转录组学(Transcriptomics)

mRNA-miRNA联合分析

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Category

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3.图像解读

Integrated m RNA and micro RNAtranscriptome analyses reveal regulation ofthermal acclimation in Gymnocypris przewalskii: A case study in TibetanSchizothoracine fish

整合mRNA和miRNA分析揭示裸鲤对高温调控机制

(2017，IF=2.77，PLOS ONE)

样本及条件设置：

Larval fish from both HS and CT groups were exposed to severe high temperature(30˚C) of 48 hours with a same heating rate of ~ 0.8˚C/h

研究的目的：

mRNA and mi RNA transcriptome sequencing to investigate regulation of thermal acclimation in larval Tibetan naked carp, Gymnocypris przewalski

研究的结果：

Regulation network of mRNA and mi RNA in thermal acclimation, and then identified differentialexpression of mi RNAs and target m RNAs enriched in metabolic and digestive pathways

The expression profiles of miRNA–mRNA of early response in genetically improved farmed tilapia (Oreochromis niloticus) liver by acute heat stress

罗非鱼对急性高温胁迫反应的肝脏mRNA和miRNA表达谱

(2017, IF=4,Scientific Report)

样本及条件设置：

We built and sequenced six miRNA libraries; three from 28°C control groups (CO-1, CO-2, and CO-3) and three from 37.5 °C-treated groups (HTS-1, HTS-2, and HTS-3)

研究的目的：

Better understanding the GIFT regulatory response to heat stress will not only help in determining the relationship between heat stress signalling pathways and adaption mechanisms, but will also contribute to breeding new high-temperature tolerant strains of GIFT.

研究的结果：

Important regulatory pathways involved in the early response of GIFT to heat stress included organism system, metabolism, and diseases.

Integrated analysis of mRNA-seq and miRNA-seq in the liver of Pelteobagrus vachelli in response to hypoxia

瓦氏黄颡鱼对缺氧反应的肝脏mRNA-miRNA整合分析

(2016, IF=4.2,Scientific Report)

样本及条件设置：

The water was deoxygenated for 30–35 min by bubbling pure nitrogen gas in order to decrease oxygen concentration from 6.8 mg/L to 0.7 mg/L. After oxygen concentration was maintained for 4 h by continuous bubbling of nitrogen gas, the experimental fish (P4 a, P4 b, and P4 c) were quickly removed for liver dissection. Samplings of control fish (P0 a, P0 b, and P0 c) and experimental fish (P4 a, P4 b, and P4 c) had three biological replicates.

研究的目的：

As a unique resource for gene expression and regulation during hypoxia and reoxygenation, this study could provide a starting point for further studies to better understand the genetic background of hypoxia stress.

研究的结果：

miRNA-mRNA pairs using bioinformatics analysis and miRNA prediction algorithms. Furthermore, we compared several key pathways which were identified as involved in the hypoxia response of P. vachelli.

Dynamic mRNA and miRNA expression analysis in response to hypoxia and reoxygenation in the blunt snout bream (Megalobrama amblycephala)

团头鲂对缺氧和恢复氧反应的动态mRNA和miRNA表达

(2017, IF=4,Scientific Report)

样本及条件设置：

Fish were randomly allocated to one of four treatment groups: hypoxia for 0 h (normoxia, control group), hypoxia for 3 h, hypoxia for 24 h and hypoxia for 24 h + 3 h recovery in normoxia.

研究的目的：

Studying the molecular mechanisms of hypoxia adaptation in fishes will not only help us to understand fish speciation and the evolution of the hypoxia-signaling pathway, but will also guide us in the breeding of hypoxia-tolerant fish strains.

研究的结果：

superoxide dismutase (SOD), catalase (CAT) and nuclear factor erythroid 2-related factor 2 (Nrf2) was signi cantly higher in M. amblycephala in response to reoxygenation 3 h.

Combined RNA-Seq with small RNA revealed ribosome biogenesis andoxidative stress associated with cadmium response in carp (Cyprinus carpioL.) Hepato-pancreas

鲤鱼体内与镉反应相关的核糖体生物发生和氧化应激的mRNA和miRNA表达

(2020, IF=3,ELSEVIER Aquaculture)

样本及条件设置：

In the control group, the fish were exposed to0 mg/ L Cd and one-third of water in the tank was replaced by de-chlorinated drinking water every day. In the Cd exposure group, thefish were exposed to 0.25 mg/ L Cd (1/25 96 h-LC50) according to theLC50 of Cd for common carp.

研究的目的：

performed transcriptome sequencing to analyze Cd responding genes and enrichment pathways.
The carp fish can live and survivein extremely toxic conditions, providing us a useful model for studying Cd response and regulation.

研究的结果：

Most of themwere involved in ribosome biogenesis. Comprehensive analysis of miRNA expression profiles in control and Cdtreated carp identified 15 known differentially expressed miRNAs. Some of them have been well studied, whichare mainly involved in cell growth and oxidative activities.

Liver Transcriptome and miRNA Analysis of Silver Carp(Hypophthalmichthys molitrix)Intraperitoneally Injected WithMicrocystin-LR

白鲢鱼腹部内腔注射微囊藻素后肝脏mRNA和miRNA表达分析

(2018, IF=3.2, Front. Physiol.)

样本及条件设置：

Silver carps were intraperitoneallyinjected with MC-LR, and RNA-seq and miRNA-seq in the liver were analyzed at0.25, 0.5, and 1 h.

The expression of glutathione S-transferase (GST) marker gene for MC-LR.

研究的目的：

Next-generation sequencing was used to analyze the effects of toxic microcystin-LR(MC-LR) on silver carp (Hypophthalmichthys molitrix).

研究的结果：

Gene Ontology (GO) term enrichment analysis suggested that 35 of the145 enriched GO terms were significantly enriched and mainly related to the immunesystem regulation network.

KEGG pathway enrichment analysis showed that 18 of the189 pathways were significantly enriched, and the most significant was a ribosomepathway containing 77 differentially expressed genes

1.摘要Abstract

1.通过mRNA-miRNA的转录组学研究热胁迫或者氧胁迫的机制；

2.样本胁迫条件和分组（对照组和实验组）；

3.测序文库构建和转录组测序；

4.mRNA共检测到的显著差异基因，主要富集的GO和KEGG通路；

5.miRNA检测到保守miRNA和新miRNA,靶基因预测；

6.mRNA-miRNA调控网络；

7.RT-qPCR验证显著相关热胁迫或氧胁迫的基因；

8.研究的意义，揭示胁迫机制和选育优良种相关。

2.前言Introduction

1.鱼类在温度，缺氧，盐度方面的特征

（如鱼类是冷血动物对温度敏感，该鱼类的正常温度范围，已有胁迫的研究）

2.miRNA的特征

（如miRNA的序列长度，miRNA的产生及功能，miRNA负调控基因表达，已有miRNA转录研究）

3.转录组测序的应用

（转录组测序可检测更多的胁迫基因表达，可以检测miRNA的表达，已有转录组在鱼类中研究）

2.前言Introduction

1.鱼类受到各种环境条件影响，水温是主要的因素

- Myrs R. A. When do environment–recruitment correlations work?, Reviews in Fish Biology and Fisher-ies. 1998; 8: 285–305.
- Wootton R.J. Fish ecology: Springer Science & Business Media; 1991.
- Lo´ pez-Olmeda J., Sa´ nchez-Va´ zquez F. Thermal biology of zebrafish (Danio rerio), Journal of ThermalBiology. 2011; 36: 91–104.

2.鱼类是冷血动物对温度敏感

- Davis, K. B. Temperature a ects physiological stress responses to acute con nement in sunshine bass (Morone chrysops × Morone saxatilis). Comp. Biochem. Phys. 39A, 433–440 (2004).
- Lu, Y. L. et al. Insight into the heat resistance of sh via blood: E ects of heat stress on metabolism, oxidative stress and antioxidant response of olive ounder Paralichthys olivaceus and turbot Scophthalmus maximus. Fish Shell sh Immun. 58, 125–135 (2016).

3.温度超过容差有坏应影响

- Long Y., Song G., Yan J., He X., Li Q., Cui Z. Transcriptomic characterization of cold acclimation in lar-val zebrafish, BMC genomics. 2013; 14: 1.

4.水温对鱼类摄食和发育的影响

- Ma, X. Y., Qiang, J., He, J., Gabriel, N. N. & Xu, P. Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (Oreochromis niloticus) reared at three di erent temperatures. Fish Physiol. Biochem. 41, 937–950 (2015).

5.免疫防御、消化酶活性、蛋白质合成和生长过程被抑制

- Miegel, R. P., Pain, S. J., van Wettere, W. H. E. J., Howarth, G. S. & Stone, D. A. J. E ect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail king sh (Seriola lalandi). Aquaculture 308, 145–151 (2010).

6.全球变暖，南海水温

Root T. L., Price J. T., Hall K. R., Schneider S. H., Rosenzweig C., Pounds J. A. Fingerprints of globalwarming on wild animals and plants, Nature. 2003; 421: 57–60.

https://doi.org/10.1038/nature01333PMID: 12511952

2.前言Introduction

1.鱼类温度研究的转录组测序

- Long Y., Song G., Yan J., He X., Li Q., Cui Z. Transcriptomic characterization of cold acclimation in lar-val zebrafish, BMC genomics. 2013; 14: 1.
- Kenkel C., Meyer E., Matz M. Gene expression under chronic heat stress in populations of the mustardhill coral (Porites astreoides) from different thermal environments, Molecular ecology. 2013; 22: 4322–4334. https://doi.org/10.1111/mec.12390 PMID: 23899402
- Long Y., Li L., Li Q., He X., Cui Z. Transcriptomic Characterization of Temperature Stress Responses inLarval Zebrafish, Plos One. 2012; 7.
- Jun Qiang1, Wen J. Bao. The expression profles of miRNA–m RNA of early response in genetically improved farmed tilapia (Oreochromis niloticus) liver by acute heat stress. Scientific Report.2017
- Zhang C, Tong C, Tian F, Zhao K (2017)Integrated m RNA and micro RNA transcriptomeanalyses reveal regulation of thermal acclimation inGymnocypris przewalski : A case study in TibetanSchizothoracine fish. PLo S ONE 12(10): e0186433.https://doi.org/10.1371/journal.pone.0186433

2.miRNA长度及调控功能

- Pillai, R. S., Bhattacharyya, S. N. & Filipowicz, W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 17, 118–126 (2007).
- Yang, R. L., Dai, Z. H., Chen, S. & Chen, L. B. MicroRNA-mediated gene regulation plays a minor role in the transcriptomic plasticity of cold-acclimated Zebra sh brain tissue. BMC Genomics 12, 605 (2011).

3.整合mRNA和miRNA研究

- Yu, J. et al. Integrating miRNA and mRNA expression pro les in response to heat stress-induced injury in rat small intestine. Funct. Integr. Genomics 11, 203–213 (2011).

4. Heat shock proteins (HSPs)

- Samples, B. L., Pool, G. L. & Lumb, R. H. Polyunsaturated fatty acids enhance the heat induced stress response in rainbow trout (Oncorhynchus mykiss) leukocytes. Comp. Biochem. Phys. 123, 389–397 (1999).

3.材料和方法
Materials and methods

1.样本通过保护协议，温度条件设置，样本随机分组为对照组和实验组

2.mRNA提取和构建文库：试剂、仪器（公司信息和参考手册）

TRIzol提取，TruSeq RSPK V2 纯化，Agilent 2000 bioanalyzer质控，Illumina Hi Seq™ 2500测序

3.mRNA 生物信息分析

原始数据，数据过滤（质控及过滤软件），高质量数据组装（软件信息），

Unigenes数据库注释（NR，GO，KEGG，KOG，Pfam，Uniprot等数据库）

4.Different Express Genes（DEGs），FPKM, DESeq2, FDR < 0.05, p < 0.05, absolute value log2Fold-Change > 1

5.miRNA提取和构建文库：试剂、仪器（公司信息和参考手册）

6.miRNA生物信息分析

原始数据过滤，RNAs (r RNA, t RNA, sn RNA and sno RNA)Blast比对到GenBank和Rfam去除，

miRBase, RNAscan数据库鉴定已知保守名字的miRNA,新miRNA(软件），miRNA靶基因预测，qPCR

4.结果Result

1. mRNA测序和分析

样品Reads，过滤数据CleanReads，高质量数据组装Unigenes，各数据库注释Unigenes

2.差异表达mRNA

FPKM, 差异表达筛选(Pvalue < 0.05, |log2FC| >1, FDR < 0.05)，上下调基因数目，volcano火山图，各样本上下调热图，差异基因GO和KEGG富集分析，与温度相关显著通路,（Top30GO的CC、MF、BP具体信息，Top30KEGG通路具体信息）

3.miRNA文库构建、测序结果，搜库鉴定

原始数据过滤，RNAs (r RNA, t RNA, sn RNA and sno RNA)Blast比对到GenBank和Rfam去除，

miRBase, RNAscan数据库鉴定已知保守名字的miRNA,新miRNA(软件）

4.miRNA靶基因预测和富集分析

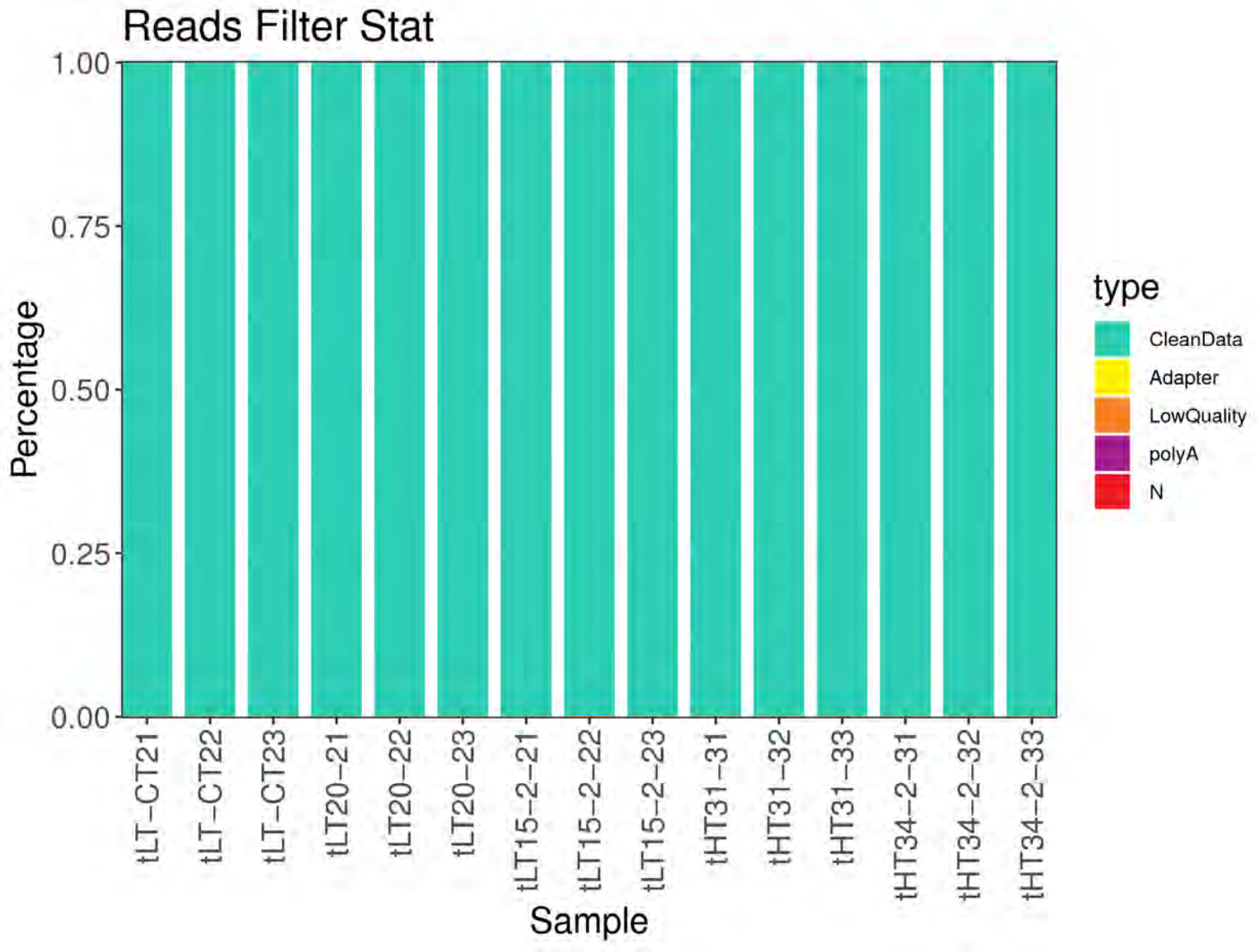
显著差异表达的miRNA，上下调miRNA，Top30GO的CC、MF、BP具体信息，Top20KEGG通路具体信息

5.mRNA-miRNA调控网络

调控网络中的基因的GO和KEGG注释

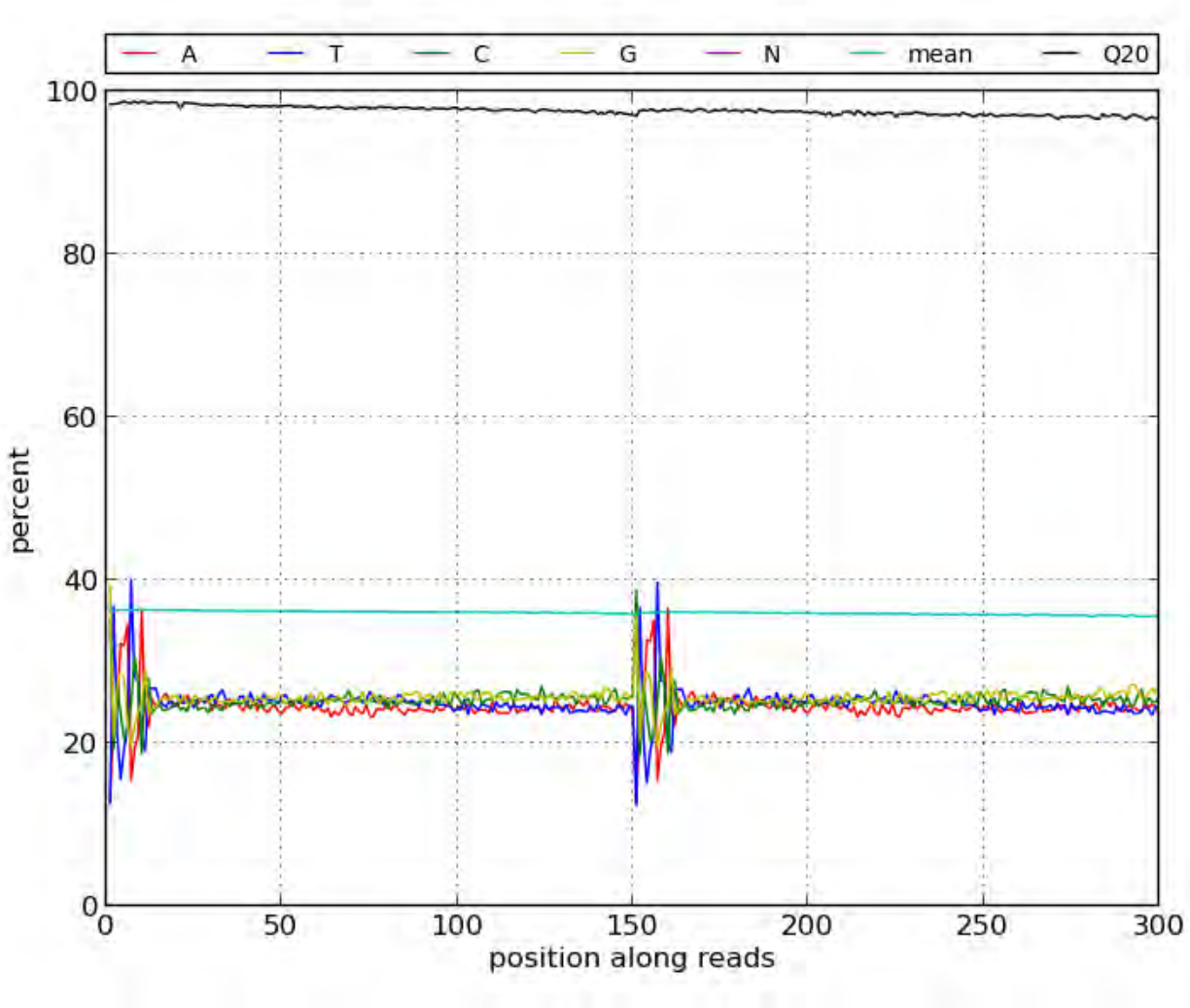
5.Reads

Clean Reads



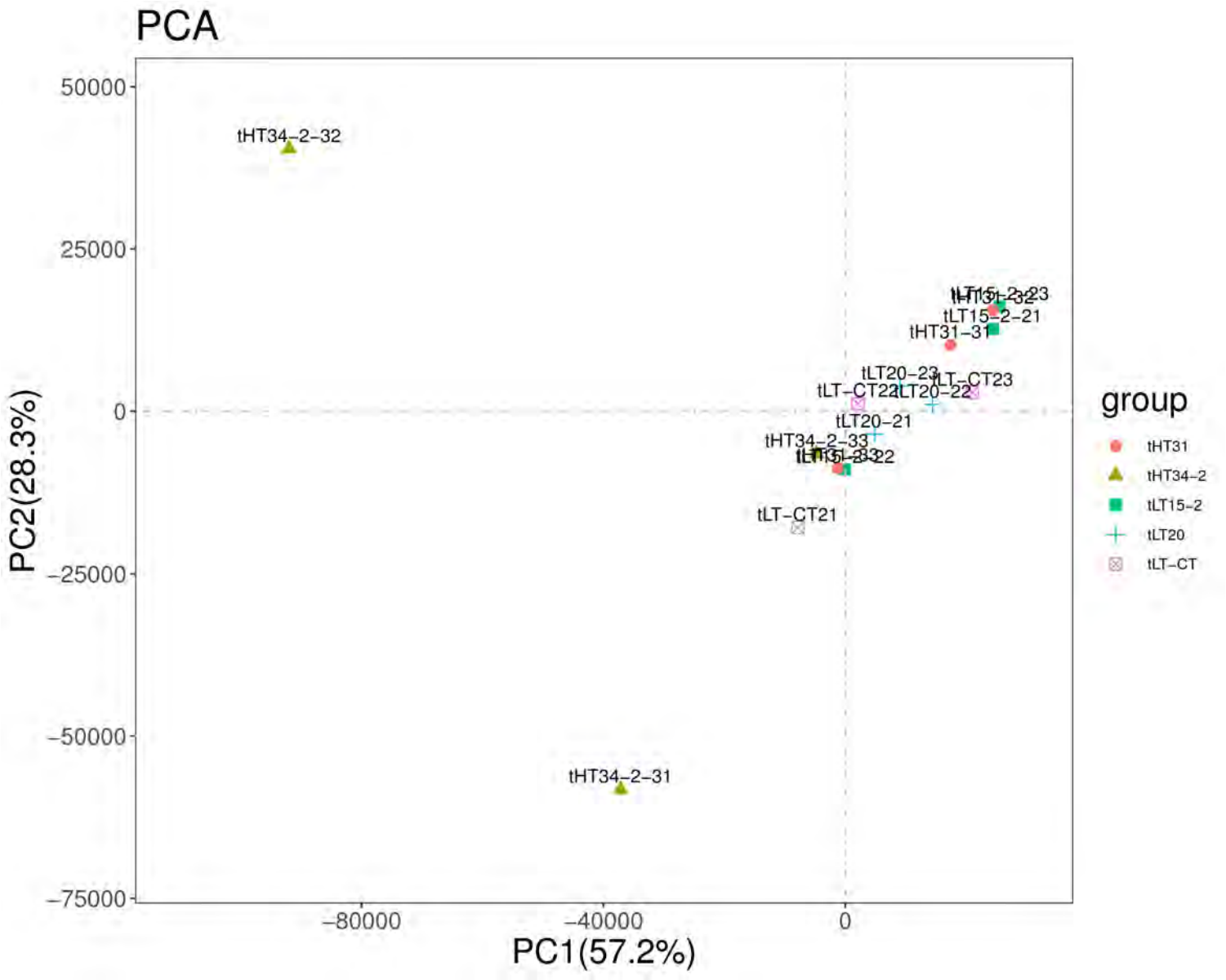
5.Reads

Bases Distribute



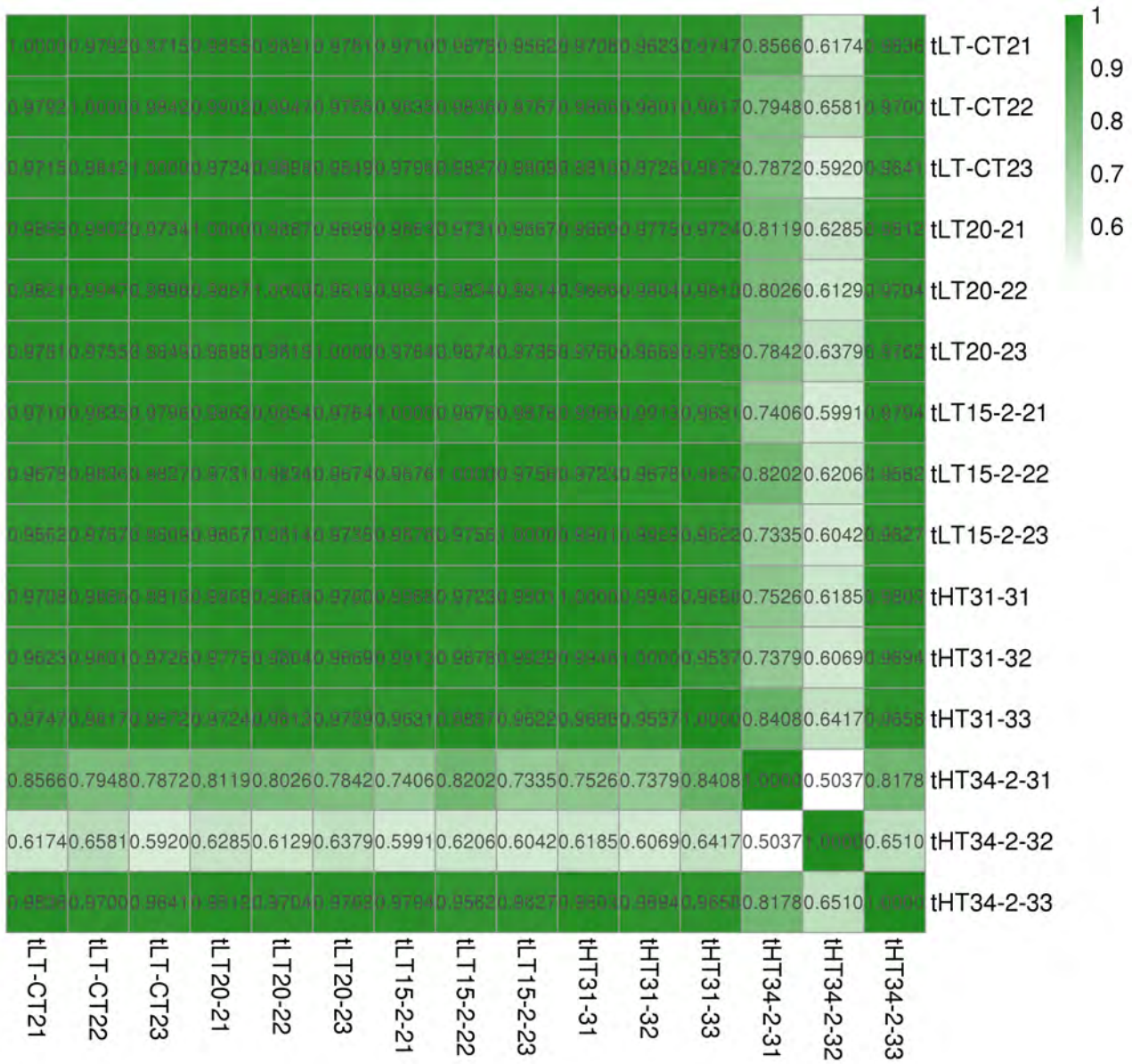
6.Simples

Simples PCA



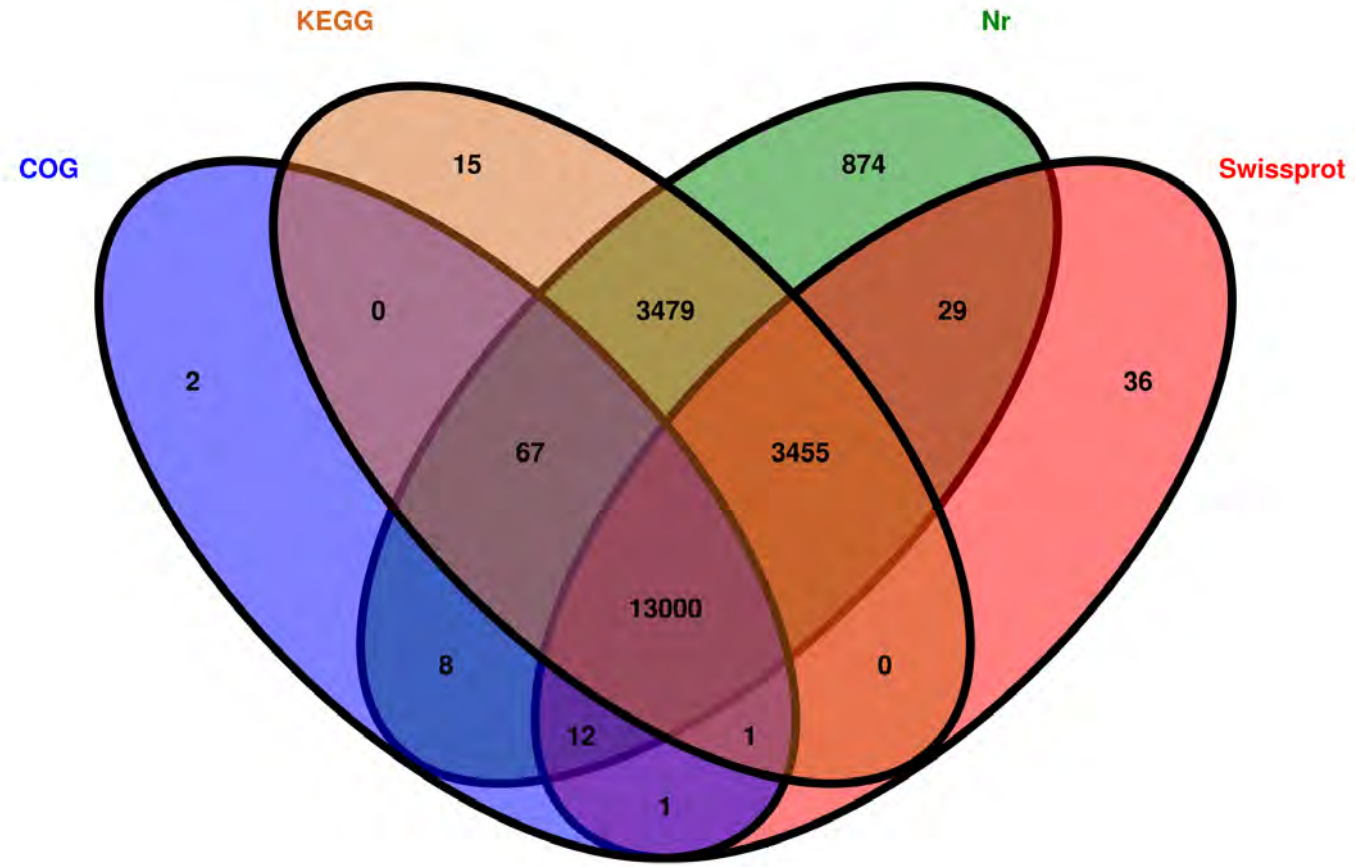
6.Simples

Simples Heatmap (pearson)



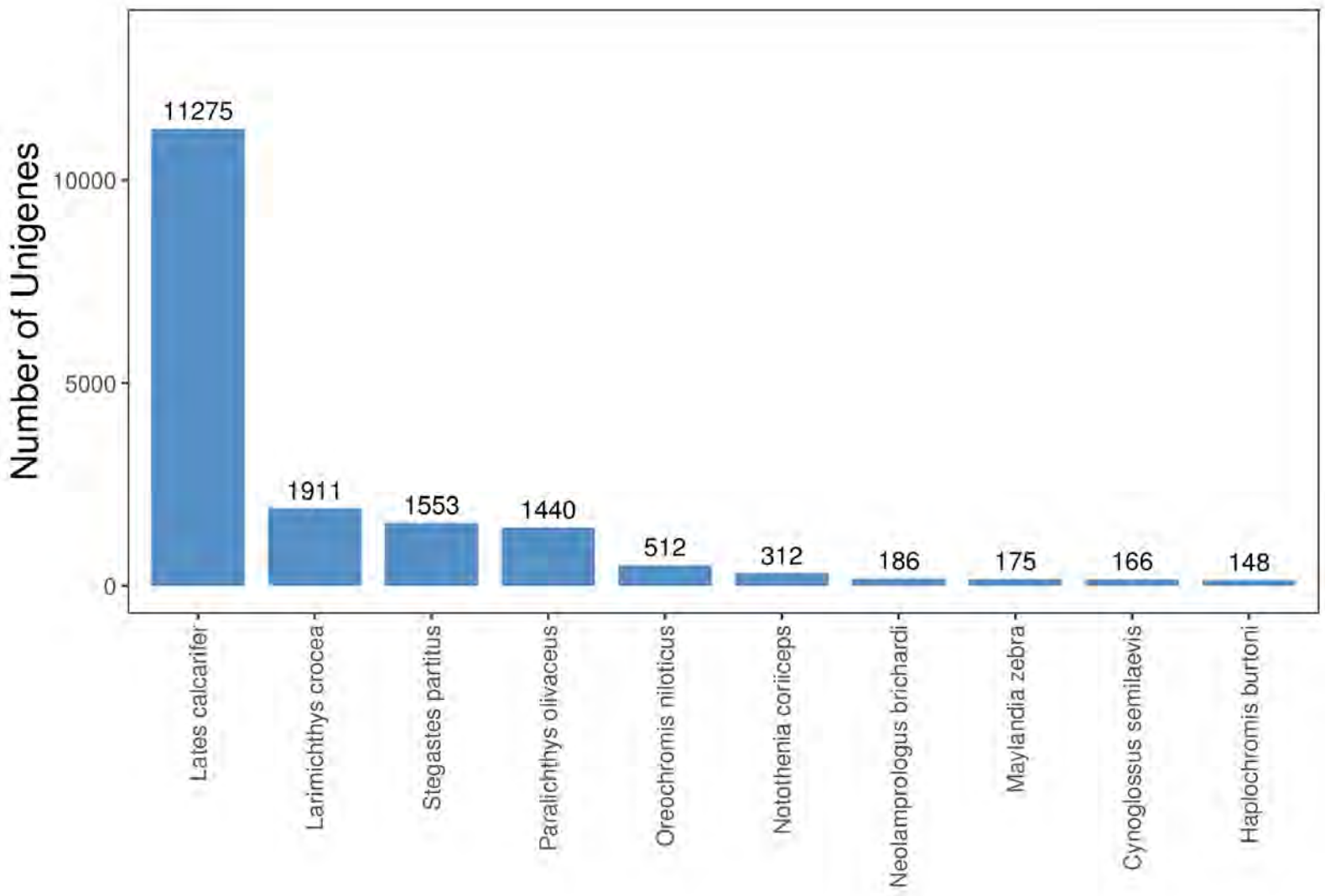
7.Unigene annotation

Unigenes Annotation Databases



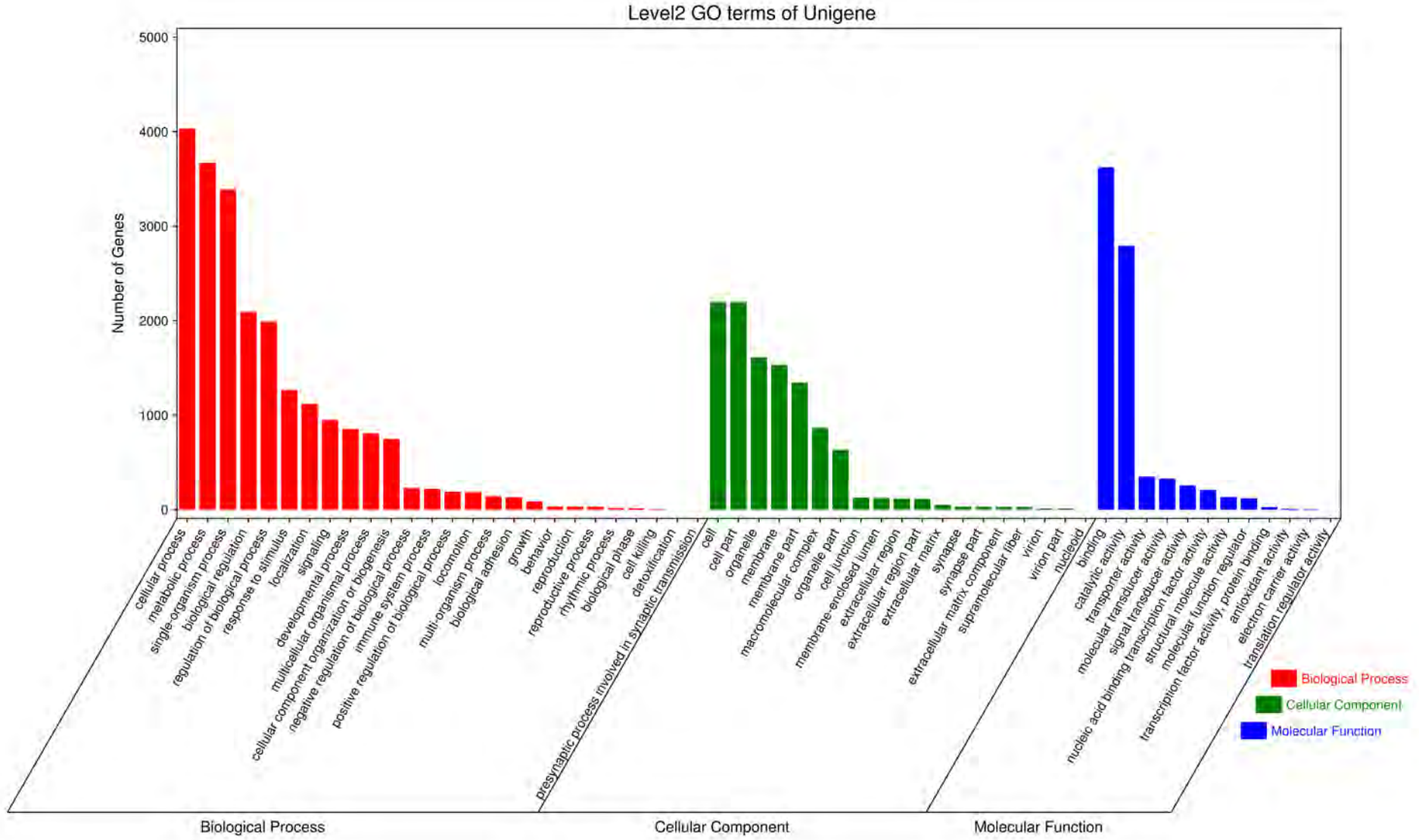
7.Unigene annotation

NR Databases



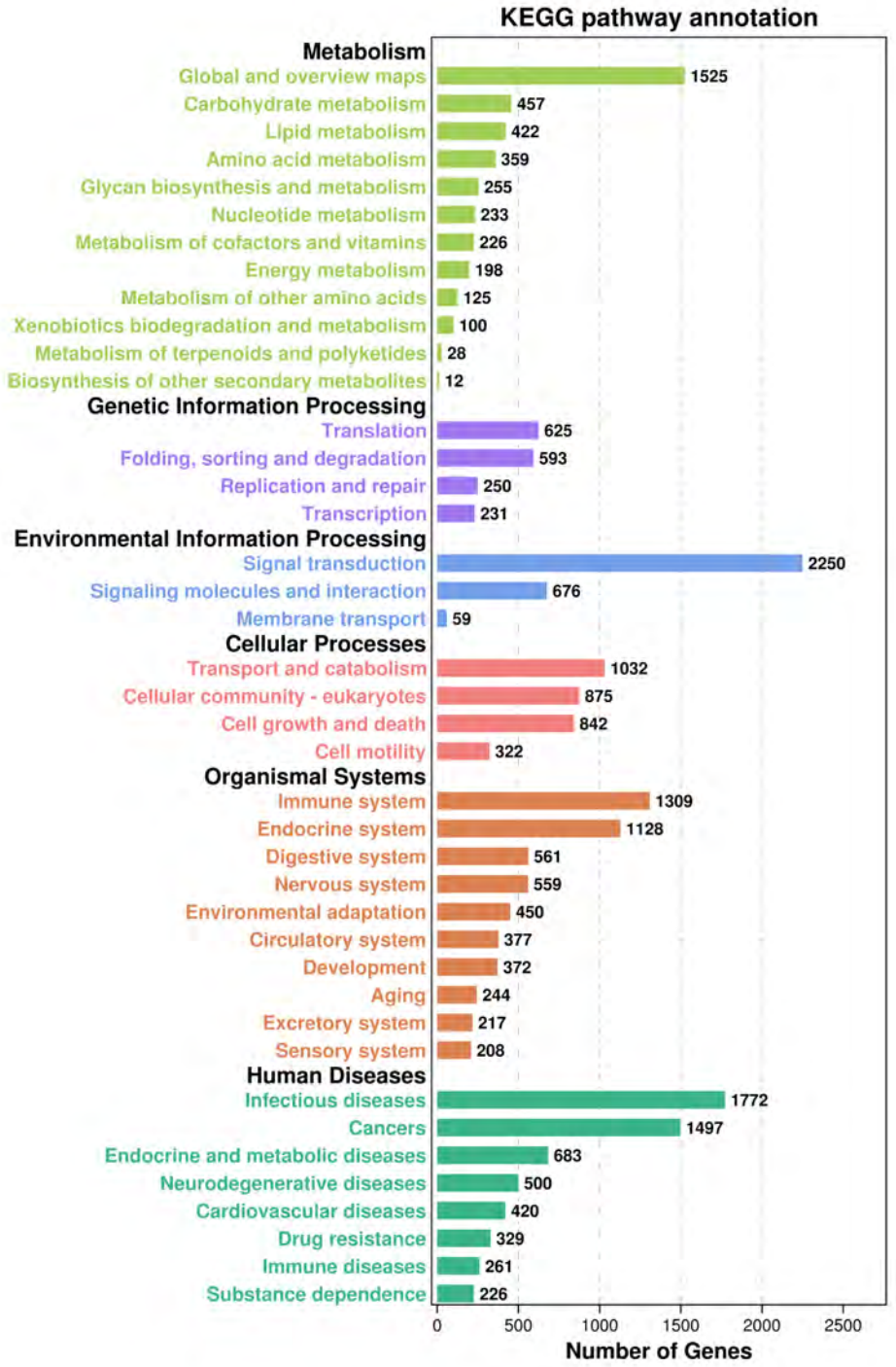
7.Unigene annotation

GO Annotation



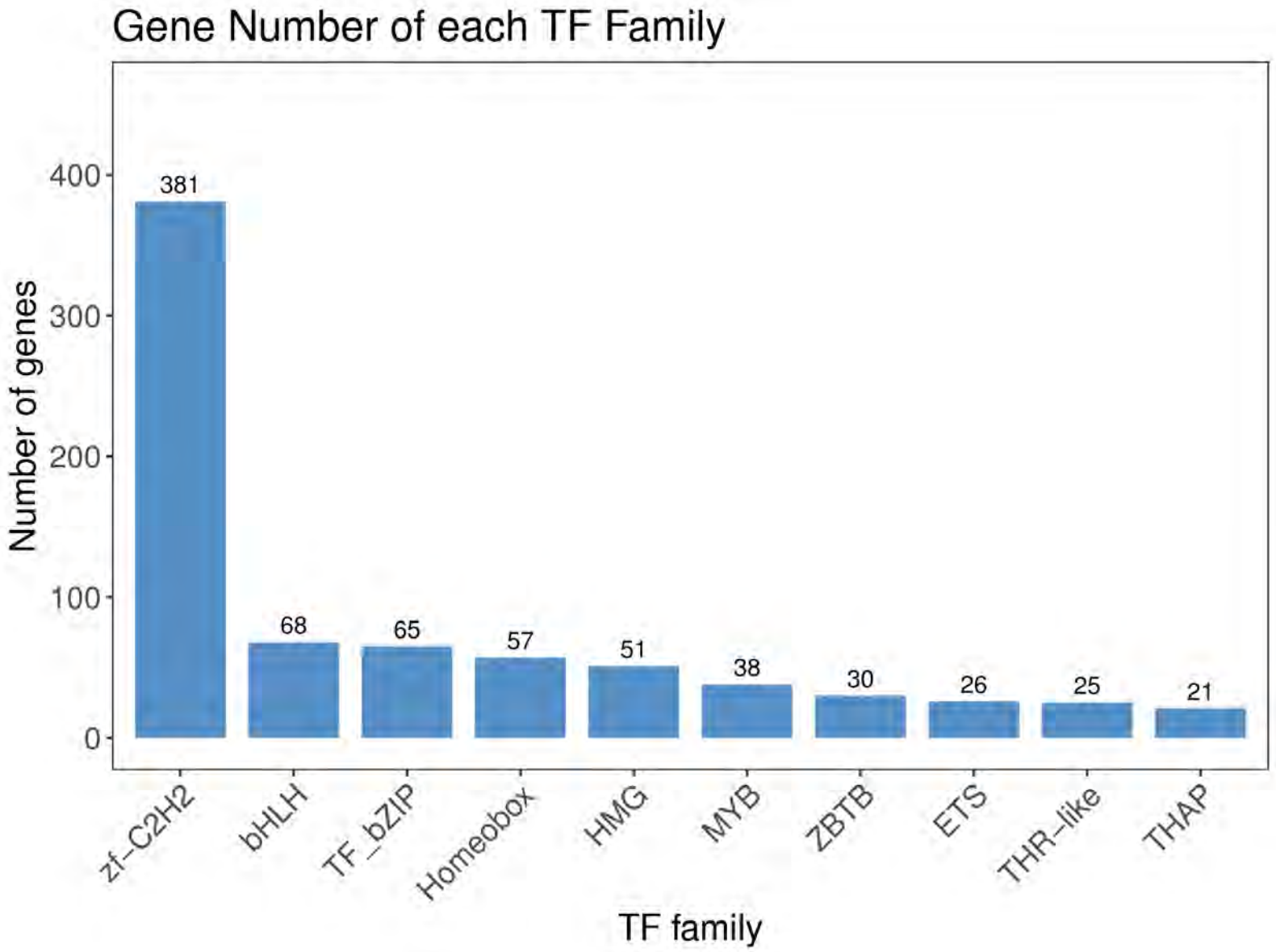
7.Unigene annotation

KEGG Annotation



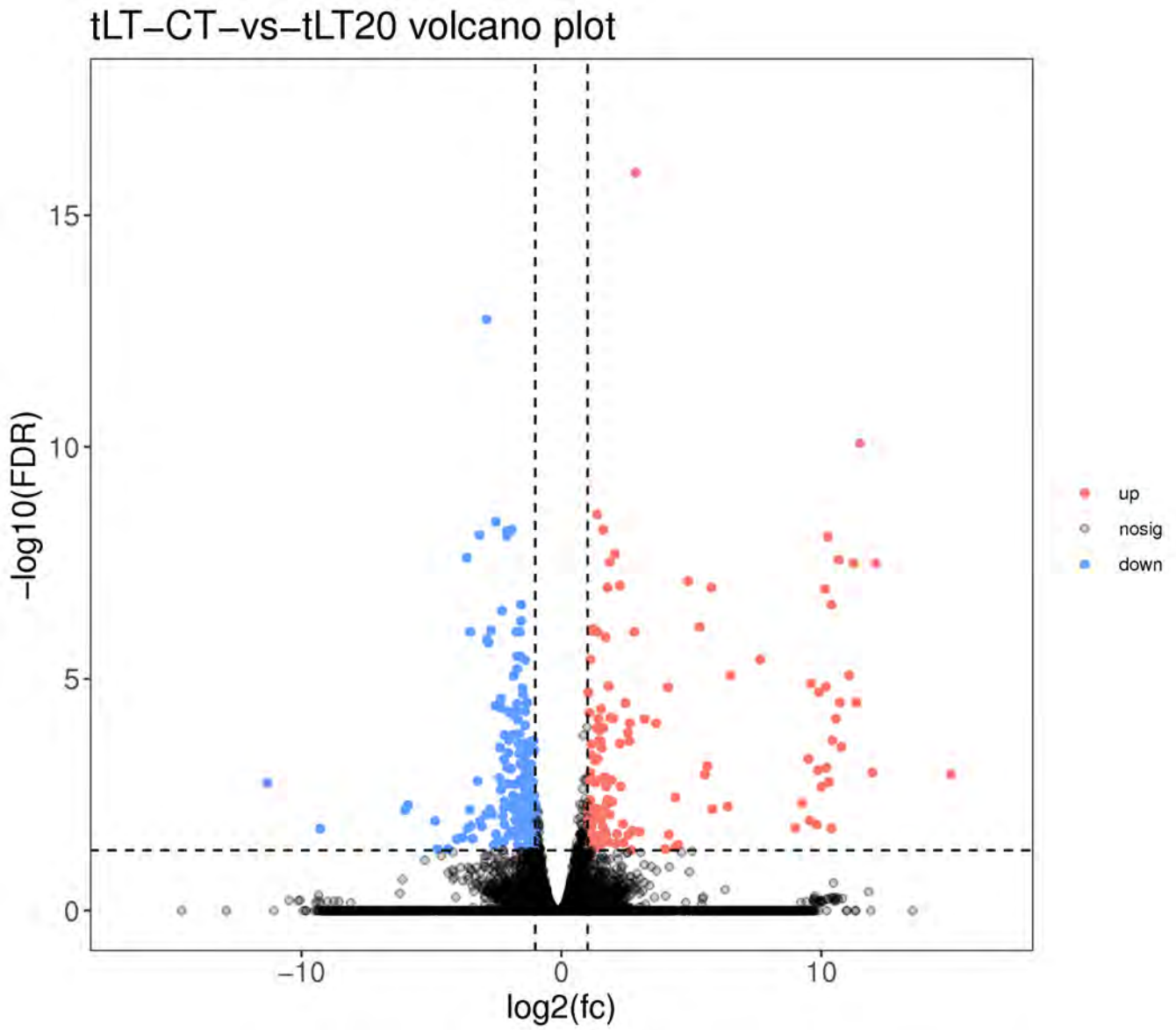
7.Unigene annotation

KOG Annotation



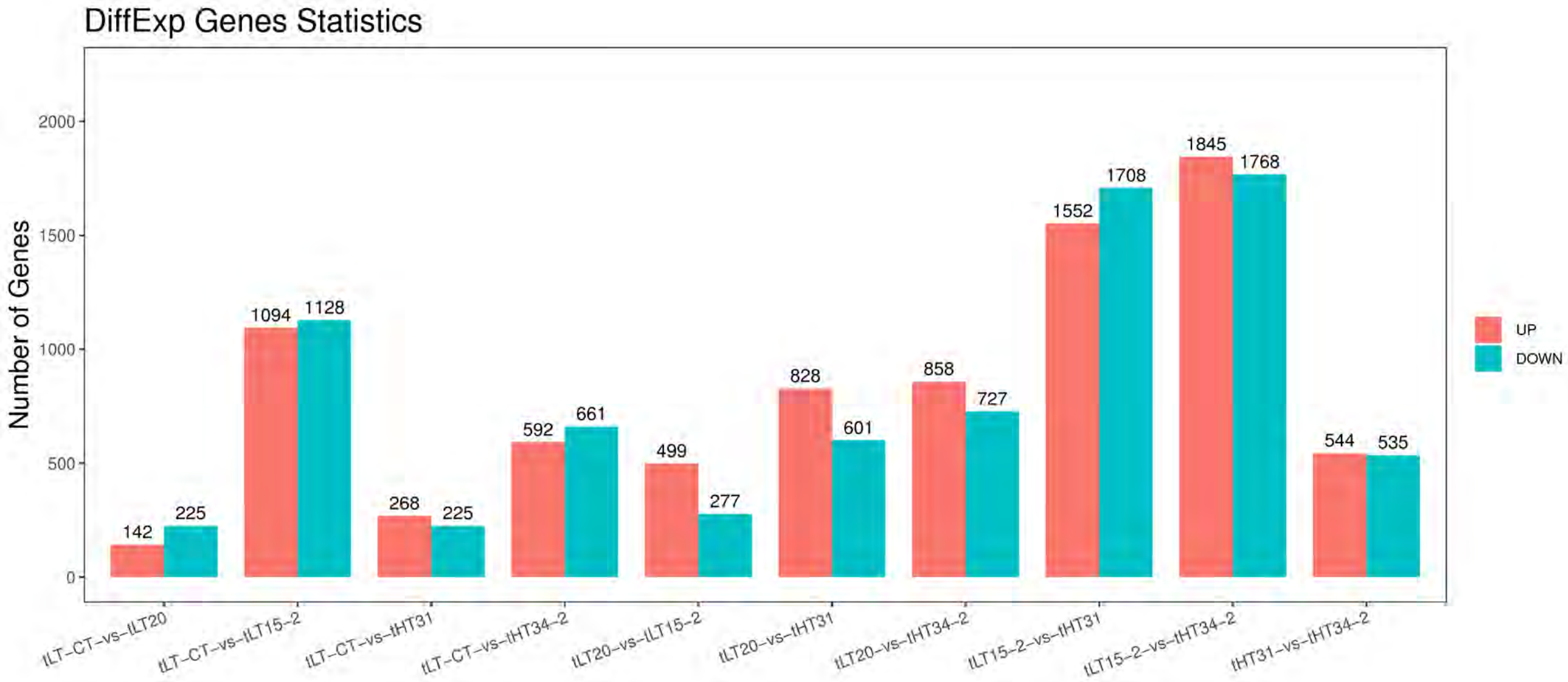
8.DEGs

Different Express Genes Volcano Diagram



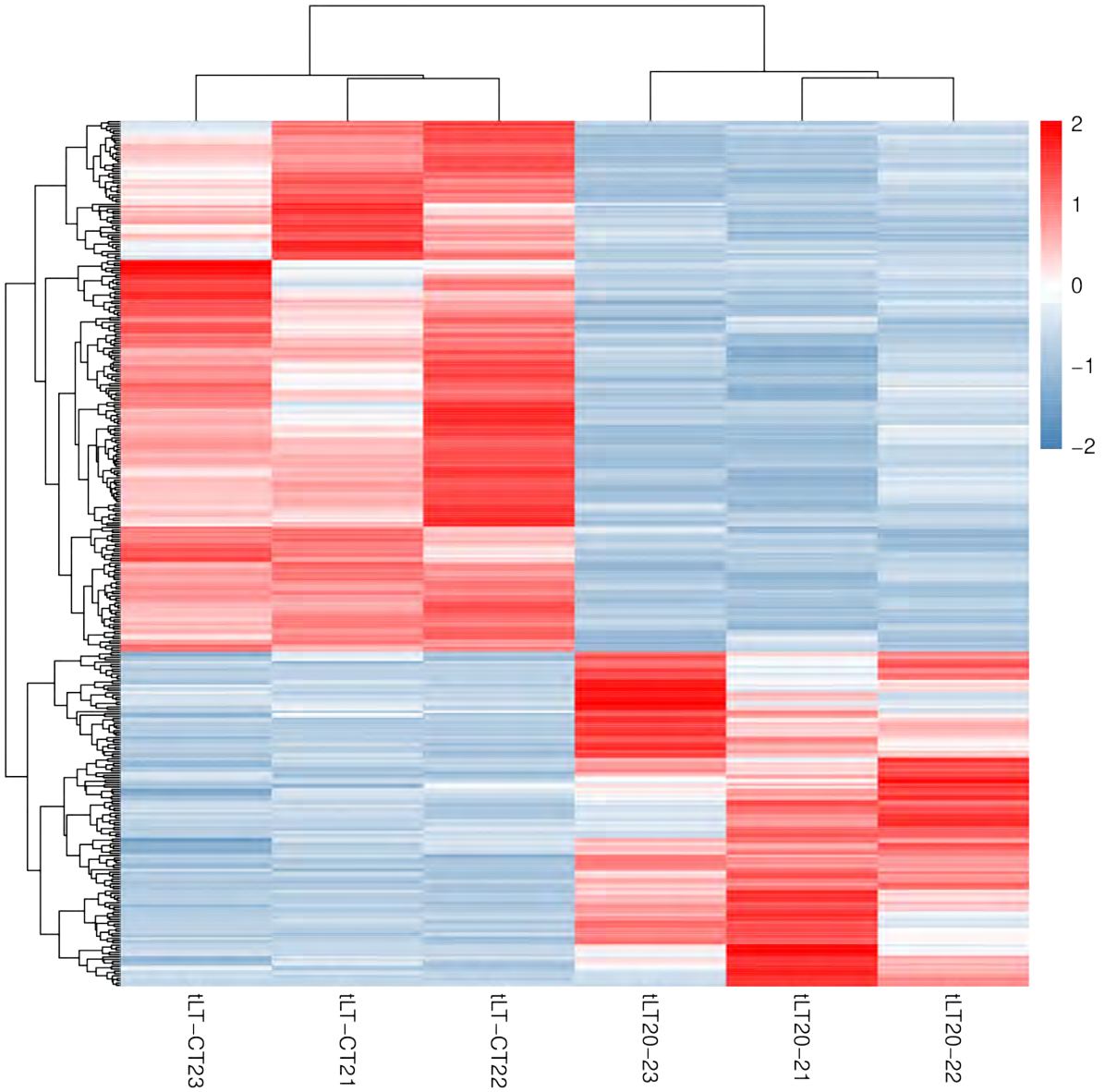
8.DEGs

Different Express Genes Statistics



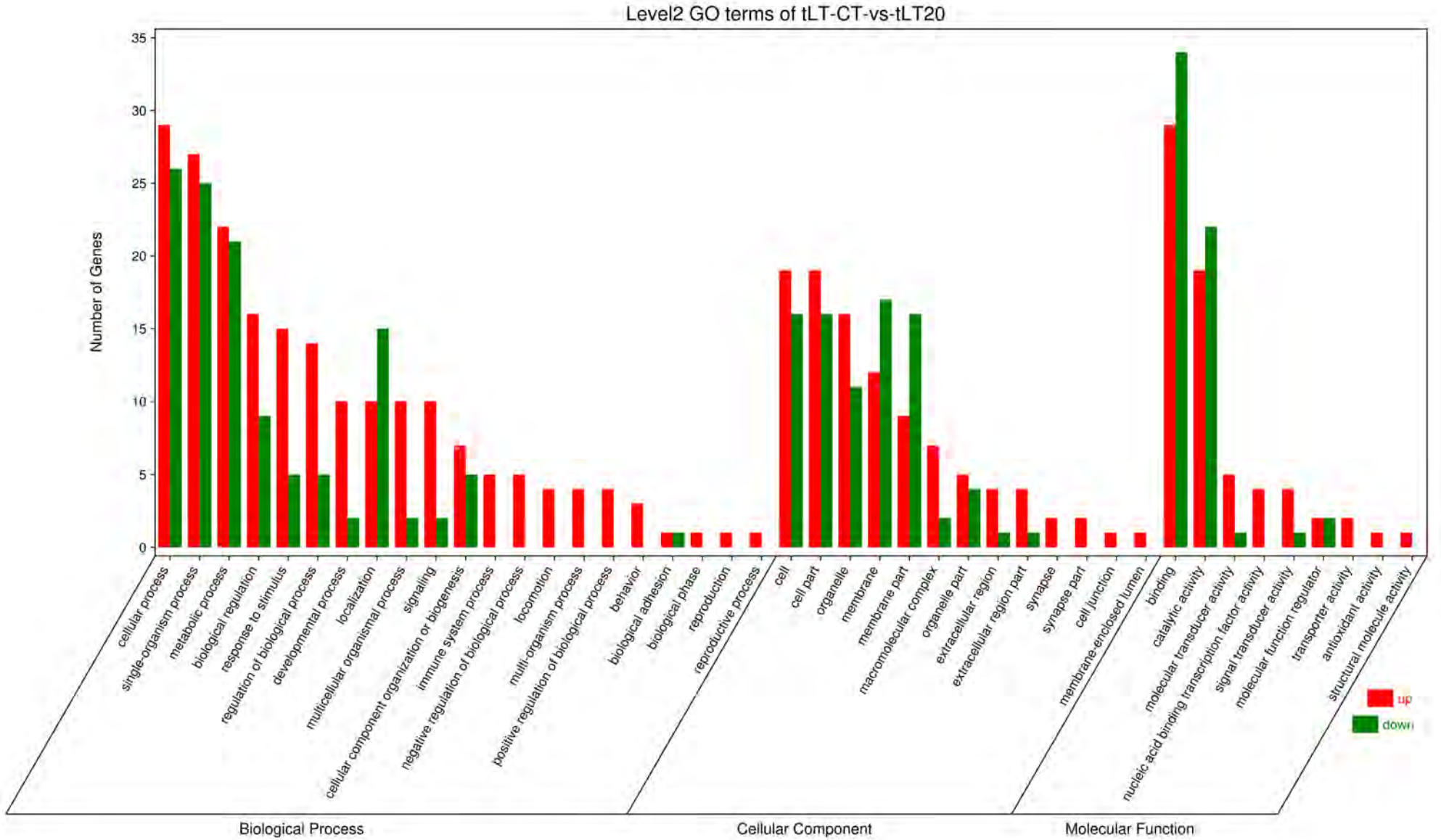
8.DEGs

Different Express Genes Heatmap Diagram



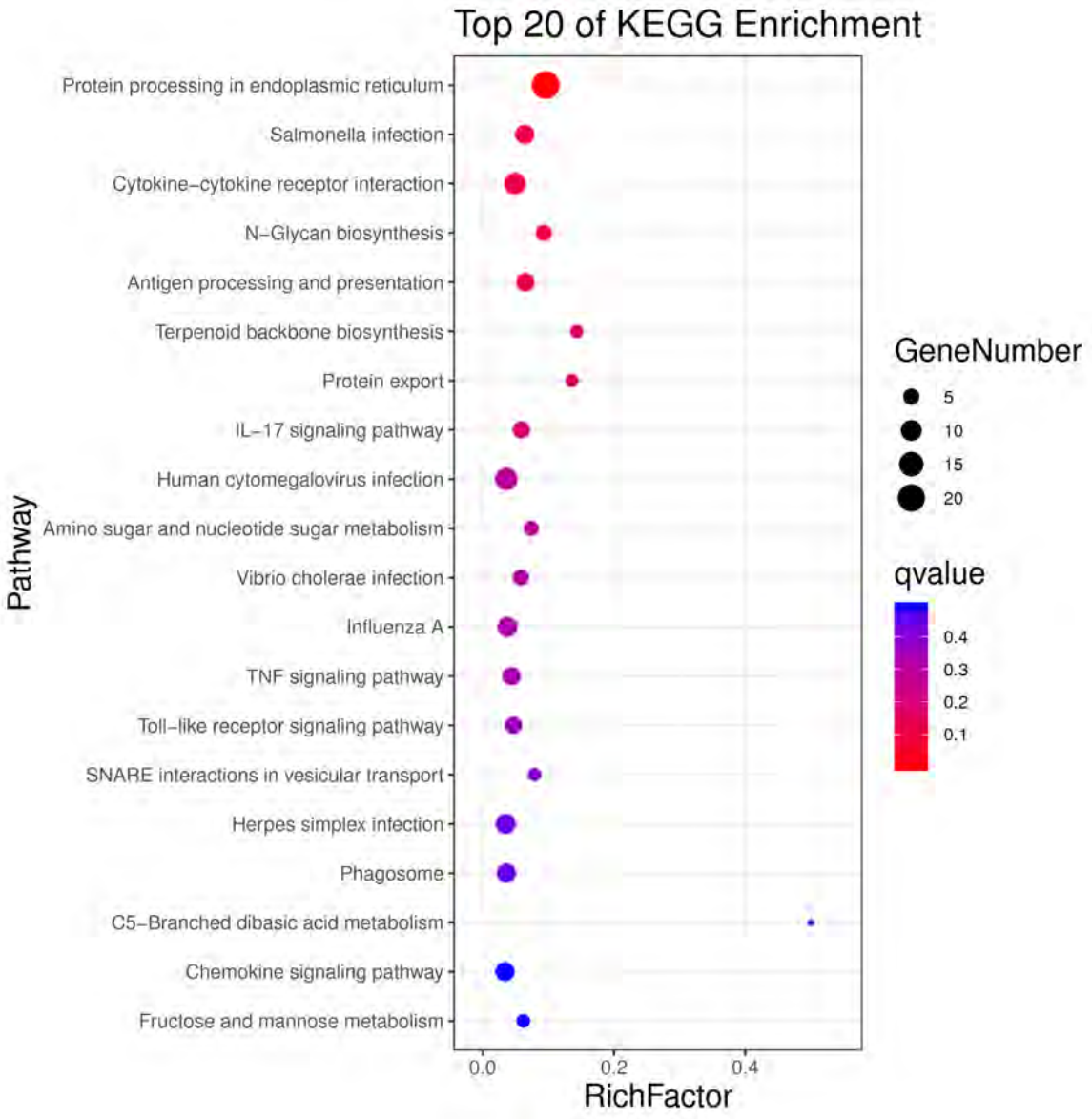
8.DEGs

Different Express Genes Go Enrichment



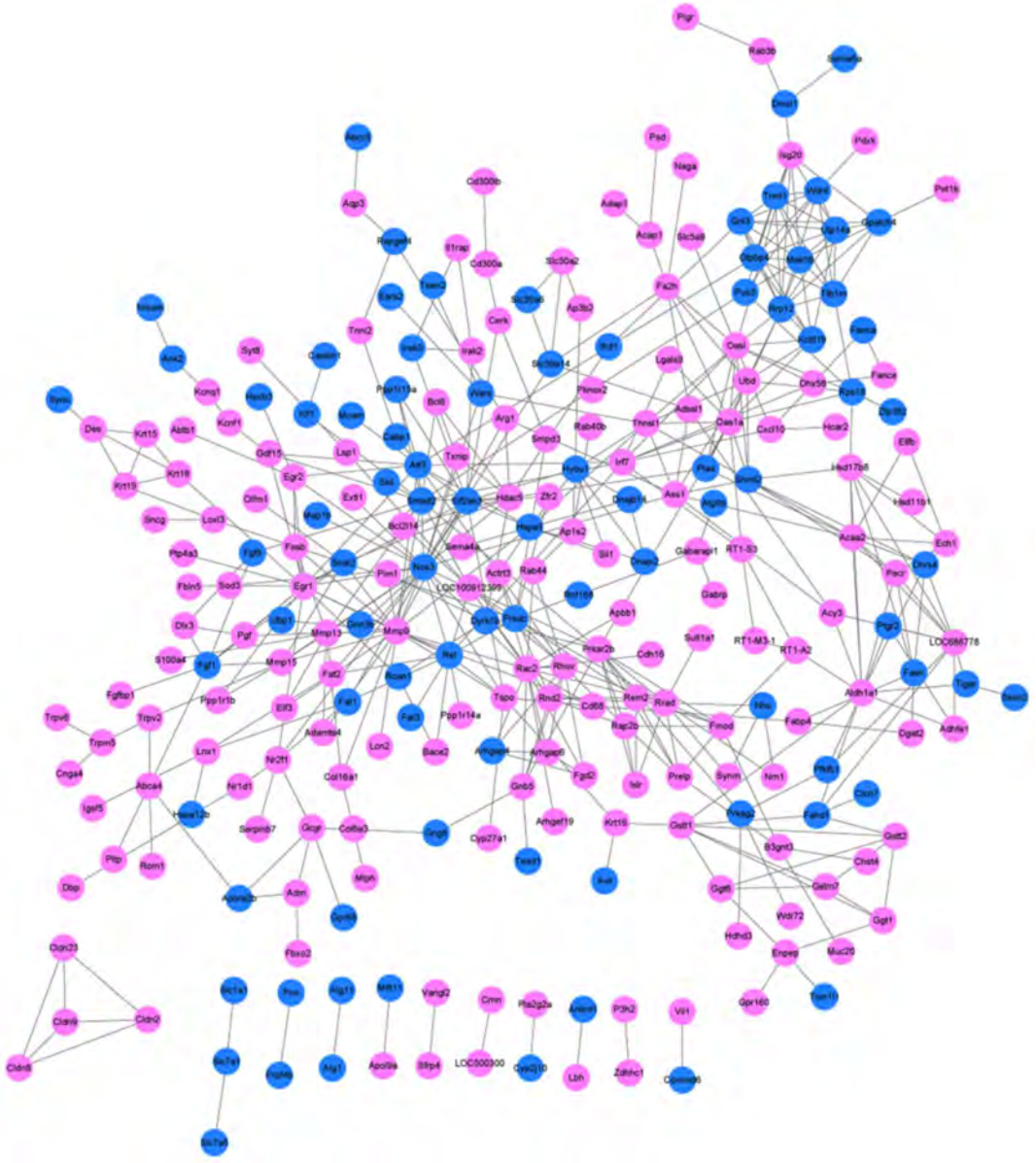
8.DEGs

Different Express Genes KEGG Enrichment



8.DEGs

Different Express Genes KEGG Enrichment



Thanks!