BMB
*reports*

# Transcript profiling of expressed sequence tags from intramuscular fat, *longissimus dorsi* muscle and liver in Korean cattle (Hanwoo)

*Dajeong Lim[1,2], Seung Hwan Lee[1], Yong Min Cho[1], Duhak Yoon[1], Younhee Shin[3], Kyu Won Kim[2], Hye Sun Park[1] & Heebal Kim[2,*]*

[1]Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, Rural Development Administration, Suwon, [2]Laboratory of Bioinformatics and Population Genetics, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, [3]Insilicogen, Inc., Suwon Chomdan Venture Valley, Suwon 441-813, Korea

**A large data set of Hanwoo (Korean cattle) ESTs was analyzed to obtain differential gene expression results for the following three libraries: intramuscular fat, *longissimus dorsi* muscle and liver. To better understand the gene expression profiles, we identified differentially expressed genes (DEGs) via digital gene expression analysis. Hierarchical clustering of genes was performed according to their relative abundance within the six separate groups (Hanwoo fat versus non-Hanwoo fat, Hanwoo muscle versus non-Hanwoo muscle and Hanwoo liver versus non-Hanwoo liver), producing detailed patterns of gene expression. We determined the quantitative traits associated with the highly expressed genes. We also provide the first list of putative regulatory elements associated with differential tissue expression in Hanwoo cattle. In addition, we conducted evolutionary analysis that suggests a subset of genes accelerated in the bovine lineage are strongly correlated with their expression in Hanwoo muscle. [BMB reports 2010; 43(2): 115-121]**

## INTRODUCTION

The field of differential expression analysis focuses on the identification of biomedically relevant target genes (1). Differentially expressed genes (DEGs) are those which show markedly different amounts of cognate mRNA among particular tissues, diseases or developmental stages. Differential expression analyses in tissues of interest can also be performed by co-expression. For this purpose, one must obtain evidence that functionally related genes are co-expressed (2, 3). Co-expressed genes are identified by their correlated expression patterns in time or space and are often controlled by common regulatory elements (e.g., transcription factors and transcription signals). Thus, co-expression analyses must be performed in concert with comparative analyses of promoter regions from co-expressed gene groups. The most popular method of differential gene expression analysis is mRNA quantification. Although mRNA is not the final product of gene expression, and is not perfectly correlated with protein abundance (4), it can be a useful indicator for gene expression profiling. Gene expression profiling uses DNA microarray technology, which is a time-consuming method with limitations related to cost and reproducibility. Moreover, DNA microarrays require sample management and can only analyze the expression profile of genes spotted on the array. Okubo *et al*. (5) reported the expression levels of genes estimated by large-scale 3′-end cDNA library sequencing. The abundance of mRNA transcript levels can be obtained from expressed sequence tag (EST) analysis. Comparison of EST sequence frequencies across several different libraries can help determine specific gene transcript profiles. This EST-based "digital gene expression profiling" has become very popular for expression studies because any gene tested can be obtained from an easily accessible database.

Cow was the first ruminant mammal to have its genome sequenced and is therefore a reference point for genomics studies whose aim is to identify domesticated animals that are well suited for a particular market or environment. The development of a functional strategy for the discovery of genes that control meat traits using quantitative trait loci (QTL) mapping can be extremely useful (6, 7). Hanwoo are indigenous cattle in Korea and are maintained as a beef cattle breed. Although cows play an important role in agriculture, the study of bovine ESTs has been limited. An EST library analysis in cattle provided muscle-specific expression data that was used to predict sets of genes whose expression was enriched in muscle and cardiac tissues (8).

Based on this approach, we present digital gene expression profiles for 1,554 genes across three tissues. We focused on

*Corresponding author. Tel: 82-2-880-4803; Fax: 82-2-873-2271; E-mail: heebal@snu.ac.kr

**Received 23 June 2009, Accepted 26 August 2009**

**Keywords:** Evolution, Gene expression, Korean cattle (Hanwoo), Quantitative trait, Transcript

the function of highly expressed genes in three tissues related to quantitative traits and characterized corresponding tissue-specific transcription factors.

## RESULTS AND DISCUSSION

### EST data sets and data analysis

We first obtained 55,213 ESTs from three cDNA libraries (fat, liver, and muscle) of Hanwoo cattle as described by Lim *et al*. (9). These ESTs were assigned to clusters corresponding to UniGene clusters of NCBI using the BLAT program (cutoff: 95% identity, 90% coverage). A total of 36,094 ESTs were included in the UniGene clusters of NCBI, and 19,119 ESTs revealed no hits in the UniGene clusters. In total, 19,119 ESTs were grouped into 1,217 clusters using the CAP3 program. New clusters identified by CAP3 are referred to as extra-clusters. We stored 5,259 clusters as 1,217 extra-clusters and UniGene clusters of NCBI, which included our ESTs. We then selected clusters containing a minimum of five identified ESTs, since the resolution of digital gene expression profiling is low if a given EST cluster is too small. Finally, 3,470 of the 5,259 clusters were involved in gene expression analysis. Bovine ESTs were extracted from the dbEST database (June 2008). To avoid variation, we removed libraries that were subtracted or normalized. Libraries were eliminated by examining their description field for the terms "subtracted*", "normaliz*" or "normalis*".

### Identification of differentially expressed genes

To generate gene expression patterns across the three tissues and to identify the overall similarities and differences between transcriptomes of different tissues, we performed digital gene expression analysis of the bovine ESTs. Expression values were computed from the number of ESTs in each gene constituting the total EST set relative to the total number of ESTs in the library (see Materials and Methods). Similarities between groups or libraries were estimated using Pearson's correlation coefficient in a pairwise manner between each row. Fig. 1 is a dendrogram that shows the complete groups of all 3,470 genes (clusters) across six tissues. DEGs were categorized into eight functional groups according to the expression patterns of each library. We focused on highly expressed gene groups in our three libraries (Hanwoo cattle versus any other cattle breed). Finally, we selected 1,554 genes from group 4 (Hanwoo fat), group 6 (Hanwoo muscle) and group 7 (Hanwoo liver) for further analysis. These data suggest that the majority of genes are upregulated compared to other bovine genes in the three tissues.

### Functional annotation of differentially expressed genes

We identified the putative function of DEGs within the three groups (group 4, 6, and 7) according to their best match in the UniProt database. We also classified genes into functional subgroups using GO. At the fifth GO level, we calculated P-values for assigned GO terms using hypergeometric tests. We found

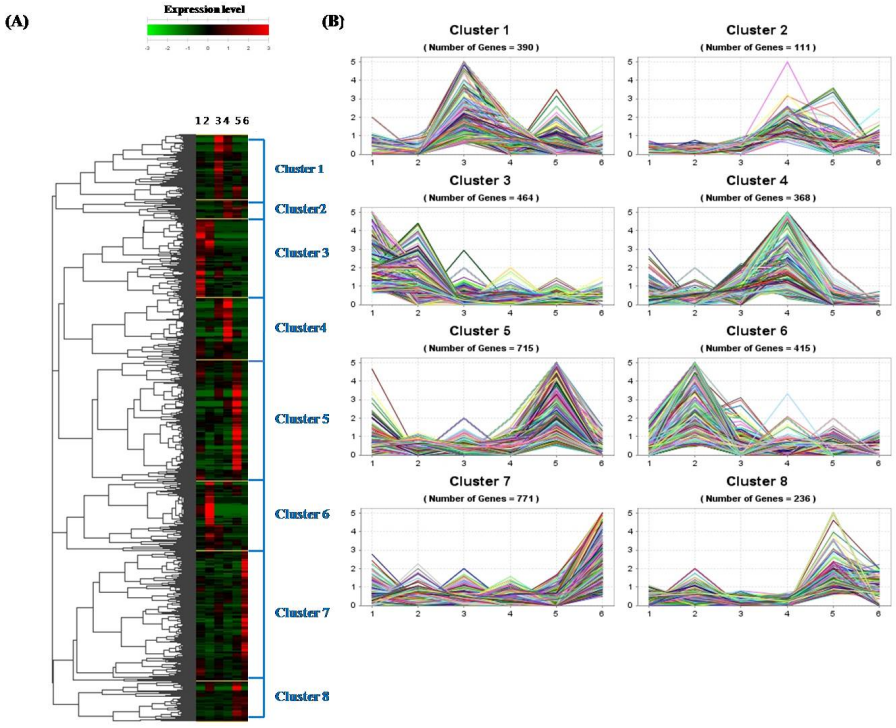



**Fig. 1.** Hierarchical clustering analysis of the gene expression in six libraries. (A) Each horizontal line represents the expression data for one gene. Columns 1 to 6 indicate non-Hanwoo muscle, Hanwoo muscle (*longissimus dorsi* muscle), non-Hanwoo fat, Hanwoo fat, non-Hanwoo liver and Hanwoo liver. Color scale ranges from saturated green for low expression to red for high expression. Numbers (groups 1 to 8) indicate defined subbranchesor groups. (B) The expression profiles of all genes in each group are displayed as groups 1 to 8. The vertical lines indicate tissues.

that 11, 12, and 11 significant GO terms were unique to the identified transcripts according to the three tissues shown in Supplementary data 1, S1. For example, the significant terms, "muscle contraction" and "contractile fiber part", are important to meat quality (10-12) because they are related to intramuscular fat tissue. Muscles vary in their physiological reaction rates, which reflect the proportion of each fiber type. Several fibers contain qualitatively different contractile proteins as well as proteinase and inhibitor systems. Jiang (1998) reported that fiber-type composition is a heterogeneous pattern of postmortem behavior between or within muscles and between adjacent cells (13). Therefore, these factors affect the meat tenderization process (13). "Muscle contraction" or "contractile fiber part" are also associated with the marbling score. The composition of muscle-fibers could possibly be used as a predictor of the marbling potential (14). These findings suggest that DEGs within the significant GO terms affect the meat quality grade via sensory characteristics of meat tenderness and juiciness.

We also determined the genomic location of cluster data sets in relation to quantitative traits. Supplementary data 2, S2 shows the distribution of genes associated with quantitative traits. Most of the genes are involved in "milk yield," "protein yield" and "protein percentage" traits. "Milk" or "protein" yields are important traits that help determine what effects breeding system has on bovines. The milking ability of a beef cow is one of the principal factors influencing the weaning weights of calves of similar breed composition (15). We then identified quantitative traits that are specifically associated with DEG groups. We found three traits in group 4, average daily gain (P = 0.0), tenderness score (P = 0.000228) and FSH (Follicle Stimulating Hormone) at castration (P = 0.00049). Regions on chromosomes 4, 5 and 15 contain loci associated with growth, meat quality and reproductive traits. We also identified four genes associated with average daily gain in group 4. In a previous study, putative quantitative traits for meat tenderness were identified on chromosomes 5 and 29 (16). Tenderness may increase as marbling score increases (dependent on the amount and type of fat), a relationship potentially important to consumers (17). The possible link between tenderness and meat composition has been debated for years, with most attention focused on fat content, especially in Korean cattle and Japanese black cattle. Increases in mass or fat occur simultaneously at several body locations, which could be important for tenderness (18). Wood (1990) studied fat cell expansion in the perimysial connective tissue, which forces muscle bundles apart and opens the muscular structure (19).

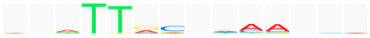
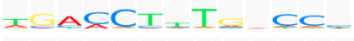
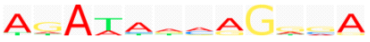
### Analysis of promoter sequences

DEGs in each group are expected to share regulatory elements. This assumption predicts that multiple TFs (transcription factors) interact with each other to achieve tissue specificity (20). We evaluated the co-occurrence of TF binding sites in DEG group promoters and defined significant TFs using Fisher's exact test. A total of 1,001 TFs were predicted and significantly different values for 4 and 12 TFs were identified in groups 4 and 7, respectively, as shown in Table 1. We also detected fat (adipocyte)-, liver- and muscle-specific TFs in the TRANSFAC database using MatchTM (Supplementary data 3, S3). Tissue-specific TFs were detected in at least two DEG groups as shown in Table 2. For example, the liver-specific profile contains matrices for the liver-enriched factors HNF-4, C/EBP and SREBP. Matrices for widely expressed TFs, both in-

**Table 1.** List of the significant transcription factors detected in each group

| Factor name | Factor ID | P-value | Factor description | Group |
|---|---|---|---|---|
| GC box | V$GC_01 | 0 | GC box elements | Group 4 |
| MZF1 | V$MZF1_02 | 0 | Myeloid Zinc Finger 1, differentiation | Group 4 |
| WT1 I -KTS | T00900 | 2.68E-05 | binding to RNA via zinc finger | Group 4 |
| HiNF-C | T00362 | 0.0001 | GC-box bindg protein/SP1 | Group 4 |
| CREB/ATF | V$CREBATF_Q6 | 0 | CRE-binding protein, Activating transcription factor | Group 7 |
| AP-2 | V$AP2_Q6_01 | 0 | Activating protein 2 | Group 7 |
| Arnt | V$ARNT_02 | 0 | AhR nuclear translocator homodimers | Group 7 |
| E2F:DP:p107 | T03218 | 0 | E2F/DP/P107 complex | Group 7 |
| HiNF-C | T00362 | 0 | GC-box bindg protein/SP1 | Group 7 |
| MAZ | V$MAZ_Q6 | 0 | MYC-associated zinc finger | Group 7 |
| WT1 -KTS | T01839 | 0 | Represses the activity of insulin-like growth factor II and platelet-derived growth factor A chain promoters | Group 7 |
| WT1 I -KTS | T00900 | 0 | Binding to RNA via zinc finger | Group 7 |
| f(alpha)-f(epsilon) | T00287 | 0 | – | Group 7 |
| GC box | V$GC_01 | 4.83E-05 | GC box elements | Group 7 |
| NRF-1 | T10331 | 9.45E-05 | Nuclear respiratory factor 1 | Group 7 |
| Churchill | V$CHCH_01 | 0.00052 | – | Group 7 |

**Table 2.** Tissue-specific TFs detected in at least two DEG groups

| Factor name | Factor ID | Factor description | Tissues | Sequence logo |
|---|---|---|---|---|
| AP1 | V$AP1_Q2_01 | Activator protein 1 | Fat, liver |  |
| C/EBP | V$CEBP_Q3 | CCAAT/enhancer binding protein | Fat, liver |  |
| C/EBPalpha | V$CEBPA_01 | CCAAT/enhancer binding protein alpha | Fat, liver |  |
| COUP direct repeat 1 | V$COUP_DR1_Q6 | Chicken ovalbumin upstream promoter transcription factor | Fat, liver |  |
| GR | V$GRE_C | Glucocorticoid response element | Fat, liver |  |
| HNF4 | V$HNF4_Q6_01 | Hepatocyte nuclear factor 1 | Fat, liver |  |
| HNF4alpha | V$HNF4ALPHA_Q6 | Hepatocyte nuclear factor 4alpha | Fat, liver |  |
| LXR,PXR,CAR,COUP, RAR | V$DR4_Q2 | Direct repeat 4 | Fat, liver |  |
| Myogenin/NF-1 | V$MYOGNF1_01 | Myogenin/nuclear factor 1 or related factors | Fat, liver |  |
| NF-1 | V$NF1_Q6_01 | Nuclear factor 1 | Fat, liver |  |
| PPAR direct repeat 1 | V$PPAR_DR1_Q2 | Peroxisome proliferator-acti vated receptors | Fat, liver |  |
| PPAR,HNF-4,COUP,RAR | V$DR1_Q3 | Direct repeat 1 | Fat, liver |  |
| STAT | V$STAT_Q6 | Signal transducer and activator of transcription | Fat, liver |  |
| SREBP | V$SREBP_Q3 | Sterol regulatory element-binding protein | Fat, liver |  |
| GATA-4 | V$GATA4_Q3 | GTP binding protein 4 | Muscle, liver |  |
| YY1 | V$YY1_Q6 | Yin Yang 1 | Muscle, liver |  |
| Ebox | V$EBOX_Q6_01 | CANNTG element | Fat, muscle, liver |  |
| Sp1 | V$SP1_Q2_01 | Promoter-specific cellular transcription factor | Fat, muscle, liver |  |
| USF | V$USF_Q6_01 | Upstream stimulatory factor | Fat, muscle, liver |  |

ducible (GR, AP1, STAT) and constitutive (Sp1, NF-1, USF, YY1), are included in this profile as sites, and they regulate the transcription of genes in the liver by cooperating with liver-specific factors (21). GC-rich housekeeping gene promoters are enriched in Sp1 binding motifs, which are also detected in the promoters and enhancers of genes expressed in hematopoietic and epithelial cells. Here, the Sp1 binding motif appears to cooperate with lineage-restricted factors in directing expression (22). CREB-H of the CREB/ATF family participates in liver-specific expression within the box-B element (23). These profiles can be applied for the regulatory regions of genes with known tissue expression. However, the mechanisms of this regulation are not completely known.

### Accelerated evolution of gene groups is upregulated in Hanwoo muscle

One of the most important measures of molecular adaptation is comparison of relative fixation rates of synonymous and nonsynonymous mutations (dN/dS). Similar to Dorus *et al*. (24), we compared the dN/dS ratios of groups 4, 6 and 7 to that of housekeeping genes in order to confirm relative acceleration of gene evolution. The evolution of all three groups was more accelerated than that of the housekeeping genes. Among the groups and housekeeping gene set, group 6, containing upregulated genes in Hanwoo muscle, shows the most accelerated evolution (Fig. 2). Currently, we are not able to present a clear explanation for the accelerated evolution of group 6. Group 6 includes 215 genes of which 132 (~61%) show significant enrichment related to metabolic processes. Genes related to metabolic processes include those related to alcohol, carboxylic acid, organic acid, lipid and amine metabolism. The accelerated evolution of the genes has been observed in numerous species, and therefore the accelerated term is not limited to Hanwoo cattle lineage. However, it is interesting that the accelerated subset of genes in the bovine lineage is upregulated in Hanwoo muscle when compared with other breeds or tissues. We speculate that the subset of genes accelerated in the bovine lineage are positively correlated with the expression level of Hanwoo muscle.

The present analysis identified 1,554 genes from the fat, muscle and liver EST libraries of Hanwoo cattle as being differentially expressed. We assume these genes share similar functions, quantitative traits and regulatory regions. Studies on variation in expression using ESTs require strict statistical tests, which have been developed recently (1). We used a previously published test (25) for analyzing differential gene expression among diverse tissues, and identified DEGs according to their relative abundance in the various EST libraries. Our ap-

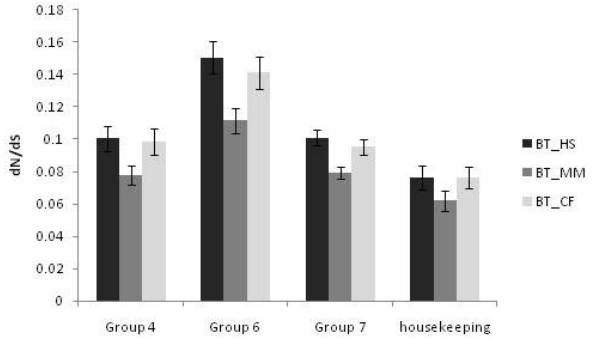



**Fig. 2.** Relative fixation rates of synonymous and nonsynonymous mutations (dN/dS) in expression groups 4, 6 and 7. The description of each group can be found in the result and discussion section. BT, HS, MM and CF stand for *Bos taurus, Homo sapiens, Mus musculus* and *Canis lupus familiaris*, respectively. Group 6 shows the most accelerated evolution compared to housekeeping genes.

proach may have several limitations shared by all EST-based analyses. For example, EST analyses are not representative of the proteome of a cell (26) as mRNA levels do not always correlate with protein abundance. These problems may be resolved, however, by expensive microarray techniques. Additionally, the abundance of detected transcripts depends on the initial number of ESTs. We therefore used the UniGene data set of NCBI to add to our current set of 55,213 ESTs. Unlike other studies (27, 28), we excluded genes represented by a single EST (singletons) and clusters with less than five ESTs, thus reducing artifacts. We believe that theycould participate in their specific expression. This information may be useful for further characterization of data from Hanwoo cattle libraries.

## MATERIALS AND METHODS

### EST data sets and data analysis

We generated 55,213 ESTs from three cDNA libraries of Hanwoo cattle (liver, intramuscular fat and *longissimus dorsi* muscle). The tissues were obtained from a 24-month-old Hanwoo steer immediately after slaughter. cDNA libraries were constructed according to the oligocapped method. Sequencing of cDNA clones and construction of EST data sets were performed as described in (9). UniGene data sets were downloaded from the *Bos taurus* (Build #92) UniGene cluster of NCBI. We also downloaded dbESTs from NCBI and stored tissue libraries containing information on ESTs. We focused on DEGs from the three Hanwoo cattle libraries. The following tissues were selected: non-Hanwoo muscle, Hanwoo muscle (*longissimus dorsi* muscle), non-Hanwoo fat, Hanwoo fat, non-Hanwoo liver and Hanwoo liver (6 columns in Fig. 1). ESTs from subtracted and normalized libraries were excluded from further digital expression analyses.

### Identification of differentially expressed genes

A total of 3,470 clusters were found to contain at least five members out of a possible 5,259, and were selected for expression analysis. A two-way matrix table of raw EST counts was constructed with rows corresponding to UniGene Clusters and columns corresponding to tissue name. Raw data were normalized using the following criteria: raw EST counts were transformed into relative expression values as defined by (29), and we calculated the ratio of ESTs in a cluster to the total number of ESTs in the corresponding pool. The expression values for each gene were then normalized to itself by dividing all gene expression values for a given gene by the median of its expression over all six tissues (25). All genes were grouped via hierarchical clustering with GenPlex™ software (ISTECH, Seoul, Korea) using Pearson's correlation as a similarity measure and complete linkage methods.

### Functional annotation of differentially expressed genes

DEGs within the groups were annotated by performing sequence similarity searches against the UniProt Human database (Swiss-Prot and TrEMBL, http://www.uniprot.org/downloads) using the BLASTX program with a cutoff of 0.00001. Next, gene ontology (GO) categorization was performed using the GO mapping file of the Gene Ontology Consortium (http://www.geneontology.org). To test for function bias in the ESTs, hypergeometric tests were used to determine statistical significance (30). Since a potential multiple test problem may have increased the likelihood of a type I error, false discovery rate (FDR) correction was performed. To determine the genomic location, genes were mapped against bovine genome sequences using the BLAT program (31). Bovine genome sequences (bosTau4) were downloaded from the UCSC Genome Browser. We obtained genomic location using 'QTL location by bp' information from the Animal QTL database (http://www.animalgenome.org/QTLdb/cattle.html) and aligned DEGs against the bovine genome. We compared the genomic locations of DEGs to those of QTL traits and assigned DEGs within QTL locations. To detect significant QTL traits, we compared the locations of all bovine genes and QTL traits, including DEGs, using fisher's exact test.

### Analysis of promoter sequences

We further analyzed the putative transcription binding sites of DEGs using the TRANSFAC® database. Upstream sequences of genes approximately 2,000 bases long were downloaded from the UCSC Genome Browser. Using a DEG genome sequence, a known or putative motif-binding transcription factor (TF) could be identified using Match™ and Patch™ in TRANSFAC® (21). Since DEG gene groups have many putative TFs in their promoters, we focused on determining which putative TFs were the most enriched, and whether this enrichment was statistically significant. The P-value was determined using Fisher's exact test (32) and each group was subjected to

FDR correction. Additionally, we characterized profiles of liver- and muscle-specific TFs with Match™ (33) using our TRANSFAC results.

### Estimating dN/dS ratio

Orthologs of *Homo sapiens, Canis lupus familiaris*, *Mus musculus* against protein IDs of groups 4, 6 and 7 of *Bos taurus,* and orthologs of *Canis lupus familiaris, Mus musculus*, *Bos Taurus* against housekeeping genes (34) of *Homo sapiens* were defined by NCBI HomoloGene (build 63). We defined the homologs group which has only one gene each species as orthologs group. We downloaded the protein and reference mRNA sequences of each ortholog group from a NCBI ftp site. The protein sequences of the orthologs were aligned pairwise (*Bos Taurus* against another species) with ClustalW2 (35). Using pal2nal (36), the protein sequence alignment and corresponding mRNA sequences were converted into codon alignment. The codeml of PAML (version 4.2a) (37) was then used to estimate the dN/dS ratio using model of estimated and F61.

### Acknowledgements

This work was supported by Agenda (200901FHT020710399) of National Institute of Animal Science, Rural Development Administration, Republic of Korea.

## REFERENCES

1. Claverie, J. M. (1999) Computational methods for the identification of differential and coordinated gene expression. *Hum. Mol. Genet.* **8**,1821-1832.
2. Eisen, M. B., Spellman, P. T., Brown, P. O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 14863-14868.
3. Spellman, P. T., Sherlock, G., Zhang, M. Q., Iyer, V. R., Anders, K., Eisen, M. B., Brown, P. O., Botstein, D. and Futcher, B. (1998) Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol. Biol. Cell.* **9**, 3273-3297.
4. Anderson, L. and Seilhamer, J. (1997) A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* **18**, 533-537.
5. Okubo, K., Hori, N., Matoba, R., Niiyama, T., Fukushima, A., Kojima, Y. and Matsubara, K. (1992) Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression. *Nat. Genet.* **2**,173-179.
6. Lee, Y., Lee, J., Lee, J., Kim, J., Park, H. and Yeo, J. (2008) Identification of Candidate SNP (Single Nucleotide Polymorphism) for Growth and Carcass Traits Related to QTL on Chromosome 6 in Hanwoo (Korean Cattle) Asian-Aust. *J. Anim. Sci.* **21**,1703-1709.
7. Lee, J., Kwon, J. and Kim, J. (2008) Multifactor dimensionality reduction (MDR) analysis to detect single nucleotide polymorphisms associated with a carcass trait in a hanwoo population. *Asian-Aust. J. Anim. Sci.* **21**, 784-788.
8. Zadissa, A., McEwan, J. C. and Brown, C. M. (2007) Inference of transcriptional regulation using gene expression data from the bovine and human genomes. *BMC Genomics* **8**, 265.
9. Lim, D., Byun, M., Cho, Y., Yoon, D., Lee, S. and Shin, Y. and Im, S. (2009) Functional analysis of expressed sequence tags from Hanwoo (Korean cattle) cDNA libraries. *J. Anim. Sci. Tech.* **51**, 1-8.
10. Bouton, P., Carrol, F., Harris, P. and Shorthose, W. (1973) Influence of pH and fiber contraction state upon factors affecting the tenderness of bovine muscle. *J. Food Sci.* **38**, 404-407.
11. Klont, R. E. and Lambooy, E. (1995) Effects of preslaughter muscle exercise on muscle metabolism and meat quality studied in anesthetized pigs of different halothane genotypes. *J. Anim. Sci.* **73**,108-117.
12. Lee, S., Panjono, Kang, S., Kim, T. and Park, Y. (2008) The effects of dietary sulfur and vitamin e supplementation on the quality of beef from the longissimus muscle of hanwoo bulls. *Asian-Aust. J. Anim. Sci.* **21**,1059-1066.
13. Jiang, S. T. (1998) Contribution of muscle proteinases to meat tenderization. *Proc. Natl. Sci. Counc. Repub. China B* **22**, 97-107.
14. Calkins, C., Dutson, T., Smith, G., Carpenter, Z. and Davis, G. (1981) Relationship of fiber type composition to marbling and tenderness of bovine muscle. *J. Food Sci.* **46**, 708-710.
15. Beal, W. E., Notter, D. R. and Akers, R. M. (1990) Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. *J. Anim. Sci.* **68**, 937-943.
16. Casas, E., Shackelford, S. D., Keele, J. W., Stone, R. T., Kappes, S. M. and Koohmaraie, M. (2000) Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *J. Anim. Sci.* **78**, 560-569.
17. Wheeler, T. L., Cundiff, L. V. and Koch, R. M. (1994) Effect of marbling degree on beef palatability in Bos taurus and Bos indicus cattle. *J. Anim. Sci.* **72**, 3145-3151.
18. Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I. and Sheard, P. R. (1999) Manipulating meat quality and composition. *Proc. Nutr. Soc.* **58**, 363-370.
19. Wood, J. D. (1990) Consequences for meat quality of reducing carcass fatness. *Reducing Fat in Meat Animals* 344-397.
20. Yu, X., Lin, J., Zack, D. J. and Qian, J. (2006) Computational analysis of tissue-specific combinatorial gene regulation: predicting interaction between transcription factors in human tissues. *Nucleic. Acids. Res.* **34**, 4925-4936.
21. Matys, V., Fricke, E., Geffers, R., Gossling, E., Haubrock, M., Hehl, R., Hornischer, K., Karas, D., Kel, A. E., Kel-Margoulis, O. V., Kloos, D. U., Land, S., Lewicki-Potapov, B., Michael, H., Munch, R., Reuter, I., Rotert, S., Saxel, H., Scheer, M., Thiele, S. and Wingender, E. (2003) TRANSFAC: transcriptional regulation, from patterns to profiles. *Nucleic. Acids. Res.* **31**, 374-378.
22. Brahmachary, M., Schonbach, C., Yang, L., Huang, E., Tan, S. L., Chowdhary, R., Krishnan, S. P., Lin, C. Y., Hume, D. A., Kai, C., Kawai, J., Carninci, P., Hayashizaki, Y. and Bajic, V. B. (2006) Computational promoter analy-

sis of mouse, rat and human antimicrobial peptide-coding genes. *BMC Bioinformatics* **7 (Suppl 5)**, S8.
23. Omori, Y., Imai, J., Watanabe, M., Komatsu, T., Suzuki, Y., Kataoka, K., Watanabe, S., Tanigami, A. and Sugano, S. (2001) CREB-H: a novel mammalian transcription factor belonging to the CREB/ATF family and functioning via the box-B element with a liver-specific expression. *Nucleic. Acids. Res.* **29**, 2154-2162.
24. Dorus, S., Vallender, E. J., Evans, P. D., Anderson, J. R., Gilbert, S. L., Mahowald, M., Wyckoff, G. J., Malcom, C. M. and Lahn, B. T. (2004) Accelerated evolution of nervous system genes in the origin of Homo sapiens. *Cell* **119**, 1027-1040.
25. Fei, Z., Tang, X., Alba, R. M., White, J. A., Ronning, C. M., Martin, G. B., Tanksley, S. D. and Giovannoni, J. J. (2004) Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. *Plant J.* **40**, 47-59.
26. Megy, K., Audic, S. and Claverie, J. M. (2002) Heart-specific genes revealed by expressed sequence tag (EST) sampling. *Genome Biol.* **3**, RESEARCH0074.
27. Hwang, D. M., Dempsey, A. A., Lee, C. Y. and Liew, C. C. (2000) Identification of differentially expressed genes in cardiac hypertrophy by analysis of expressed sequence tags. *Genomics* **66**, 1-14.
28. Bortoluzzi, S., d'Alessi, F. and Danieli, G. A. (2000) A computational reconstruction of the adult human heart transcriptional profile. *J. Mol. Cell Cardiol.* **32**, 1931-1938.
29. Schmitt, A. O., Specht, T., Beckmann, G., Dahl, E., Pilarsky, C. P., Hinzmann, B. and Rosenthal, A. (1999) Exhaustive mining of EST libraries for genes differentially expressed in normal and tumour tissues. *Nucleic. Acids. Res.* **27**, 4251-4260.
30. Draghici, S., Khatri, P., Martins, R. P., Ostermeier, G. C. and Krawetz, S. A. (2003) Global functional profiling of gene expression. *Genomics* **81**, 98-104.
31. Kent, W. J. (2002) BLAT–the BLAST-like alignment tool. *Genome Res* **12**, 656-664.
32. Siegel, S. (1988) *Nonparametric methods for the behavioral sciences,* 2nd ed., pp. 127-136, McGraw-Hill, New York, NY, USA.
33. Kel, A. E., Gossling, E., Reuter, I., Cheremushkin, E., Kel-Margoulis, O. V. and Wingender, E. (2003) MATCH: A tool for searching transcription factor binding sites in DNA sequences. *Nucleic. Acids. Res.* **31**, 3576-3579.
34. Eisenberg, E. and Levanon, E. Y. (2003) Human housekeeping genes are compact. *Trends Genet.* **19**, 362-365.
35. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948.
36. Suyama, M., Torrents, D. and Bork, P. (2006) PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic. Acids. Res.* **34**, W609.
37. Yang, Z. (2007) PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24**, 1586-1591.