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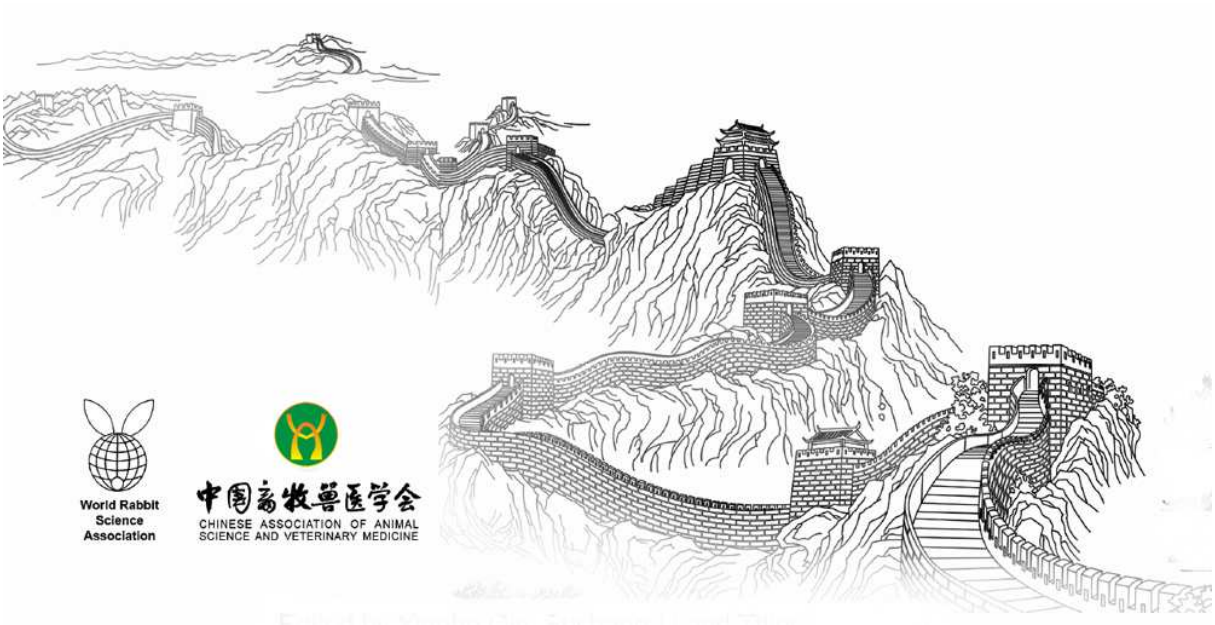

TRANSCRIPTOME ANALYSIS OF COAT COLOR GENES IN REX RABBIT

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ABSTRACT

In order to identify differently expressed genes in Rex Rabbit skin of different color (White and Beaver), we selected 1 White and 1 Beaver Rex Rabbit of full sibs both aged 120 days, and total mRNA in their ear tissues were sequenced and analyzed using Illumina HiSeq™ 2500 sequencing platform. The experiment found 12,408 differently expressed genes, among which 4,904 genes were up-regulated and 7,504 genes were down-regulated. GO analysis indicated that these altered genes were mainly associated with cellular process, metabolic process, cellular part, catalytic, binding and other processes. KEGG analysis found 13 signal pathways in which differently expressed genes were significantly enriched ($P<0.05$). Some important genes were identified in the pathway of xenobiotics by cytochrome P450, which might be useful as candidate genes for coat color in Rex Rabbit.

Key words：Rex Rabbit, coat color, gene expression, transcriptome sequence, GO and KEGG analysis.

INTRODUCTION

Rex Rabbit is a world-famous raising breed for fur. There are numerous coat color of Rex Rabbit, including beaver, blue, purple blue, red and other more than 90 colors (Pang *et al.*2011). Currently, the requirements of fur quality are improving constantly in foreign and domestic markets. Color separation in Rex Rabbit production may affect the fur quality to a certain extent. Therefore, it is meaningful to study the coat color related genes.

The coat color of mammals is decided by pigment in their skin, which is mainly tyrosine, i.e. melanin and its derivatives. At the present, there have been more than 300 loci and 150 coat color related genes identified, and these genes affect the formation and transformation of pigments by different pathways (Yang, 2014). In domestic rabbit, it is now proved that at least 8 loci controlled its coat color (Pang *et al.*, 2008). Except for the interactions between genes, environmental factors as micronutrient levels, illumination, temperature, altitude, even feeding method may all affect expression of melanin, then change coat color.

RNA sequencing is a large-scale sequencing method for cDNA of given tissue or given period, which can identify differential gene expression patterns of different tissues, different periods and different individuals, and acquire the results of gene expression, alternative splicing and new-found genes (Zhou *et al.*, 2012; Yue *et al.*, 2012). Transcriptome sequencing has the advantages of high throughout, low cost, high sensitivity and no need of reference genome information, so it is widely used in animal and plant researches.

In this study, the gene expression patterns in different coat color Rex Rabbit skin were analyzed with Illumina HiSeq™ 2500 sequencing platform, and presented differently expressed genes, gene functions were annotated.

MATERIALS AND METHODS

Experimental animals and tissue samples

All rabbits used came from the Experimental Rex Rabbit farm of Institute of Animal Husbandry and Veterinary, Shanxi Agricultural Academy, Taiyuan. Two full-sib Rex Rabbits (1 white and 1 beaver) both aged 120 days were selected. All the rabbits raised separately with double row type three hutch, the cage area were $0.025m^2$. They were fed by the same breeder at 8:00 AM and 5:00 PM at the same day with the same forage. The ear tissues from those 2 rabbits of around $0.5cm^3$ were obtained and were snap-frozen and stored in liquid nitrogen until use.

cDNA library construction and HiSeq™ sequencing

Total RNA was extracted using Trizol reagent following the manufacturer's protocol (Invitrogen, US). We used 1% agarose gel electrophoresis to test degradation of total RNA. RNA integrity and concentration was assessed by Agilent 2100 RNA Nano Assay Kit (Agilent Company, US). After passing the quality detection, mRNA was assembled, fragmented, reverse transcripted and added adaptor at both of their ends. The mRNA sample was prepared to construct cDNA library. The length of average inserted fragments is 250bp.

The original sequences acquired by HiSeq™ were assembled by Trinity (Version: 20140717), then TransDeorder (Version: 20140717) was used to predict ORF of assembled sequences. Trinotate (Version: 20140717) was used to annotate assembled sequences by referring database Uniprot, Swiss-prot, GO, KEGG and so on.

Identification of differentially expressed genes (DEGs) and pathway analysis

The expression levels of Unigene were quantified by standard RPKM method (Mortazavi *et al.*, 2008). The gene expression levels calculated can be used directly to compare differential gene expression between two samples. Using DEGseq software (Wang *et al.*, 2010) to compare samples of two groups (White and Beaver). The differentially expressed genes of both up-regulated and down-regulated were calculated based on the results above, the threshold was $(|\log_2 Ratio| \geq 2, q < 0.01)$ .We did multiple testing to correct p value using FDR method (Benjamini and Hochberg, 1995). Differentially expressed genes were mapped to each GO term and a histogram was drawn according to GO analysis. Pathways which DEGs enriched were analyzed based on the KEGG pathway analysis (http://www.genome.jp/kegg/).

RESULTS AND DISCUSSION

Differentially expressed gene(s) in Rex rabbit skin

The expression level of gene expression was affected by environment, space-time, tissues, and developmental stages of animal. Compared gene expression level between two samples using DEG-seq software, 12,408 DEGs were acquired, with 4,904 genes up-regulated and 7,504 genes down-regulated.

All the DEGs were annotated by Uniprot, Pfam, GO and KEGG databases to get functional information. Fig.1 is the histogram of GO statistical results. From all the up-regulated genes, 818 DEGs were classified into cellular process (61.50%), 610 were classified into metabolic process (45.86%); all the down-regulated genes, 1044 were attributed to cellular process (59.04%) and 825 were classified into metabolic process (46.66%).

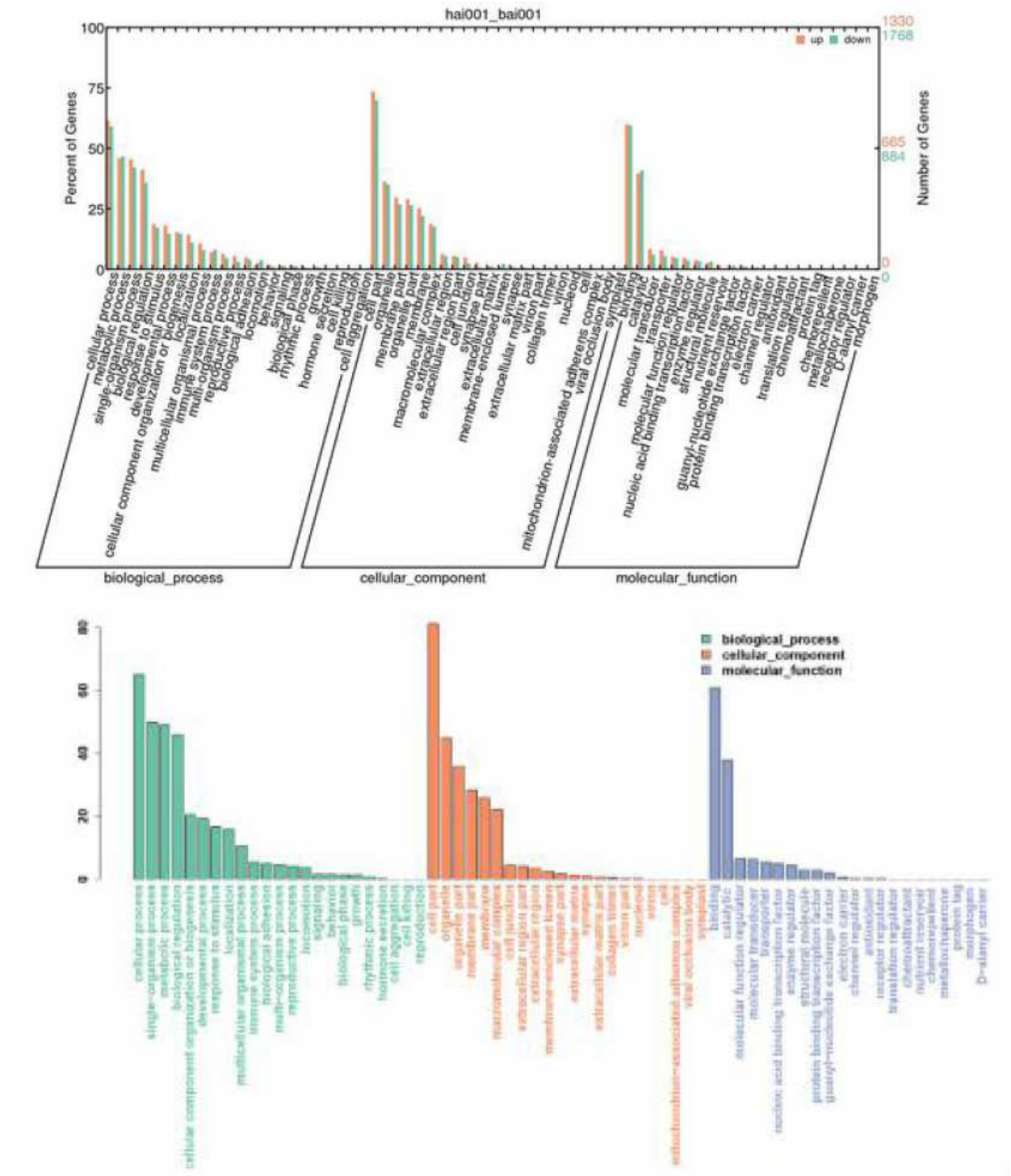

Figure 1：Histogram presentation of DGEs ontology classification
Red color represents up-regulated DGEs and green color represents down-regulated DGEs.

KEGG pathway analysis

The annotated sequences were mapped to the reference pathways in KEGG. All the DGEs were assigned to 315 metabolic pathways, of which 13 were significantly enriched, including glycol-metabolism, linoleic acid metabolism, RNA transfer, cytochrome metabolism and so on. We analyzed DEGs in the cytochrome P450 pathway (map00980) and found 8 genes that expressed differently, among which GSTM4 and ARK72 were up-regulated; CP2F1, UD11, ADH6, GSTA4, CP2BB and ST2A1 were down-regulated. GSTM4 (glutathione transferase) shows up-regulation in most of the cytochrome P450 pathways, and only CP2F1 and CP2BB are members of cytochrome

P450 gene family which all involved in the pigment synthesis. Uehara *et al.* (2009) reported GSTA4 was associated with melanocyte differentiation in mouse cochlear. Christopher *et al.* (2013) summarized loci that affect coat color of dog and cat, including classical genes as MITF, PMEL, TYRP1, MLPH, MC1R, ASIP, KIT and PSMB7, CBD103, TAQPEP and so on, while the genes found in this study are not included. This may due to the sample limit of the experimental design or may find new genes related to coat color. The effects of those genes and the pathways they act on rabbit coat color need further research.

We only used 1 White and 1 Beaver Rex Rabbit in this experiment, this is not only because relatively higher cost of biological repeating in RNA-Seq, but also individual difference and genetic background difference will also affect the results when we mix multiple samples before sequencing. Therefore, we selected full sibs and restricted the threshold that differentially expressed genes could be found, and guaranteed the accuracy of the final results.

CONCLUSIONS

In this study, the DGEs in ear tissue of Rex rabbit of different colors were analyzed by RNA-Seq. In all, 12,408 DGEs were found, which mainly associated with metabolism process, cellular part, catalytic reactions. Through KEGG pathway analysis, 8 candidate genes were found in metabolic of xenobiotics by cytochrome P450, they might involve into the process of cytochrome synthesis and metabolism process, and finally affect coat color of Rex Rabbit. The functions of these candidate genes need to be further verified, and developed molecular markers, which can be used in early selection of Rex Rabbit group and also mechanisms of coat color.

ACKOWLEDGEMENTS

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