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Differential expression profile of long non-coding RNA in PPRV-Vaccinated sheep monocytes

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Abstract
Peste-des-petits ruminants (PPR) disease is a highly contagious viral disease of major economic importance in sheep and goats caused by the PPR virus (PPRV). The virus is grouped in the Morbillivirus genus under the family *Paramyxoviridae*. Antigenically it has a close relationship with Rinderpest (RP) disease in cattle. Following the successful eradication program of RP and considering the severity and importance of this disease, the PPR- Global Control and Eradication Strategy (PPR-GCES) was adopted by FAO and OIE in 2015 for the effective control and eradication of PPR. For the efficient implementation and evaluation of such eradication programs, we require an extensive knowledge of molecular mechanisms involving both genetic and epigenetic players of host and virus involved in the epidemiology and pathogenesis of the disease. Earlier studies on the transcriptome profile of host cells infected or vaccinated with PPRV both *in vivo* and *in vitro* revealed various dysregulated immune factors and also an altered miRNA profile suggestive of the role of non-coding RNA during PPRV infection. However, the long noncoding RNAs (lncRNAs) regulation of PPRV infection in PBMC cell subsets has not yet been reported. In this study, we have identified differentially expressed lncRNAs in monocytes, a $CD14^+$ PBMC cell subset, of sheep vaccinated with PPRV. On their functional analysis, out of 82 up and 198 downregulated differentially expressed lncRNAs, 13 lncRNAs were found to be responsible for the generation of immune response in sheep and all were downregulated. The key lncRNAs may serve as potential molecular markers in PPRV vaccination. It can also help us to understand the molecular mechanism of occurrence and development of the disease in sheep.

Keywords: Long non-coding RNA, Monocytes, Peste-des-petits ruminants virus

Introduction
PPR is a highly contagious viral disease of major economic importance in sheep and goats. It is closely related to Rinderpest and is caused by the Peste-des-petits ruminants virus (PPRV). It is more prevalent in Africa, Asia, and the Middle East, which are home to 80% of the total sheep and goat population in the world (Edo *et al*., 2017) [1]. The antigenic relationship between the RP virus and PPR virus was given in 1956 (Mornet *et al*., 1956) [3] and the PPRV grouped in the order Mononegavirales and family *Paramyxoviridae* in the year 1979 alongside RP, measles, and canine distemper viruses under Morbillivirus genus (Gibbs *et al*., 1979) [2]. The overall morbidity can reach up to 90% with a high case fatality rate in acute cases (Pope *et al*., 2013) [4]. It is a highly immunosuppressive disease-causing interfering with interferon signaling in hosts thereby evading the host's anti-viral immune response. Although few of these immune suppression strategies mediated by PPRV structural and non-structural proteins are known, there are still many unknown mechanisms that are yet to be elucidated. Considering the severity and importance of this disease, PPR- Global Control and Eradication Strategy-GCES has been adopted by FAO and OIE in 2015 for the effective control and eradication of PPR taking inspiration from the success story of RP eradication (Njeumi *et al*., 2020) [5]. For the efficient implementation and evaluation of such eradication programs, we require an extensive knowledge of molecular mechanisms involving both genetic and epigenetic players of host and virus involved in the epidemiology and pathogenesis of the disease. One such genetic player is long non-coding RNA, abbreviated as lncRNA. LncRNAs are defined as non-coding linear transcripts longer than 200 nucleotides and share common characteristics with mRNAs. The majority of lncRNAs are usually transcribed by RNA polymerase II, capped at 5′ end, and spliced; most of them are polyadenylated at the 3′ end and have promoter regions. Compared to the protein-coding gene mRNA, lncRNAs lack of open reading frame (ORF), contain fewer exons (~2.8 exons in lncRNAs compared to 11 exons for

protein-coding gene), are expressed in low abundance, and are more tissue-specific (Derrien *et al*., 2012) [6]. Therefore, the present study aims to identify differentially expressed lncRNAs (DE-lncRNAs) in the PPRV-vaccinated sheep monocytes ($CD14^+$) cell subset and predict their possible role in antiviral immunity.

Materials and Methods

Previous data from our lab on sheep-vaccinated monocytes ($CD14^+$) with SRR12362062 of the control group and SRR12362063 of the vaccinated group were retrieved from the NCBI database. The vaccinated data of the sheep was on the 5th day of post-vaccination (dpv) with vaccine strain Sungri/96. Quality checking of reads was done in FASTQC (Andrews, 2010) [7]. All the reads from all samples had no adapter content and were of good quality. Therefore, bowtie2 (Langmead and Salzberg, 2012) [8] was used to align the input FASTQ reads to known sheep rRNA sequences, and only unmapped reads were retained. The latest sheep (*Ovies aries*) [ARS-UI_Ramb_v3.0 (GCF_016772045.2)] and its' corresponding annotation files were downloaded from the NCBI. The reference genome was indexed and the retained reads were then mapped against the genome using STAR v2.7.3a with default parameters (Dobin *et al*., 2013) [10] and subsequently, the bam files were generated using Samtools (Danecek *et al*., 2021) [9]. Stringtie v2.1.1 (Pertea *et al*., 2015) [12] was used for ab initio transcriptome assembly from the BAM files generated in the previous step, and a custom transcriptome (GTF) of sheep was obtained for each sample. Individual GTF files were merged into a single transcriptome that was compared against the reference sheep annotations using cuff-compare (Trapnell *et al*., 2010) [13] to remove potential errors (class codes c, e, p, s). FASTA sequences of the filtered transcriptome were extracted using gffread v0.11.7 (Pertea and Pertea, 2020) [11], and subsequently indexed in and used for transcript-level quantification in Salmon v0.12.0 (Patro *et al*., 2017) [14]. After quantification, the annotated transcriptome of sheep was used for lncRNA filtering steps based on its length (>200), class codes (u, i, o, and x selected), ORF length (less than 100), and coding potential score by CPC2 (Kang *et al*., 2017) [15]. Finally, all the transcripts that were remaining after the steps were considered lncRNA. The count matrices of transcript per million (TPM) were prepared from Salmon output using abundance_estimates_to_matrix.pl script of the Trinity RNA-Seq package (Grabherr *et al*., 2011) [16]. Differential expression analysis of lncRNA was done using DESeq2. Similarly, DE-mRNAs were also identified by the RSEM-STAR package along with DESeq2. Subsequently, we identified the *cis*-target genes that are located 500kb upstream and downstream of DE-lncRNA. A custom R script was used for the same. The functional annotation of *cis*-target genes was done by Gene Ontology (GO) enrichment analysis using g: Profiler (Raudvere *et al*., 2019) [17]. GO terms and KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways with p-values < 0.05 were considered to be significantly enriched. GO terms related to the immune process were selected to filter the immune genes associated with the study. Finally, these immune genes that were found common in the DEGs list from mRNA analysis were considered for GO enrichment analysis related to the immune system process and interaction network formation. The network was constructed using Cytoscape v3.10.1 (Shannon *et al*., 2003) [18].

Results and Discussion

A total of 512 lncRNAs and 813 mRNAs were identified to be significantly (based on $\log_2$FC of ±1 and $p<0.05$) and differentially expressed in PPRV-vaccinated sheep monocytes ($CD14^+$) in comparison to the control group. The volcano plots in Figures. 1a and 1b show the number of up (103) and downregulated (409) DElncRNAs and the number of up (392) and downregulated (421) DEGs in the particular cell type. To reduce the number of differentially expressed lncRNAs and mRNAs, a threshold value of $\log_2$FC of ±2 and $p<0.05$ were considered to obtain significant molecules i.e. 82 up and 198 down regulated DE-lncRNAs and 133 up and 157 down regulated DE-mRNAs. A total 3418 number of *cis*-target genes in sheep $CD14^+$ cell subset was predicted, classified, and described according to biological processes (BP), cell composition (CC), and molecular functions (MF). Figure 2 shows gene ontology (GO) classification results of the differentially expressed lncRNAs target genes based on the top ten most significant (P-value <0.05) GO terms of BP, CC, and MF category at the 5th day of post-vaccination by PPRV vaccine strain. Biological processes like regulation of the biological process, regulation of the cellular process, response to stimulus, organo-nitrogen compound metabolic process, cellular response to stimulus, and multicellular organismal process were prominent in the cell subset used in this study. Not only BP, but other terms related to the CC and MF i. e. cytoplasm, membrane, cell periphery, cytosol, endomembrane system, vesicle, intracellular vesicle, etc., and protein binding and ligase activity were also predicted in the cell subset. Gene count of a particular GO term, mentioned at the end of each horizontal bar is the number of genes within our query that were annotated to the corresponding GO term.

Finally, significant processes and pathways related to the immune system process were chosen to get the immune genes involved in a particular process. A total of 515 immune process-related genes were involved in the sheep $CD14^+$ cell subset. We found 13 common genes between these immune genes and DEGs that were taken for further GO enrichment analysis to predict immune-related processes (Figure 3). Similar types of processes and pathways were activated in vaccinated pigs with the vaccine strain of classical swine fever virus (CSFV) (Pathak *et al*., 2017) [19] and porcine reproductive and respiratory syndrome virus (PRRSV) on 3rd day of vaccination (Islam *et al*., 2016) [20], chicken with Newcastle disease virus (NDV) on 4th dpv (Guo *et al*., 2021) [21]. In the vaccinated sheep monocytes ($CD14^+$), the *IL10* gene was found to be downregulated and the protein encoded by this gene is a cytokine, produced primarily by monocytes and to a lesser extent by lymphocytes. It protects the host from tissue damage during acute phases of immune responses. IL10 also enhances B cell survival, proliferation, and antibody production. This cytokine can block NF-kappa B activity and is involved in the regulation of the JAK-STAT signaling pathway. Our result is supported by the survey of both non-vaccinated and double-vaccinated individuals infected with the delta variant of SARS-CoV-2. The expression of *IL10* was decreased in COVID-19 patients irrespective of their vaccination status (Biswas *et al*., 2023) [22]. These 13 genes and their corresponding 13 lncRNAs involved in forming interaction networks between them (Figure 4).

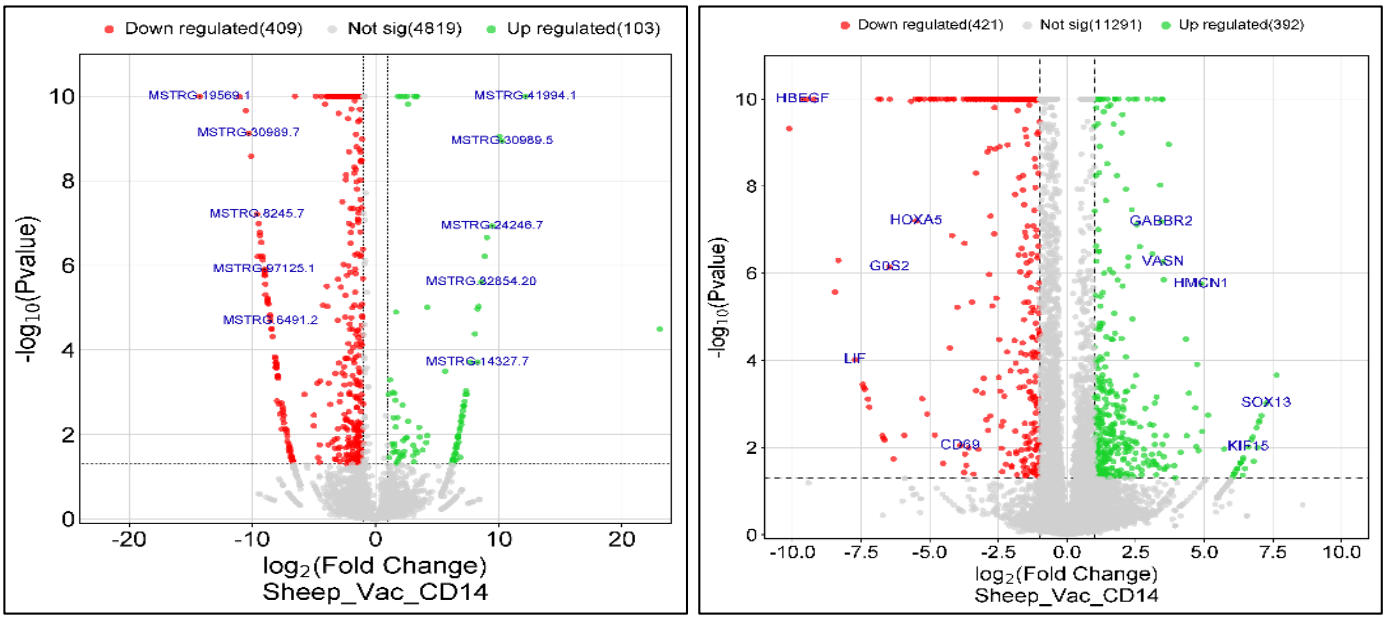

Fig 1: Volcano plot depicting significant (a) DE-lncRNAs and (b) DE-mRNAs in the CD14 subset of sheep vaccinated with PPRV (strain Sungri/96) based on based on $log_2FC > \pm 1$ and $p<0.05$

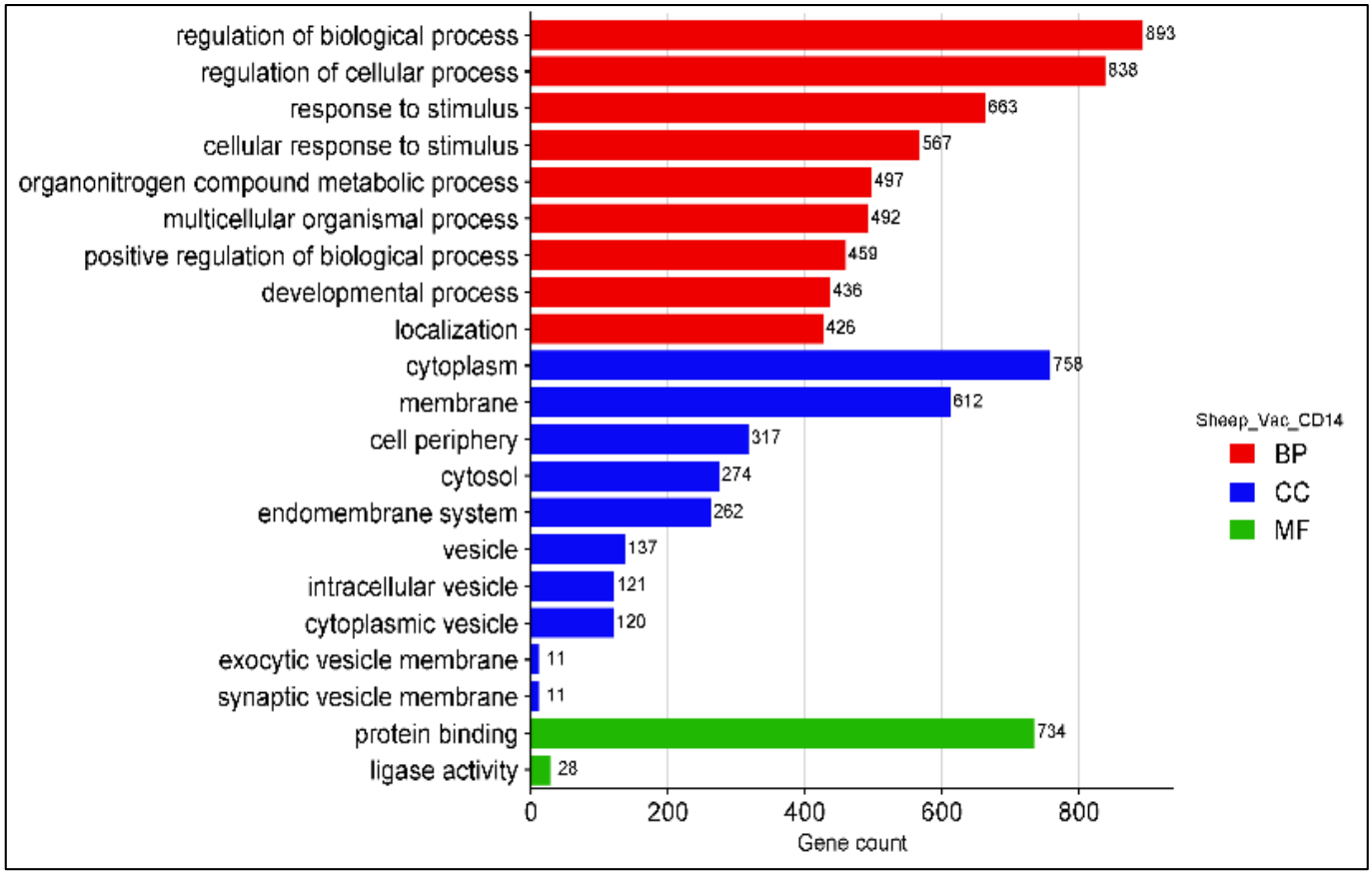

Fig 2: Gene Ontology analysis for *cis*-target genes of DE-lncRNAs of the CD14 subset in the sheep-vaccinated group.

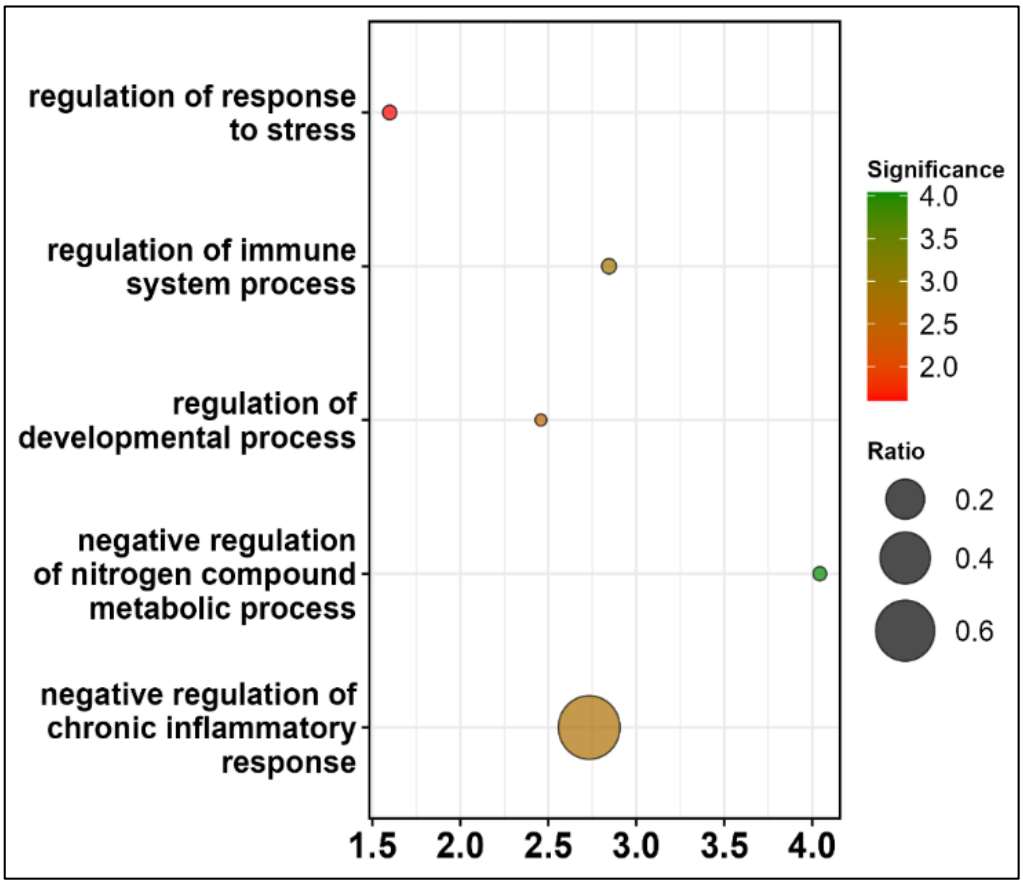

Fig 3: Gene ontology (GO) and pathway enrichment analysis for common genes (immune process-related *cis*-target genes found in DE-mRNAs) identified in the CD14 subset of vaccinated sheep. The green color and the red color represent the most and least significant processes and the size of the bubble denotes the gene involved in the process.

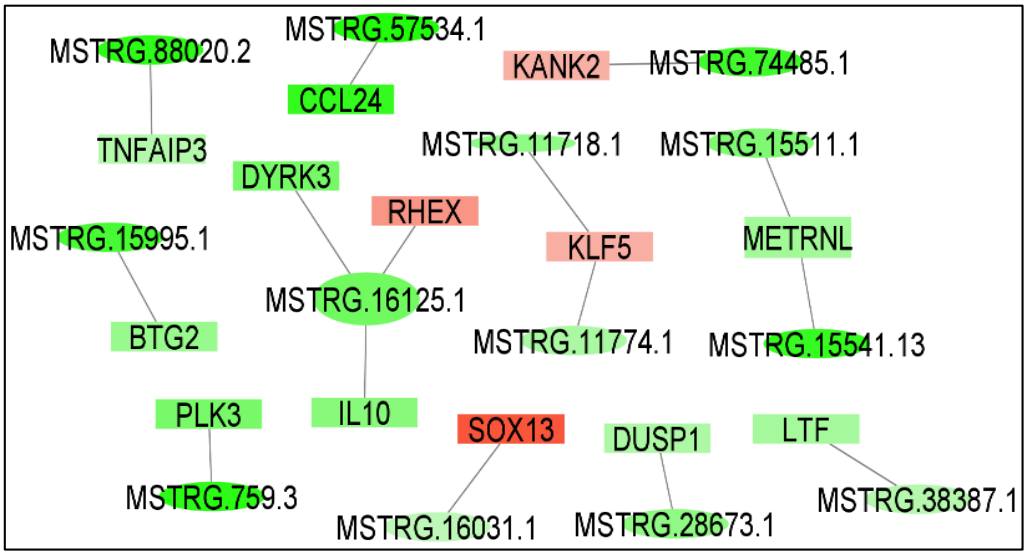

Fig 4: Interaction networks among common genes (immune process-related cis-target genes found in DE-mRNAs) and their corresponding DE-lncRNAs in the CD14 subset of vaccinated sheep. The elliptical nodes represent the differentially expressed lncRNAs, the rectangular nodes represent the co-expressed mRNAs. The green color and red color represent downregulated and upregulated molecules respectively.

References

1. Edo T, Deneke Y, Abdela N. Peste Des Petits Ruminants and its Economic Importance. Glob. Vet. 2017;18(4):256-268.
2. Gibbs EP, Taylor WP, Lawman MJ, *et al*. Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. Intervirology 1979;11(5):268-274.
3. Mornet P, Orue J, Gilbert Y, Thiery G, *et al*. Peste des Petits ruminants en Mrique occidentale francaise : ses rapports avec la Peste bovine. Rev. Elev. Med. Vet. Pays Trop. 1956;9:313-342.
4. Pope RA, Parida S, Bailey D, Brownlie J, Barrett T, *et al*. Early events following experimental infection with Peste-Des-Petits ruminants virus suggest immune cell targeting. PLoS One. 2013;8(2):e55830.
5. Njeumi F, Bailey D, Soula JJ, Diop B, Tekola BG. Eradicating the Scourge of Peste Des Petits Ruminants

from the World. Viruses. 2020;12(3):313.
6. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, *et al*. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 2012;22(9):1775-1789.
7. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data, 2010. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
8. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357.
9. Danecek P, Bonfield JK, Liddle J, Marshall J, *et al*. Twelve years of SAMtools and BCFtools. Gigascience 2021;10(2):giab008.
10. Dobin A, Davis CA, Schlesinger F, Drenkow J, *et al*. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15-21.
11. Pertea G, Pertea M. GFF utilities: gffRead and GffCompare. F1000Research. 2020, 9.
12. Pertea M, Pertea GM, Antonescu CM, Chang T, *et al*. StringTie enables improved reconstruction of transcriptome from RNA-seq reads. Nat. Biotechnol. 2015;33(3):290-295.
13. Trapnell C, Williams BA, Pertea G, Mortazavi A, *et al*. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 2010;28(5):511-515.
14. Patro R, Duggal G, Love MI, Irizarry RA, *et al*. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods. 2017;14(4):417-419.
15. Kang YJ, Yang DC, Kong L, Hou M, *et al*. CPC2: a fast and accurate coding potential calculator based on sequence intrinsic features. Nucleic Acids Res. 2017;45(W1):W12-W16.
16. Grabherr MG, Haas BJ, Yassour M, Levin JZ, *et al*. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011;29(7):644-652.
17. Raudvere U, Kolberg L, Kuzmin I, Arak T, *et al*. g: Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 2019;47(W1):W191-W198.
18. Shannon P, Markiel A, Ozier O, Baliga NS, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-2504.
19. Pathak SK, Kumar A, Bhuwana G, Sah V, *et al*. RNA Seq analysis for transcriptome profiling in response to classical swine fever vaccination in indigenous and crossbred pigs. Funct. Integr. Genomics. 2017;17(5):607-620.
20. Islam MA, Große-Brinkhaus C, Pröll MJ, Uddin MJ, *et al*. Deciphering transcriptome profiles of peripheral blood mononuclear cells in response to PRRSV vaccination in pigs. BMC Genomics. 2016;17(1):641.
21. Guo LX, Nie FR, Huang AQ, Wang RN, *et al*. Transcriptomic analysis of chicken immune response to infection of different doses of Newcastle disease vaccine. Gene. 2021;766:145077.
22. Biswas B, Roy S, Banerjee I, Jana S, *et al*. IL-10/IL-6 ratio from nasal and oral swab samples acts as an inflammatory indicator for COVID-19 patients infected with the delta variant. Heliyon. 2023;9(6):e16985.