was created in Interactive Tree of Life (iTOL; <https://itol.embl.de/itol.cgi>). (Hall 2013).

Results

Chromosomal mapping analysis

The results displayed that most genes were located on chromosomes 2 and 4 in cattle while on 2 and 8 in buffalo. Fifty growth and development genes were found on almost all chromosomes except chromosomes # 1, 8, 9, 26 and 27 in cattle and 50 in buffalo on 24 chromosomes except chromosome # 1 and 10. In cattle, Ch#4 had maximum 5 genes CFTR, CRHR2, IGFBP3, LEP and SMO. Ch#2 had second largest concentration of genes of trait with four genes MSTN, NEB,



R3HDM1 and CREB1. Ch#2 in buffalo had six genes of trait MSTN, CREB1 and NEB. ELOVL2, SMIM13 and R3HDM1. Ch# 8 had 5 genes in buffalo but none in cattle. GH1 was located on Ch#19 in but while Ch#2 in Bb. Majority of genes had the same mapping regions and Ch# in both organisms. Chromosome #2 was the largest chromosome in cattle and buffalo among all mapped chromosomes (Figure 1A&1B).

Gene structure analysis

By comparison of sequence and CDS, we determined and examined the structural variation in introns and exons. There were 45 and 38 genes structures analyzed in cattle and buffalo respectively (Figure 2A&2B). Red segment showed the gap region while red thin line indicated mismatch and blue line for sequence shift. In cattle, DGAT1, IGFBP3, MMP1 and MYOZ1 had long gap regions. CRTC2 and GH1 had spliced introns. NEB had very complex structure with large number of small size exons followed by introns. In buffalo, ALAS1, CHRNE, MSTN, SNC 29 and CRTC3 possessed long gap regions. DGT1 and LHX6 had two introns separated by small exotic regions. ALDH2, RORA and STAT6 had single long introns. Each of DGAT1 and IGFALS contained 3, ABCG2, ADMST4, CAST ELOVL2, IGFBP3 had 2 and CPN2, GPX8 SIRT1, STAT6 and WRD7 had 1 mismatch region. CAST, CRTC3, DGAT1, STAT6 and TCF12 contained one additional codon. The number of exons and introns in cattle of almost all genes were greater as in buffalo.

Motifs analysis

This analysis depicted the motif location, motif consensus and graphical representation of motif. Conserved motifs are involved in action of growth and developmental proteins. The top 10 motifs were predicted. Motif 1 was most prominent out of the top 10 motifs. (Shown figure 5). Motif 1 was highly expressed in

buffalo and *Bos taurus* having frequency 222 and 217 respectively. Motif analysis revealed that the top 10 motifs are located on 25 genes. In cattle, motif-1 was located on 6 gene namely. Motif-10 had the least f value 12 and was present on ADMST4, TG and NEB. Motif -5 was located on just two genes ADMST4 and NEB. In buffalo, Motif-1 was located on 9 genes CHRNE, NEB, SIRT1, STAT6, MYF5, HSD17B7, WRD7, LHX6 and CAST. This motif was present in these six genes in cattle also. Motif-9 and MOTIF-10 had the least frequency 2. Motif-9 was present on IGFALS, CFTR, NEB and STAT6 while motif -10 on NEB only. Motif-5 was present on PLTP in addition to NEB in place of ADMST4 as in cattle. STAT6 had 3 motifs (motif-1, 6 and 10) but only one in cattle (motif-3). Motif -7 was present only on NEB both in cattle and buffalo. TG had two motifs (motif-9, 10) in cattle but none in buffalo. NEB had all motifs showed it is highly conserved for production (Figure 3A&3B).

Gene expression analysis

Heatmapper was used to analyze the expression of growth and development genes in several organs such as the brain, colon, heart, kidney, liver, lung, skeletal muscles, spleen, and testis (Bakhtiarizadeh *et al.*, 2018). In the heat map red colour showed up regulation and blue colour to down regulation. Results revealed NEB up-regulation in skeletal muscle tissues (high expression). The top Up-regulation of CFL1 was seen in brain, lungs, spleen, colon and testis. ALDH2 showed up regulation in kidney and liver and ACTC1 in heart (Figure 4A&4B).

GO annotation analysis

This analysis results revealed annotation. Plotted graph (Figure 5A) showed that fifteen genes CRTC3, IGFBP3, TCF12, PAX6, RORA, SIRT2, FOXO1, GH1, CREB1, MYOD1, CAPN2, LHX6, STAT6, ABCG2, MYF5 were



involved in GO terms for nucleus with P-Value 0.0059 E-21 followed by cellular compartment. Plotted graph (Figure 5B) showed that nine genes ADAMTS4, GH1, TG, SMO, MSTN, MMP1, IGFBP3, LEP and CHRNE were involved in disulfide bond with P-Value 0.0057 E-21 followed by functional category. Plotted graph (Figure 5C) showed that five CREB1, LHX6, PAX6, RORA, FOXO1 genes were involved in sequence-specific DNA binding for cytokine activity with P-Value 0.0239 E-22 followed by molecular function. The plotted graph (Figure 5D) revealed that eight genes, CRT3, SMO, TCF12, STAT6, RORA, SIRT1, SIRT2, FOXO1 played a part in positive transcription regulation from the RNA polymerase II promoter with P-Value 0.0011E-20, followed by biological process. Blue bar indicated gene count while Black lines indicated the P-Value in graphs.

Protein-protein association

Protein control biological function solely or by association with other proteins. Empty nodes indicated the unpredicted 3D structure while filled structure indicated predicted 3D structure like TG consists of predicted 3D structure (filled node). The thickness of line showed degree of interaction of one gene with others. TG showed first shell of interaction with ten predicted functional partners that were included MB, LRP2, TTR, NKX2-1, SLCA5, TSHR, ASGR2, ASGR1 and ALB genes in cattle (Figure 6A). Strongest interaction indicated in cattle than buffalo. TG gene had high association with the LRP2 gene. All genes were involved in first shell interaction also showed strong second shell interaction. In buffalo, TG gene showed first shell of interaction with ten predicted functional partner TTR, LRP2, CD44, PDIA4, ASG, ASGR2, MB, TSHR, TPO and DIO2. TTR, LRP2, ASG, ASGR2, TSHR, TPO and DIO2 nodes also showed second shell of interaction. TTR, LRP2,

ASG, ASGR2, TSHR, TPO and DIO2 showed the strong interaction with TG. (Figure 6B). TG gene had most association with the LRP2 gene. Filled nodes consisted of known 3D protein structures found in PDB database.

Synten Analysis

In synten analysis, ninety-six gene pairs showed duplication of gene pair in cattle and buffalo. Synteny revealed amino acids sequence duplication on paralogous as well as orthologous chromosomes. All ninety-six showed segmental duplication in synteny graph. Genes located on Bt 4 chromosome were duplicated on Bb8 chromosome while genes present Bt5 were duplicated on Bb 4 (Figure 7). For gene expansion mechanisms of cattle and buffalo species in evolution from diploid to tetraploid, gene duplication events analyzed in mastitis resistant genes. Tandem duplication arose resulting because of tight linking of gene to the same chromosome (Wei *et al.*, 2007; Zhang *et al.*, 2011).

Phylogenetic Analysis

Phylogenetic genetic analysis showed the evolutionary link between the Cattle and Buffalo. The evolutionary link between these genes in buffalo and cattle was expressed through phylogenetic trees, which was found by constructing a phylogenetic tree (Figure 8). Protein sequence of growth and development related genes belonging to different families were used to construct phylogenetic trees and to measure the evolutionary relationship between growth and development related genes in buffalo and cattle. Phylogenetic tree showed the 4 clades consist of 50 genes. Most of genes were in first clade while clade #4 contains only two genes. Resembling genes were grouped in same clade while diversified in different clades. Genes at first nodes e.g. BtIGFALS and BbCHRNE were conserved



and important to show evolutionary relationship

Discussion

Bubalus bubalis and *Bos taurus* are economically important due to meat production. We used chromosomal mapping to find location of genes affecting meat production. Our result also revealed that *Bubalus bubalis* and *Bos taurus* have similarity in mapping regions. This enabled us to develop genetic markers that could be used to induce positive mutations via gene editing. Genetic changes are viewed for growth regulation (Vaughn *et al.*, 1999). Based on genetic studies, Atchley and colleagues came to the same conclusion. (Vaughn *et al.*, 1999) This genome scan method enables us to identify genes that influence body composition and meat production features. (Gordo *et al.*, 2012)

Gene structure analysis was performed to elucidate different functional portions (exon, intron, mismatch, codon etc.) as activity of gene is attributed to its parts mainly exon. There were 45 and 38 genes structures analyzed in cattle and buffalo respectively. All genes in cattle showed a greater number of exons and introns as compared to corresponding gene in buffalo. NEB exhibited an extremely complicated and distinctive structure in cattle, with multiple exons and introns. Conserved sections are most likely found in this sort of gene. Our findings also revealed that *Bubalus bubalis* and *Bos taurus* are related. Batzoglou *et al.*, (2000) used analysis to predict the degree of similarity in the number, size, and sequence of exons and introns in humans and mice. Although it has long been considered that numerous genes govern meat output and quality, there is growing evidence that single genes account for a significant amount of variance in some respects (Warner *et al.*, 2010). Myostatin is highly expressed in the

developing somite and skeletal muscles of mice. (Maccatrozzo *et al.*, 2001).

The goal of motif analysis was to find conserved domains. Variations in conserved motifs had the potential to be fatal. The number of conserved motifs in NEB was the highest. The presence of all ten motifs in NEB exhibited it as unique and highly conserved. Interestingly TG had two motifs in cow and none in buffalo probably may be due to its specialty toward species. Existence of motif-1 on six common genes CAST, LHX6, WRD7, HSD17B7, MYF5 and NEB showed that these genes remained conserved over time. Motif 1,5,7 and 8 had about same value in Bt and Bb showed they were more conserved than others. The STAT motif region is an important transcriptional mediator of GH's sex-dependent effects in the mouse liver (Zhang *et al.*, 2012). SOCS2 inhibits the action of growth hormone in vitro and in vivo. In SOCS2-deficient mice, the magnitude of organ and tissue growth in response to GH was significant in SOCS2-deficient mice. SOCS2 levels and timing are a GH-controlling factor. (Greenhalgh *et al.*, 2005). AP-1-like motif regulates insulin-like growth factor I (IGF-I). The STAT gene is involved in growth hormone signaling (Herrington *et al.*, 2000). The PKC motif may possibly be implicated in GH action signaling, as well as STAT transcription activators. The human IGF-I gene is also a target for estrogen regulation, and disorders like osteoporosis may be linked to the development of the regulating machinery. The pufferfish (*Fugu*) genome has been classified as a compact vertebrate genome (Brenner *et al.*, 1993). Because it contains around 90% of all mammalian genes and is largely conserved among mammalian species and chicken, the Pufferfish genome, *Fugu rubripes*, was chosen as a model of the mammalian genome (Brenner *et al.*, 1993).

NEB was found to be upregulated in skeletal muscle tissues (high expression).



CFL1 was up regulated in the brain, lungs, spleen, and testis and colon. ALDH2 was regulated in the kidney and liver and ACTC1 in the heart. Cattle cloning using somatic cell nuclear transfer (SCNT) is a common agricultural practice that also serves as a model system for researching mammalian development and gene expression (Biase *et al.*, 2016). Gene expression can be utilized to detect molecular changes in bovine muscle that are related with marbling (Wang *et al.*, 2009). Furthermore, the genes shown to be significantly expressed during the formation of marbling could be exploited to generate genetic indicators or biomarkers to improve beef production tactics (Wang *et al.*, 2009). Both myostatin1 and myostatin2 expression peaked at the 140-g stage, indicating that muscle growth in poultry may be restricted at this period (Johansen and Overturf 2005). Collagen isoforms are expressed during bovine adipogenesis (Tahara *et al.*, 2004). Adipocyte fatty acid binding protein (FABP4), an adipogenesis-related gene, is continuously expressed in buffalo and is involved in intracellular transport and fatty acid metabolism, as well as differentiation (Rump *et al.*, 1996) and signal transduction (Glatz *et al.*, 1993; Veerkamp *et al.*, 1993). (Borchers and Spener 1994).

Our GO analysis showed that MYOD1 and MYF5 positively regulated skeletal muscle fiber development, SMO, MYOD1 skeletal muscle fiber development, and SMO, MYOD1, GH1, CREB1 and SMO in positive regulation of multicellular organism growth, IGFBP3, RORA, and SIRT1 were involved in the regulation of the glucose metabolic process. Hepatocyte growth was regulated by CREB1 and SIRT2. The transcription from the RNA polymerase II promoter was positively regulated by CRTC3, SMO, TCF12, STAT6, RORA, SIRT1, SIRT2, and FOXO1. IGFBP3, MYOD1, MYF5 in positive regulation of myoblast differentiation, and MSTN, MYOD1 in

negative regulation of myoblast proliferation. RORA, SIRT1 and SIRT2, FOXO1 in negative regulation of fat cell differentiation, IGFBP3, MYOD1, MYF5 in positive regulation of myoblast differentiation, and MSTN, MYOD1 in negative regulation of myoblast proliferation. Brown fat cell differentiation was aided by LEP and SIRT1, while transcription was regulated by PAX6, STAT6, RORA, and FOXO1. HSD17B7 and CFTR are involved in the biosynthesis of cholesterol. SIRT2 regulated negatively autophagy, LEP, MSTN, GAA had effect in muscle cell cellular homeostasis and LEP, RORA, SIRT1 in angiogenesis. MYOD1, CAPN2 regulated myoblast fusion, MYOD1, MYF5 positively regulated myoblast fusion, SIRT1, PLTP positively regulated cholesterol and TG, MYOD1, MYF5 negatively regulate myoblast fusion, MYOD1, MYF5 negatively regulate myoblast fusion, MYOD1, MYF5 negatively regulated myoblast fusion, MYOD1, MY DGAT1, SIRT1 fatty acid homeostasis, SIRT2 myelination Nucleus formation and function was controlled by CRTC3, IGFBP3, TCF12, PAX6, RORA, SIRT2, FOXO1, GH1, CREB1, MYOD1, CAPN2, LHX6, STAT6, ABCG2, MYF5. In DNA binding, CREB1, LHX6, PAX6, RORA, and FOXO1 were involved. The AMPK signaling pathway includes CREB1, LEP, SIRT1, FOXO1, and CFTR. SOCS2, GH1, LEP, and STAT6 were involved in the Jak-STAT signaling pathway; TG, CREB1, and GPX8 were involved in thyroid hormone synthesis; PAX6, STAT6, RORA, and FOXO1 were involved in transcription; and CHRNE, KCNK2, and CFTR were ion channels. Multi gene expression was involved in the majority of biological processes and structures. A single gene can influence multiple traits. GH was discovered as a potential gene that affects goose growth and development after a transcriptome investigation of the pituitary gland (Tang *et al.*, 2020).



Protein association analysis was used to look into gene interaction. Genes usually work together to produce a phenotype. Our findings revealed that TG is the most important gene in gene networking. In beef cattle, the association of the thyroglobulin gene with other genes was investigated for carcass and meat qualities (Gan *et al.*, 2008). The thyroglobulin gene is connected to other genes that regulate meat output in beef cattle. Multi-breed and multi-trait co-association analyses were used to investigate meat production features in three beef cow breeds. Caldas *et al.*, (Caldas *et al.*, 2016). Three genes, DGAT1, TG, and FABP4, were studied to see if they were linked to varying amounts of IMF. There was no indication of a link between DGAT1 and FABP4 in this study (Nasser *et al.*, 2021). Polymorphisms in the leptin and thyroglobulin genes have been linked to beef cow meat and carcass qualities (Carvalho *et al.*, 2012). The LEP gene has been related to CFT in beef cattle (Li *et al.*, 2013). In beef cow populations, the genes DGAT1, leptin, SCD1, CAPN1, and CAST show a high connection. In beef cattle, the LEP, SCD1, and CAPN1 genes demonstrate networking in meat characteristics (Li *et al.*, 2013). Commercial tests for TG5, CAPN, and CAST gene association with marbling are currently available under the GeneStar trademark (Sosnicki and Newman 2010).

Synteny analysis was performed to elucidate syntenic regions between Bt and Bb. Results showed that there is strong similarity and conserved regions. This is due to strong evolutionary relationships of Bt and Bb. Strong segmental duplication is due to high evolutionary relationships. Genes present on same chromosome in Bt and Bb are highly conserved while present on different chromosomes depict variations over time. The GAPD-IGF1 loci are substantially conserved in chickens and other vertebrates, according to genetic maps of the insulin-like growth factor 1

gene (Amills *et al.*, 2003). Synteny conservation between humans and pigs is three times higher than between humans and mice, resulting in fewer but larger conserved segments (Rettenberger *et al.*, 2003).

Synteny Using the Mouse Genome Database (MGD), Genome Database (GDB), and Online Mendelian Inheritance in Man (OMIM) websites, a systematic review identified approximately 400 mouse mutations, reviewed approximately 250 of these for vertebral phenotypes, and assessed 45 of these for synteny conservation between mouse and man. Some mouse mutants show traits that are similar to human phenotypes (Cooper *et al.*, 1998).

The evolutionary relationship between these genes in buffalo and cattle was determined by constructing a phylogenetic tree. Genes paralogues were close to each other in tree. paralogues emerging from the same node had structural similarity and known as sister paralogues for example BtGH1 and BbGH1 were paralogues. Distance of node from centre showed that during the evolutionary process, these genes underwent greater diversification in the progenitor species. Most of the genes of buffalo and cattle grouped together revealed their strong evolutionary relationship. The buffalo HSP gene family was found to be more closely linked to *Bos taurus*, goats, and sheep, as well as the common clade of horse and camel, when each HSP family gene was clustered together in one group (Figure 2). Buffalo is also distantly related to other mammals such as pigs, dogs, rats, mice, and zebra fish (Nadeem *et al.*, 2020). The role of MSTN in this model organism (zebrafish) must be investigated further (Acosta *et al.*, 2005). Many of the disparities between zebrafish and mammalian studies could be explained by the combined influence of zfMSTN-1 and -2, which diminish MSTN-1 mRNA levels in fish (Kerr *et al.*, 2005) while raising MSTN expression in humans



and rats (Shmelkov *et al.*, 2005). This was previously seen as evidence of differences in expression regulation between fish and mammals. The myostatin (MSTN 1, 2)-null phenotypes in mammals and zebrafish are characterized by dramatic increases in skeletal muscle mass, also known as "double muscling" (Kerr *et al.* 2005). There is no apparent phylogenetic relationship between IGFBPs and IGFBP-rPs, nor between the three different IGFBP-rPs, which contradicts their evolutionary relationship. (Rodgers *et al.*, 2008).

Conclusions

With the increasing pressure of quality food with population explosion, the world has moved towards JWAS for study and introduction of constructive mutations in a short span. This research was conducted to elucidate the integrated role of various genes. Each gene has a unique structure and discrete role regarding growth. GH affected directly on growth and development. TG associated with other genes in complex network to stimulate growth as depicted in association analysis. Results of heatmap, motif and gene structure analysis showed NEB had highly conserved domains for structural and functional role in skeletal muscle growth and development. CFL1 had a role in growth and development of brain, lungs, spleen and testis. ALDH2 showed up regulation in kidney and liver and ACTC1 in heart. *Bubalus bubalis* and *Bos taurus* had strong evolutionary relationships as shown by synteny and phylogenetic analysis. This study may be helpful to resolve the problem of under nutrition. This will lead to entry into a new era of artificial selection. Pakistan has a wealth of cattle genetic resources, with tremendous phenotypic and genetic variety. Efforts to effectively manage and use these resources, on the other hand, are limited due to a lack of understanding as well as the fragility of government institutions. Animal scientists could use this information to design genetic

markers to boost cattle output (Wu *et al.*, 2015). To better understand the structure and function of NEB in buffalo, further research is required.

Conflict of interest statement

This research was not funded by anyone. There was no involvement of a corporation in the study design, data collection, analysis, or interpretation, or the decision to submit the paper for publication. There are no other financial or personal links between the authors that could improperly affect or distort the content of the study. This is entirely and equally for the benefit of all human beings.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi: ...

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Table 1

Agreement between the ELISA antibody test results reported using a semi-quantitative evaluation scheme and canine parvovirus (CPV) hemagglutination titers/canine distemper (CDV) serum neutralization titers performed at a reference laboratory.

	CPV r^2 (n)	CDV r^2 (n)
Point-of-care testing using manufacturer-recommended protocol ^a	0.67 (199)	0.71 (200)
Point-of-care testing using extra wash	0.64 (103)	0.73 (104)
Synbiotics laboratory-performed testing using manufacturer-recommended protocol	0.56 (153)	0.72 (153)
Optical density (OD) measurement	0.51 (153)	0.63 (153)

^a As per Synbiotics TiterCHEK CDV/CPV direction insert

Figure legends

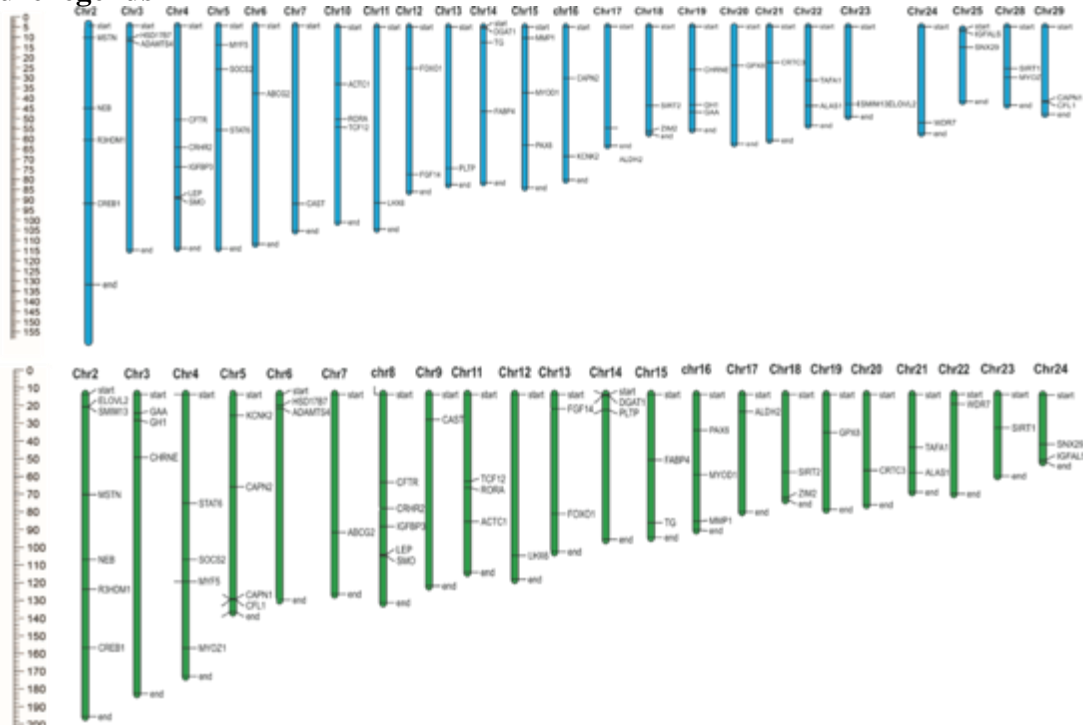


Fig. (1): chromosomal mapping in buffalo (A) and cattle (B).

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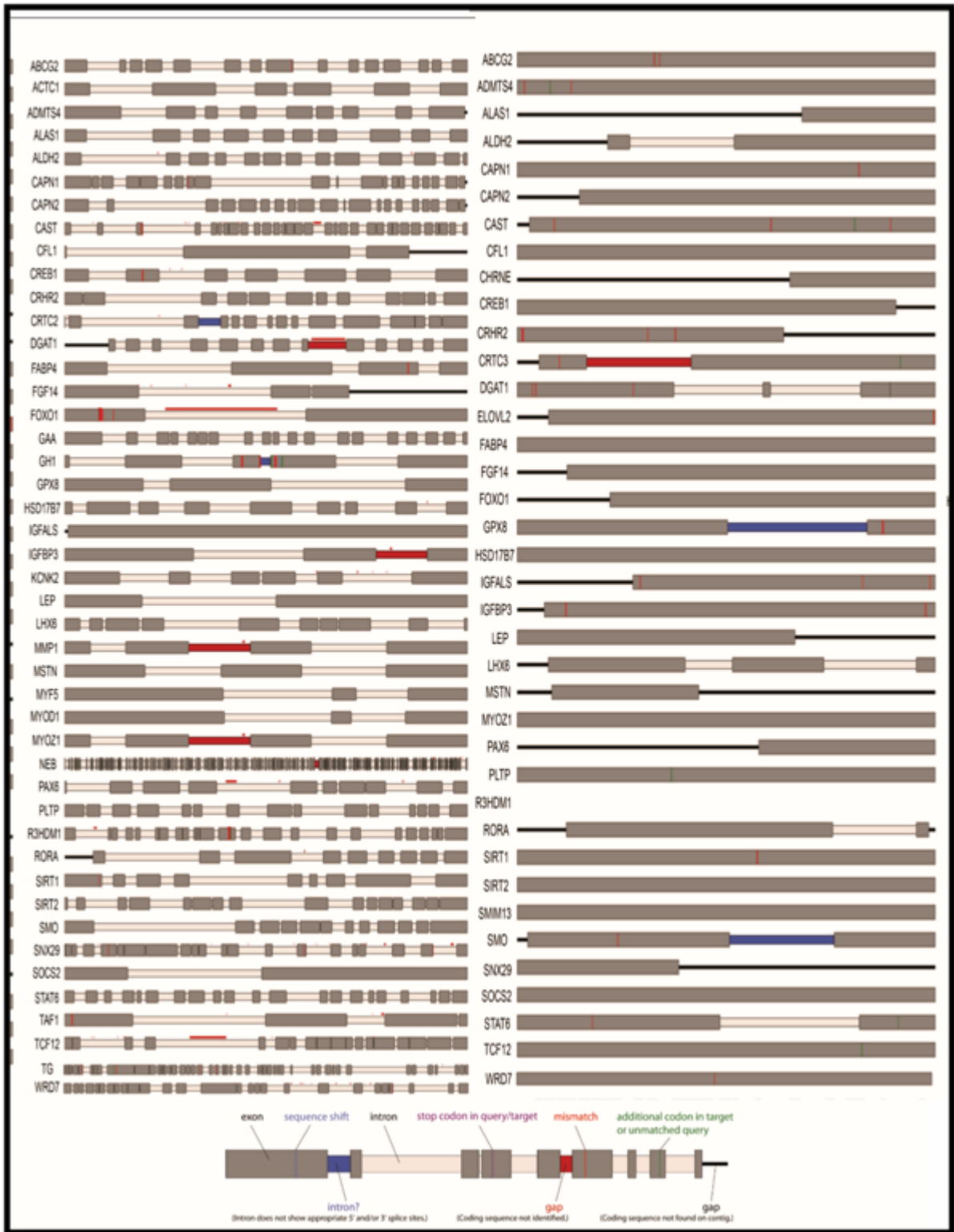
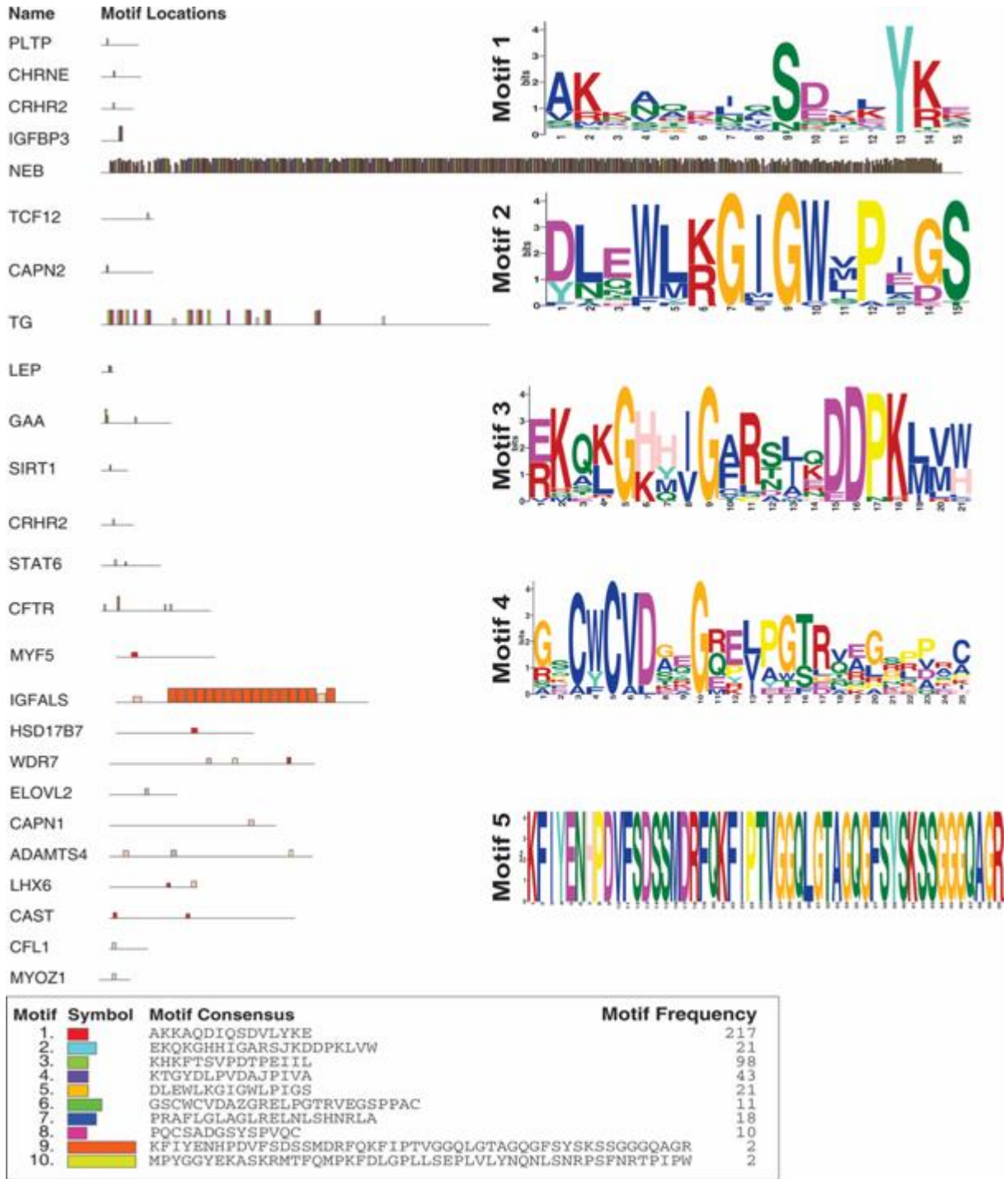


Figure (2): (A) and (B) gene structure analysis of growth and development genes in cattle and buffalo respectively.

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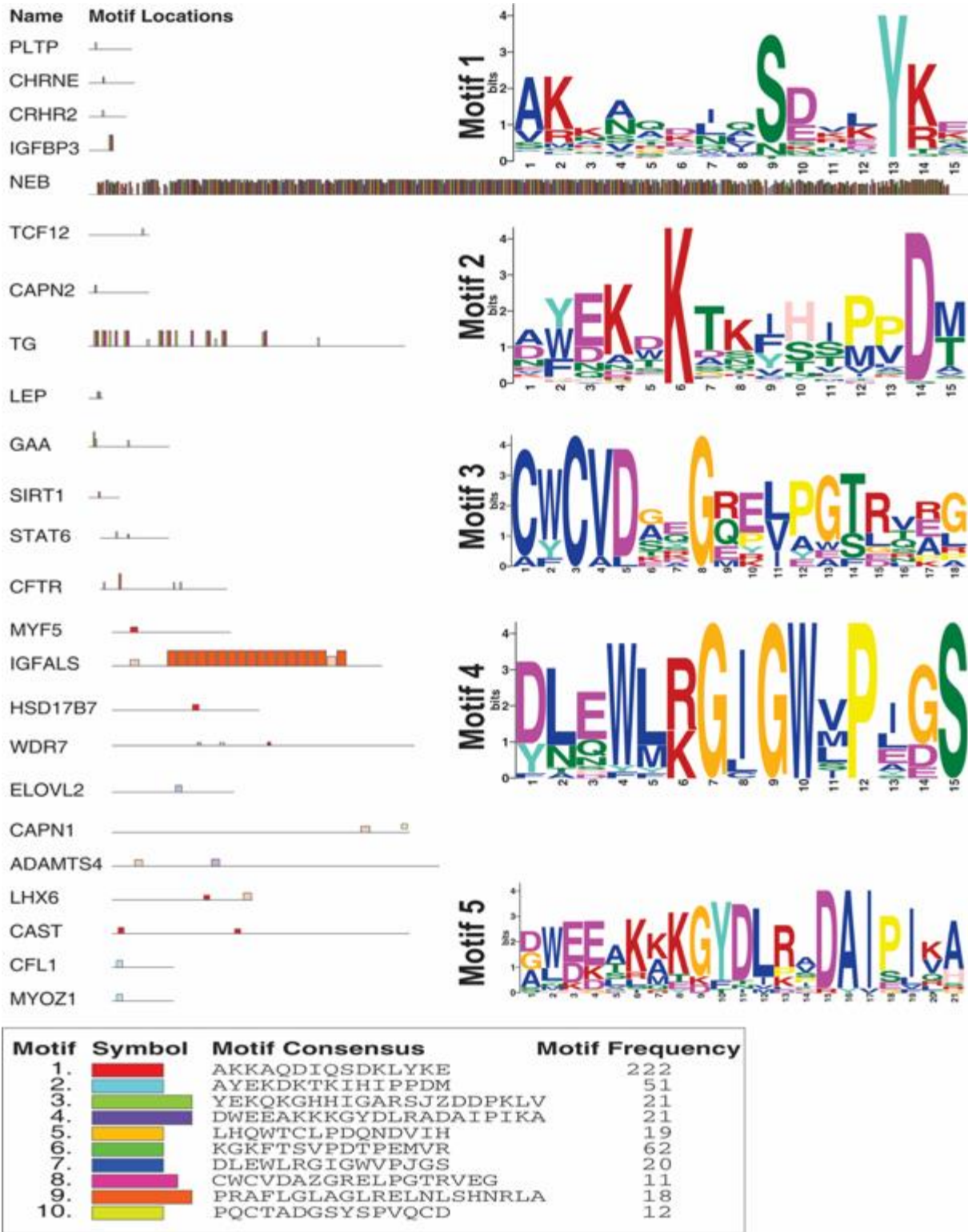


Figure (3): (A) and (B) were motif analysis of growth and development genes in cattle and buffalo respectively.

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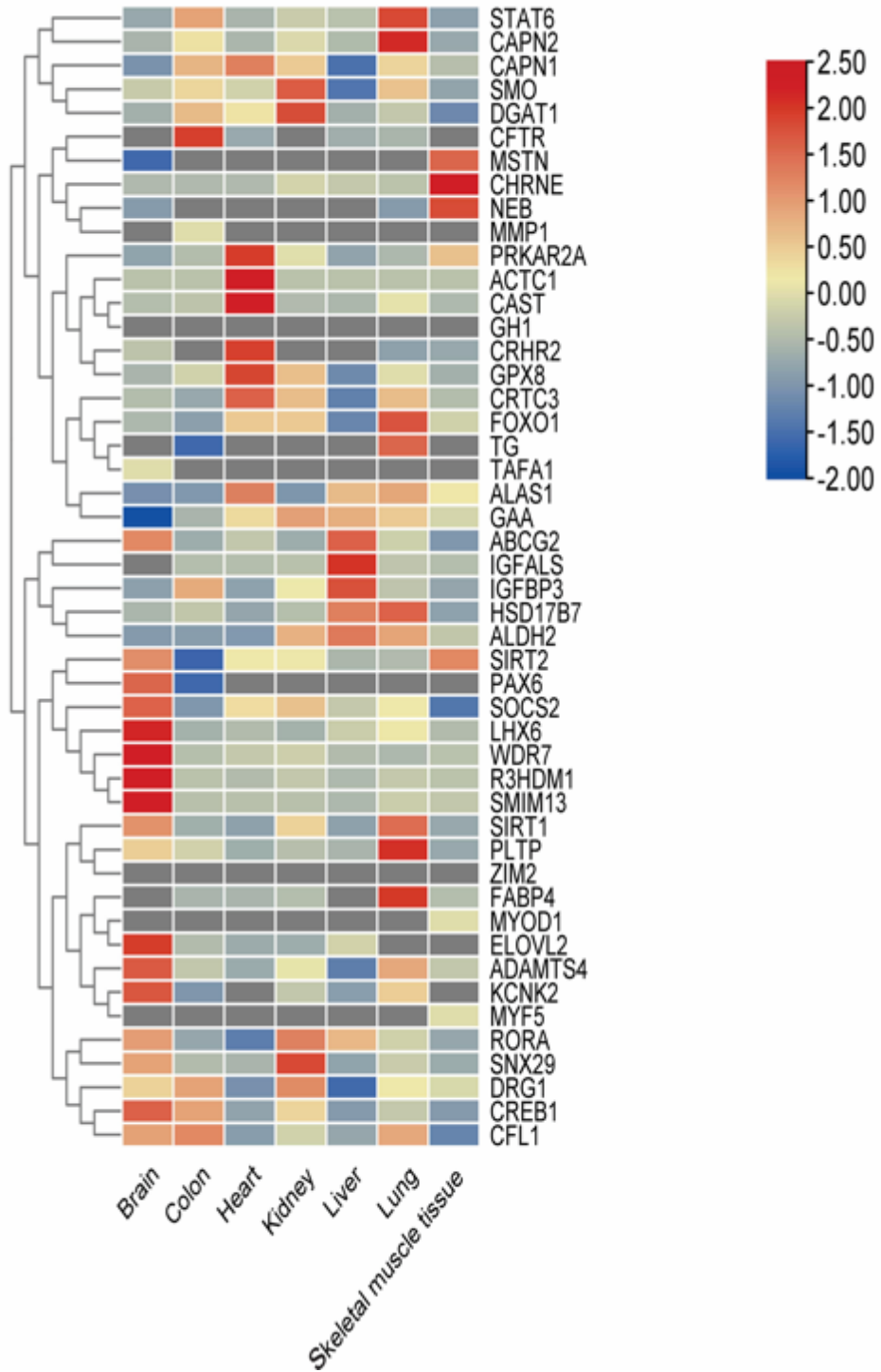


Figure (4): Heat map of growth and development related genes in different tissues of cattle

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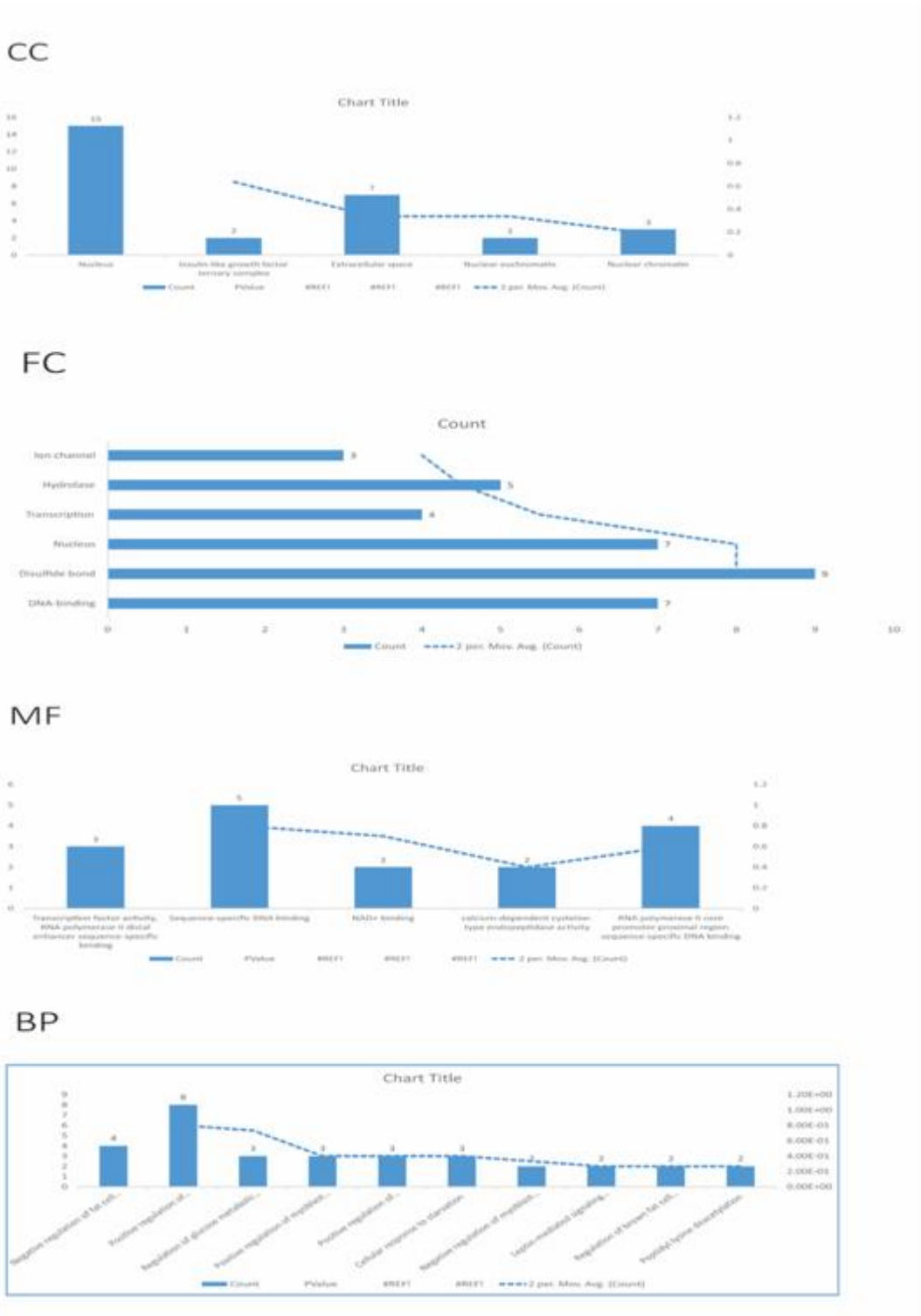


Figure (5): (A), (B), (C), and (D) graphs of Cellular component, Functional category, Molecular function and Biological process respectively that show gene annotation result.

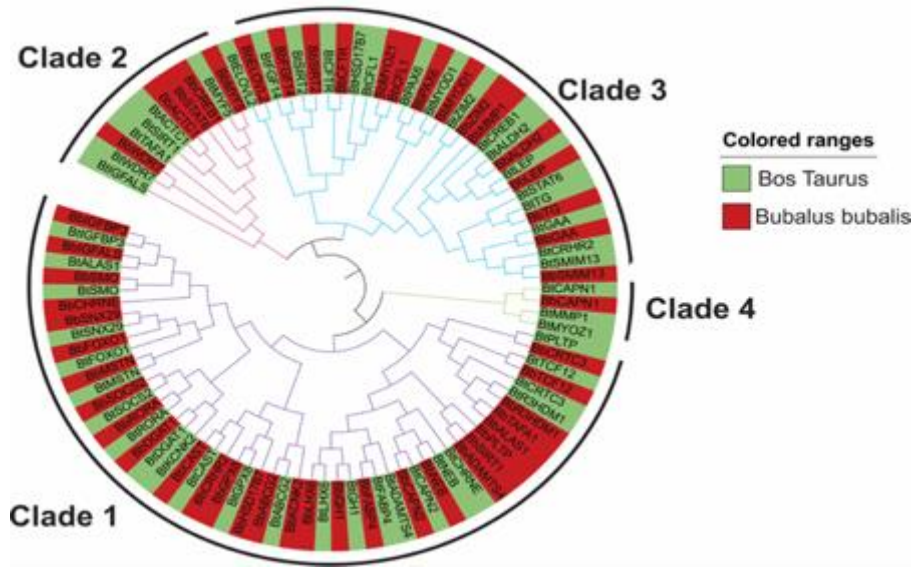


Figure (8): Phylogenetic analysis of buffalo (Bb) and cattle (Bt). The amino acid sequence was aligned by CLUSTALW and newick file format was prepared with the help of MEGA V.5.0 and then the phylogenetic tree was made using the neighbor-joining method using iTOL.



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