Transcriptome profiles and novel lncRNA identification of *Aedes aegypti* cells in response to dengue virus serotype 1.

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Abstract

Dengue virus (DENV) is a single-stranded, positive-strand RNA virus that belongs to the family of *Flaviviridae,* and it is mainly transmitted by the mosquito *Aedes aegypti* (*Ae. aegypti*). Understanding the interaction of the virus with mosquito vector is vital for devising new strategies for preventing virus transmission. Although protein-coding genes have been the central focus, many reports indicated that long non-coding RNAs (lncRNAs) were also involved in virus-host interaction. Recently, the latest version of *Ae. aegypti* genome (AaegL5) was released, and the assembly was up to chromosome level. This prompted us to perform lncRNA identification and characterization using the latest genome release as reference. In this study, we investigated the transcriptome profiles of both protein-coding and lncRNA genes in *Aedes aegypti* cells upon DENV infection. By combining RNA-seq libraries generated in this study with publicly available datasets, we identified a total of 7,221 novel lncRNA transcripts, of which 3,052 and 3,620 were intronic and intergenic respectively, while 549 were antisense to the reference genes. A total of 2,435 differentially expressed transcripts, of which 956 of them were lncRNAs. Overall, the distribution of lncRNA expression and fold change upon virus infection were lower than that of protein-coding genes. We found that the expression of immune-related genes involved in IMD and MAPK signaling pathways were altered. In addition, the expression of major genes involved in RNA-interference (RNAi) pathway that confers antiviral resistance in mosquitoes were found to be unchanged upon DENV infection. Gene ontology analysis suggests that differentially expressed transcripts, either upregulated or downregulated, generally belong to the same functional categories or working in similar signaling pathways. Taken together, besides providing a new set of lncRNA repertoire, the outcomes of our study offer better understanding of *Ae. aegypti* responses to DENV infection at gene level.

Author Summary

Dengue virus (DENV), a single-stranded and positive-strand RNA virus of the family *Flaviviridae*, is primarily transmitted by *Aedes aegypti* (*Ae. aegypti*) mosquitoes. There are four closely related but antigenically different serotypes of dengue virus namely DENV1-4. Our understanding on the interaction of each serotype of DENV with its mosquito vector is still very limited. Since vector-borne viruses pose significant burden to public health, knowledge on the virus-host interaction at the molecular level is essential, especially in developing effective strategies to control virus transmission. In this study, we embarked on investigating the transcriptional response of long non-coding RNAs (lncRNAs) and protein-coding genes upon dengue virus serotype 1 (DENV1) infection. Besides, we also generate a comprehensive list of novel lncRNAs identified from the latest and improved genome version of *Ae. aegypti*. Similar to protein-coding genes, we discovered that the overall expression of lncRNA was significantly altered, suggesting that lncRNAs were involved in virus-host interaction. The results of this study provide basic understanding on the interaction between DENV1 and *Ae. aegypti* vector at the transcriptional level.

Introduction

Dengue virus (DENV) is a single-stranded, positive-strand RNA virus that belongs to the family of *Flaviviridae* [1]. There are four serotypes of dengue virus (DENV1-4), which are categorized based on the variation of antigens present on the viral particles [1]. Symptoms of the dengue infection include fever, headache, muscle, joint pains and rashes. In some cases, the disease develops into a life-threatening dengue hemorrhagic fever (DHF), which results in bleeding, low levels of blood platelets, and extremely low blood pressure [2] All four serotypes of DENV are transmitted by *Aedes aegypti* (*Ae. aegypti)* mosquito, which is the primary vector.

Ae. aegypti, a blood-sucking mosquito, is the principal vector responsible for replication and transmission of dengue virus. Due its pathological effects and mortality worldwide, this mosquito-borne virus is a serious threat to human health. Although virus infections of the vertebrate host are acute and often associated with disease, the maintenance of these pathogens in nature generally requires the establishment of a persistent non-lethal infection in the insect host. This is also true in the context of dengue virus infection. While dengue-infected humans show serious symptoms of illnesses, interestingly, infected *Ae. aegypti* mosquitoes are generally asymptomatic, suggesting that these vectors possess intrinsic antiviral immunity to resist or tolerate the virus infection

A number of studies have shown that, upon flavivirus infection, major transcriptomic changes occurred within *Ae. aegypti* [3–5]. Such changes involved a wide range of host genes including genes related to immunity, metabolic pathways, and trafficking. Beside protein-coding genes, non-coding RNA (ncRNA) including small and long ncRNA (lncRNA) of *Aedes* mosquitoes have been shown to undergo massive changes in transcription following virus infection [6,4].

Here, we aimed to investigate the transcriptome profiles in *Ae. aegypti* cell (Aag2) in response to DENV serotype 1 (DENV1) infection. Detailed characterization of transcriptomic changes in adult mosquitoes is convoluted by the complexity of different tissue types with unique niche and functions.

Therefore, we decided to use an immune-competent Aag2 cells in our study as it provided a more homogenous system composed of clonal cells of the same niche. To provide a global overview of transriptomic changes occuring within mosquito cells, we analyzed the expression of both protein-coding genes and lncRNAs. The latest version of *Ae. aegypti* (AaegL5) genome has been released [7], and the assembly is composed of chromosome-length scaffolds, which is more contiguous than the previous AaegL3 and AaegL4 assemblies [8]. We realized that previous lncRNA identification done in *Ae. aegypti* was based on AaegL3 assembly [6]; hence, this motivated us to perform re-annotation and identification of lncRNA using AaegL5 genome as reference. The results generated in this study provide better understanding of the mosquito-virus interaction at the molecular level, offering insights into devising strategies to inhibit viral replication within mosquitoes.

Identification of lncRNA

To perform lncRNA prediction, we used a total of 117 datasets of Illumina high-throughput sequencing derived from *Ae. aegypti* mosquito and Aag2 cell, a widely used *Ae. aegypti* derived cell line [9]. An overview of lncRNA identification pipeline can be found in Fig 1A. The pipeline developed in this study was adapted with few modifications from previous reports [6,10,11]. Briefly, each dataset (both paired-end and single-end) was individually mapped using HISAT2 [12]. The resulting alignment files were used for transcriptome assembly, and the assemblies were merged into a single unified transcriptome. Both transcriptome assembly and merging were performed using Stringtie [13]. Then, we used Gffcompare to annotate and compare the unified transcriptome assembly with reference annotation (AaegL5.1, VectorBase). We classified lncRNA transcripts based on their position relative to annotated genes derived from AaegL5.1 assembly (VectorBase). We only selected transcripts with class code “i”, “u”, and “x” that denote intronic, intergenic, and antisense to reference genes for downstream analysis.

To get confident lncRNA transcripts, we performed multiple steps of filtering transcripts having coding potential or open-reading frame (Fig 1A). The steps involved TransDecoder [14], CPAT [15] and finally BLASTX. Detailed description on the prediction analysis and parameter used can be found in Material and Methods section. Using this pipeline, we identified a set of 7,221 lncRNA transcript isoforms derived from 6,577 loci. Of these 7,221 lncRNA transcripts, 3,052 and 3,620 were intronic and intergenic respectively, while the remaining 549 transcripts were antisense to reference genes. Currently, AaegL5.1 annotation catalogs 4,155 lncRNA transcripts. Here, we provided another set of 7,221 lncRNA transcripts, making a total of 11,376 lncRNA in *Ae. aegypti*, which made up of 27.28% of the *Ae. aegypti* transcriptome.

Characterization of lncRNA

To examine whether lncRNAs identified in this study exhibit typical characteristics observed in other species [11,16–18], we analyzed features such as coding potential, sequence length, GC content and sequence conservation with closely related species. Since lncRNAs were strictly defined by their inability to code for protein, we determined coding probability of our newly identified lncRNAs and compared them with known lncRNA, 3'UTR, 5'UTR, and protein-coding mRNA. We discovered that, similar to other non-coding sequence such as known lncRNA, 3'UTR, and 5'UTR, our novel lncRNA transcripts have extremely low coding probability when compared to protein-coding mRNA (Fig 2A). Besides that, we found that both novel and known lncRNAs (provided by AaegL5.1 annotation) were shorter than protein-coding transcripts. Mean length of novel and known lncRNAs was 825.4 bp and 745.6 bp respectively, while protein-coding mRNA has an average length of 3330 bp.

Similar to previous reports [6,11], we observed that lncRNAs identified in this study had slightly lower GC content compared to protein-coding mRNAs (Fig 2C). For instances, mean GC content of novel lncRNA and mRNA was 40.1% and 46.4% respectively. Known lncRNA, on the other hand, had

relatively similar mean GC content to novel lncRNA (40.8%), while average GC of 5'UTR and 3'UTR sequence was 43.1% and 34.6% respectively. Overall, GC content of non-coding sequence was relatively lower than coding sequence.

To determine the *Ae. aegypti* lncRNAs that were highly conserved to other related insect species, we perform BLASTN against other insect genomes including *Ae. albopictus, C, quinquifasciatus*, *An, gambiae* and *D. melanogaster*. Similar to previous study [6], we applied the same BLASTN e-value cut-off (10-50) to identify highly conserved lncRNAs. By grouping these highly conserved lncRNAs, we found that most of them only shared high sequence similarity with *Ae. albopictus* (Fig 2D), suggesting that they were presumably genus specific. Although *Ae. aegypti* lncRNAs shared high sequence similarity with *Ae. albopictus*, compared to total lncRNAs, the fraction of conserved lncRNAs was relatively lower. For example, only 11% (1,258 out of 11,376 lncRNAs) showed high sequence similarity with *Ae. albopictus*.

Identification of differentially expressed lncRNAs following DENV1 infection

We next assessed whether lncRNA transcripts in Aag2 cell undergo differential expression upon DENV1 infection. To answer this question, we generated paired-end RNA-seq libraries derived from triplicates of Aag2 cells infected with DENV1. To avoid bias in library size which affects fold change calculation, we performed differential expression analysis of protein-coding genes and lncRNAs together in a single transcriptome reference using Salmon v0.9 [19] followed by edgeR [20]. A total of 2,435 transcripts were differentially expressed (P-value < 0.05), of which 956 of them were lncRNAs, while the remaining 1,479 were protein-coding (Supplemental Table 3).

In general, we found that across all six samples (3 uninfected and 3 DENV1-infected), expression of lncRNA was lower than protein-coding mRNAs (Fig 3A). Besides, distribution of fold change (FC) of differentially expressed lncRNAs (P-value < 0.05) were much lower than that of mRNAs (Fig 3),

suggesting that unlike mRNA, lncRNA expression was less responsive to viral infection. For example, log2 FC of mRNA can extend to more than 10 fold in either direction (-10 and 10 fold), but in lncRNA, log2 FC ranged from -3 to 5. Beside, most of the differentially expressed lncRNAs (952 out of 956) transcripts had log2 FC of less than 2 in either direction.

Differential expression of protein-coding genes

We next analyzed the protein-coding transcriptome of DENV1-infected Aag2 cells. A total of 1,479 transcripts that derived from 1,272 genes were found to be differentially expressed (|log2 FC| > 0, P-value < 0.05). In subsequent analysis of protein-coding transcriptome, we only focused on transcripts having log2 FC of more or less than 1. The number of upregulated transcripts of more than one fold log2 FC was 141. Meanwhile, the number of downregulated transcripts having less than -1 log2 FC was 167.

Previous studies highlighted the importance of immune-related genes upon infection with bacteria and viruses. Thus, we examined our differentially expressed transcripts for the presence of immune-related genes. We found that key players of immunodeficiency (IMD) pathway such as NF-kappa B Relish-like transcription factor (AAEL007624-RB) and I kappa B kinase gamma-subunit (AAEL005079-RB) were downregulated [21]. Furthermore, transcript encoding defensin anti-microbial peptide (AAEL003857-RA) was also downregulated. Meanwhile, Clip-domain serine protease (AAEL002601-RA) that functions in insect innate immunity including Toll receptor activation [22] was found to be upregulated. We also found that several components of MAPK signaling pathway [23], an important pathway in immunity, were both upregulated and downregulated. For example, MAPKKK5 (AAEL008306-RG), GTP-binding protein alpha (O) subunit, gnao, (AAEL008641-RE, AAEL008641-RK), rho GTPase activator (AAEL009157-RE), eph receptor tyrosine kinase (AAEL010711-RE), and regulator of G protein signaling (AAEL010676-RB) were discovered to be upregulated. On the other

hand, GTP-binding protein alpha subunit, gna, (AAEL008630-RD) and MAPKKK (AAEL010835-RC) were downregulated.

Beside immune-related genes, we also looked at the expression of key genes in RNA-interference (RNAi) pathways. It has been reported that one of the most important immune responses against viruses known in insects is the RNAi system that make use of three major conserved small RNAs – microRNA (miRNA), small-interfering RNA (siRNA) and PIWI-interacting RNA (piRNA) [24–26]. These small RNA pathways require the binding of Argonaute/Piwi (AGO/PIWI) proteins to execute their functions [24]. We discovered that transcripts encoding AGO/PIWI proteins were not represented in our differentially expressed transcript list. To validate this observation, we performed qRT-PCR on *AGO/PIWI* genes using primers from previous studies [25]. In agreement with our RNA-seq data, qRT-PCR result showed that the expression of *AGO/PIWI* genes were largely unaffected upon DENV1 infection (Fig 4).

We next asked if there was any correlation between differentially expressed lncRNAs and protein coding genes. Co-expression analyses of lncRNAs and their adjacent protein-coding genes have been reported to provide insights into the potential roles of lncRNAs in various biological processes [27,28]. Therefore, in this study, we determined the relative distance of differentially expressed lncRNAs (956 transcripts) with the nearest protein-coding genes *in cis*, resulting in a total of 1409 lncRNA-mRNA pairs formed by a total of 1156 and 956 transcripts of mRNAs and lncRNAs respectively. We found that 68% of these pairs were located within 10kb upstream and downstream from each other. However, only 39 mRNA transcripts within this 10kb range underwent differential expression upon differential expression.

Gene ontology analysis

To investigate the gene ontology (GO) associated with the differentially expressed transcripts, we submitted both the downregulated and upregulated transcripts (|log2FC|>1) to Blast2GO for GO

analysis [29]. In GO analysis of upregulated transcripts, Blast2GO recognized 38, 31, 29 GO terms in biological process, molecular functions and cellular components respectively. Meanwhile, in downregulated transcripts, Blast2GO identified GO terms of 61 in biological process, 33 in molecular function, and 24 in cellular components. We found that in biological process category, similar GO terms such as signal transduction (GO:0007165), cellular process (GO:0009987), and cellular protein modification process (GO:0006464) were represented in both upregulated and downregulated GO analysis (Fig 5). In fact, transcripts that could be assigned to those GO terms were among the largest in number.

Similar observation could be found in other GO categories namely molecular function and cellular component. For example, in molecular function category, similar GO terms such as binding (GO:0005488), protein-binding (GO:0005515), metal ion binding (GO:0046872) were represented in both upregulated and downregulated analysis (Fig 5). Meanwhile in cellular component category, in both cases, GO terms such as membrane (GO:0016020) and cytoplasm (GO:0005737) were among the largest (Fig 5).

This observation led us to speculate that, upon DENV1 infection, the differentially expressed transcripts, either upregulated or downregulated, generally perform relatively similar function inside the cells. Therefore, we then searched GO IDs that overlapped in both upregulated and downregulated transcripts based on GO categories namely biological process, molecular function and cellular component. We found that most GO IDs in each category were overlapped in both upregulated and downregulated data (Fig 6), suggesting that upon DENV1, genes with relatively similar functional categories or working in similar signaling pathways experienced changes in expression.

Discussion

The field of lncRNA has become increasingly important in many areas of biology particularly infectious disease, immunity, and pathogenesis [6,11,17,30]. High-throughput sequencing combined with bioinformatics enable scientists to uncover comprehensive repertoire of lncRNA in many species. Here, we present a comprehensive lncRNA annotation using the latest genome reference of *Ae. aegypti* (AaegL5). Previous study [6] reported that a total of 3,482 intergenic lncRNA (lincRNA) was identified in *Ae. aegypti*. However, the identification was performed using previous version of *Ae. aegypti* genome (AaegL3), and only lncRNAs residing in intergenic region were retained.

Due to the recent release of *Ae. aegypti* genome (AaegL5) equipped with dramatically improved gene set annotation [7], we decided to perform lncRNA identification using this latest genome reference. Unlike previous study on *Ae. aegypti* lincRNA, we also annotated lncRNAs residing within the introns (intronic lncRNA) and antisense to reference genes (antisense lncRNA), resulting in the identification of 3,052 and 549 intronic and antisense lncRNA respectively. A total of 7,221 lncRNA transcripts were identified in this study, which was more than previously reported lncRNAs in *Ae. aegypti.* This observation was presumably due to the high-quality assembly of AaegL5 genome and larger RNA-seq datasets with high sequencing depth being used in lncRNA prediction pipeline.

Similar to previous reports, we discovered that lncRNAs identified in our study exhibited typical characteristics of lncRNAs found in other species including vertebrates [10]. Such characteristics are lower GC content, shorter in length, and low sequence conservation even among closely related species. Upon DENV1 infection, we observed that both protein-coding and lncRNAs experienced overall changes in expression level. However, the overall level of fold change displayed by lncRNAs was not as high as protein-coding genes. This observation however, did not necessarily negate the possibility of lncRNAs having potential roles in immunity. For example, RNAi-mediated knockdown of one lncRNA candidate

(lincRNA_1317) in *Ae. aegypti* resulted in higher viral replication [6]. Therefore, intensive investigation using loss or gain of function approach on lncRNAs is crucial in dissecting their functions in mosquito-virus interaction.

Transcriptome analysis of protein-coding genes showed that cellular response to DENV infection involved numerous pathways and functional gene classes, suggesting that virus infection resulted in substantial changes on cell physiology. Many viruses including DENV suppress immune signaling pathways to ensure the success of their replication. For instance, infection of *Ae. aegypti* mosquitoes with Sindbis virus (SINV) resulted in the inhibition of Toll signaling pathway [31]. Likewise, infection of mosquito cells with Semliki Forest virus (SFV) led to the reduced activation of Toll, JAK-STAT, and IMD pathways [32]. In agreement with previous studies done in *Ae. aegypti* [33], we observed that the expression level of several genes implicated in immunity was simultaneously up- and down-regulated upon DENV1 infection. For example, core components of IMD signaling pathways such as NF-kB and I kappa B kinase gamma-subunit were transcriptionally repressed.

The IMD pathway plays important role in immunity by promoting the production of anti-microbial peptides (AMP) such as defensin and cecropin [21]. Due to reduced expression of NF-kB, we discovered that the expression of defensin in DENV1-infected cell was also reduced. On the other hand, genes involved in Toll and MAPK signaling, both of which are intimately connected to JAK-STAT pathways [23], experienced up- and down-regulation upon infection with DENV1 in Aag2 cells. This simultaneous up- and down-regulation of immune-related genes that function in similar signaling pathways suggest that there is a physiological arm race in survival between host cell and DENV1.

In summary, we provided a comprehensive list of lncRNA in *Ae. aegypti* using the latest genome version. We then explored the transcriptome of both lncRNA and protein-coding genes upon infection with DENV1. Results generated in this study provide a global picture of transcriptional response in *Ae. aegypti* cell during virus infection; thereby offering a fundamental understanding of virus-host interaction.

Materials and Methods

Cell culture and virus

Ae. aegypti Aag2 cell and *Ae. albopictus* C6/36 cell (ATCC: CRL-1660) were cultured in Leibovitz's L-15 medium (Gibco, 41300039), supplemented with 10% Fetal Bovine Serum (FBS, Gibco, 10270) and 10% Tryptose Phosphate Broth (TPB) solution (Sigma, T9157). Both Aag2 and C6/36 cells were incubated at 25ºC without CO_2. Vero E6 cells (ATCC:CRL-1586) were cultured at 37ºC in Dulbecco's modified Eagles Medium (DMEM, Gibco, 11995065) supplemented with 10% FBS (Gibco, 10270), and 5% CO_2. DENV1, Hawaiian strain was propagated in C6/36 cells and titered using Vero E6 cells. Determination of DENV1 titer was done using 50% tissue culture infectious dose – cytopathic effect (TCID50-CPE) as previously described [34]. DENV1 used in this study was a gift from Dr. David Perera, University Malaysia Sarawak. Aag2 cell line was a kind gift from Dr. Ronald P. Van Rij from Radbound Institute for Molecular Life Sciences, Netherlands.

Virus infection, RNA isolation and sequencing

Aag2 cells were infected with DENV1 at multiplicity of infection (MOI) of 0.5. At 72 hours post infection (hpi), the culture medium was removed, and RNA isolation was carried out using miRNeasy Mini Kit 50 (Qiagen, 217004) according to the manufacturer's protocol. Total RNA was then subjected to next-generation sequencing. The RNA-sequencing libraries were prepared using standard Illumina protocols and sequenced using HiSeq2500 platform generating paired-end reads of 150 in size.

Verification of DENV1 infection

Total RNA of both uninfected and DENV1-infected samples were subjected to cDNA synthesis using Tetro cDNA synthesis kit (Bioline, BIO-65042). Reverse primer for DENV1 was included in the reaction

310 set-up. This followed with PCR and gel electrophoresis using forward and reverse primers of DENV1.
311 Primers used for DENV1 verification were taken from previous study [35].
312
313 **RNA-seq data preparation**
314 Publicly available datasets were downloaded from NCBI Sequence Reads Archive (SRA) and
315 ArrayExpress with accession numbers SRP041845, SRP047470, SRP046160, SRP115939, E-MTAB-
316 1635, SRP035216, SRP065731, SRP065119, SRA048559, PRJEB13078 [4,36–41]. Adapters were
317 removed using Trimmomatic version 0.38 [], and reads with average quality score (Phred Score) above
318 20 were retained for downstream analysis.
319
320 **lncRNA identification**
321 Each library (both paired-end and single-end) was individually mapped against *Ae. aegypti* genome
322 (AaegL5) using HISAT2 version 2.1.0 [12]. The resulting alignment files were used as input for
323 transcript assembly using Stringtie version 1.3.2 [13]. We used reference annotation file of AaegL5
324 (VectorBase) to guide the assembly only without discarding the assembly of novel transcripts. Here, we
325 set the minimum assembled transcript length to be 200 bp. Then, the output gtf files were merged into a
326 single unified transcriptome using Stringtie merge [13]. Only input transcripts of more than 1 FPKM and
327 TPM were included in the merging. Then, we compared the assembled unified transcript to a reference
328 annotation of AaegL5 (VectorBase) using Gffcompare (https://github.com/gpertea/gffcompare). For the
329 purpose of lncRNA prediction, we only retained transcripts with class code “i”, “u”, and “x”. The
330 transcripts were then subjected to coding potential prediction. We used TransDecoder [14] to identify
331 transcripts having open-reading frame (ORF), and those having ORF were discarded. The remaining
332 transcripts were then subjected to a coding potential assessment toll (CPAT) [15]. We set the same cut-
333 off as previous study in *Ae. aegypti* which is less than 0.3 [6]. Transcripts having coding potential more

than 0.3 were discarded. To exclude false positive prediction, we used BLASTX against Swissprot database, and transcripts having E-value of less than 10-5 were removed.

Identification of differentially expressed transcripts

We used Salmon version 0.10.1 [19] to quantify the expression of transcripts. Read count for each transcript was subjected for differential analysis using edgeR [20] in R/Bioconductor environment.

Quantitative real-time PCR (qRT-PCR)

cDNA synthesis was done using QuantiNova Reverse Transcription Kit (Qiagen) followed by qRT-PCR with QuantiNova SYBR Green PCR kit (Qiagen) according to manufacturer's protocol. *RPS17* was used as housekeeping gene [42], and the experiment was performed using Applied Biosystems Step One PlusTM Real-Time PCR System.

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Figure Legend

Fig 1 lncRNA identification

(A) Overview of lncRNA identification pipeline. (B) Overview number of transcripts in *Ae. aegypti*

Fig 2 Characterization of *Ae aegypti* lncRNA

(A) Coding probability of lncRNA, 3'UTR, 5'UTR and mRNA. (B) Sequence length distribution of lncRNA and mRNA. (C) GC content. (D) Venn Diagram showing lncRNA transcripts that share high sequence similarity with other insect species (BLASTN cut-off < 10-50).

Fig 3 Differentially expressed lncRNA

(A) Overall expression of lncRNA and protein-coding mRNA. (B) Volcano plot of lncRNA. (C) Violin plot of log2 FC distribution. (D) Distribution of distance of differentially expressed lncRNA to neighboring protein-coding genes.

Fig 4 qRT-PCR of *AGO/PIWI* genes

qRT-PCR analysis of *AGO/PIWI* genes in uninfected and DENV1-infected in Aag2 cells. Error bars represent standard error of the mean across three replicates of uninfected and DENV1-infected samples.

Fig 5 GO of upregulated and downregulated transcripts

Fig 6 Shared GO IDs found in both upregulated and downregulated genes

List of Supporting Informations

S1 Table. Genomic coordinates of lncRNA candidates identified in *Ae. aegypti*

S2 Table. List of differentially expressed lncRNA

S3 Table. List of differentially expressed protein-coding transcripts

S4 Table. List of GO IDs

S5 Table. List of primers used

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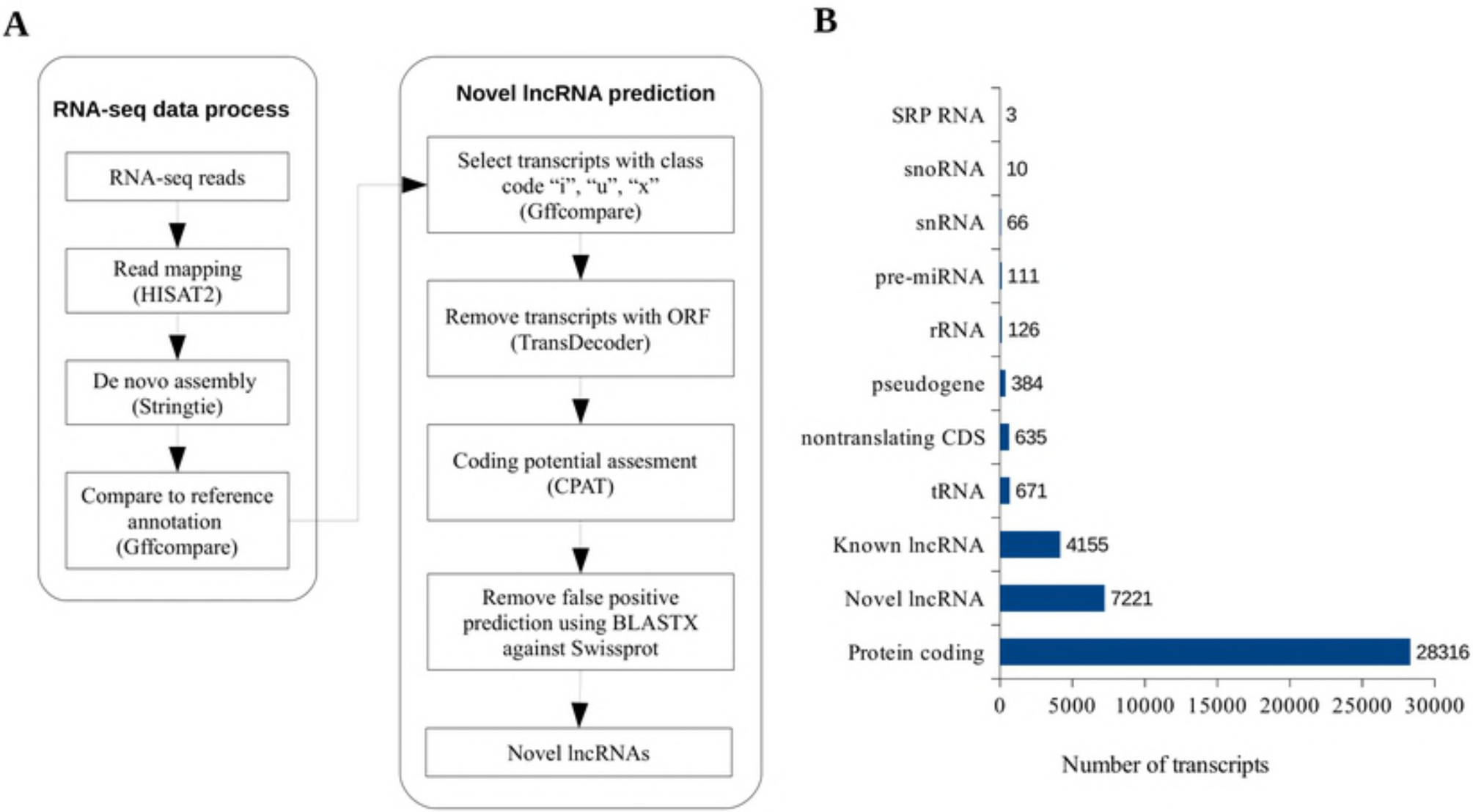
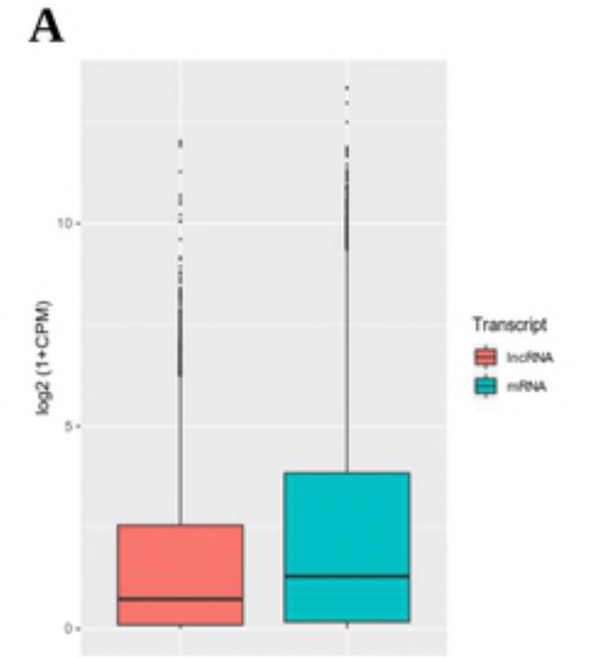
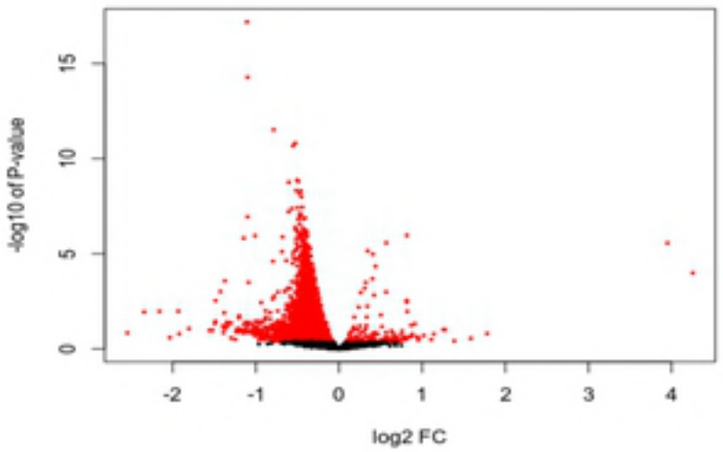
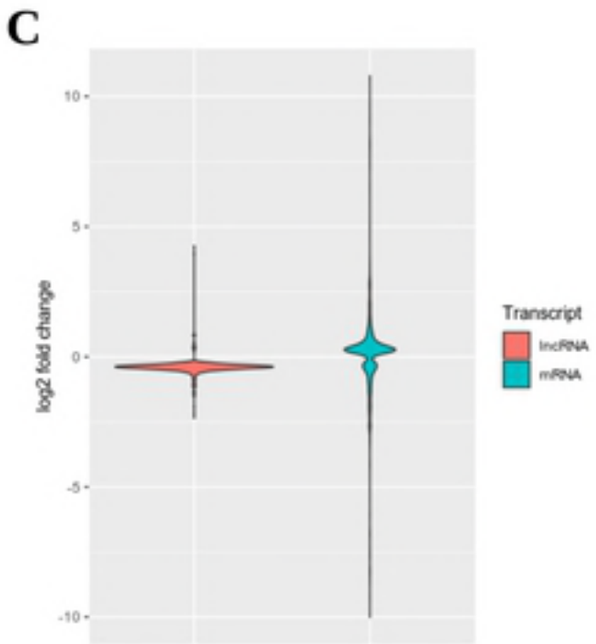
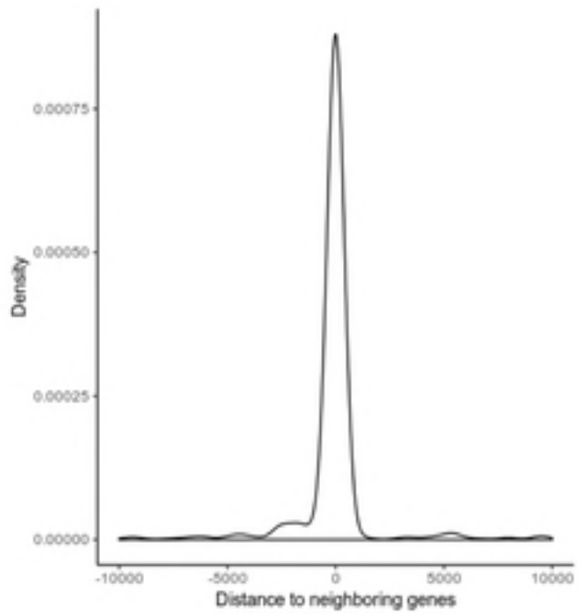
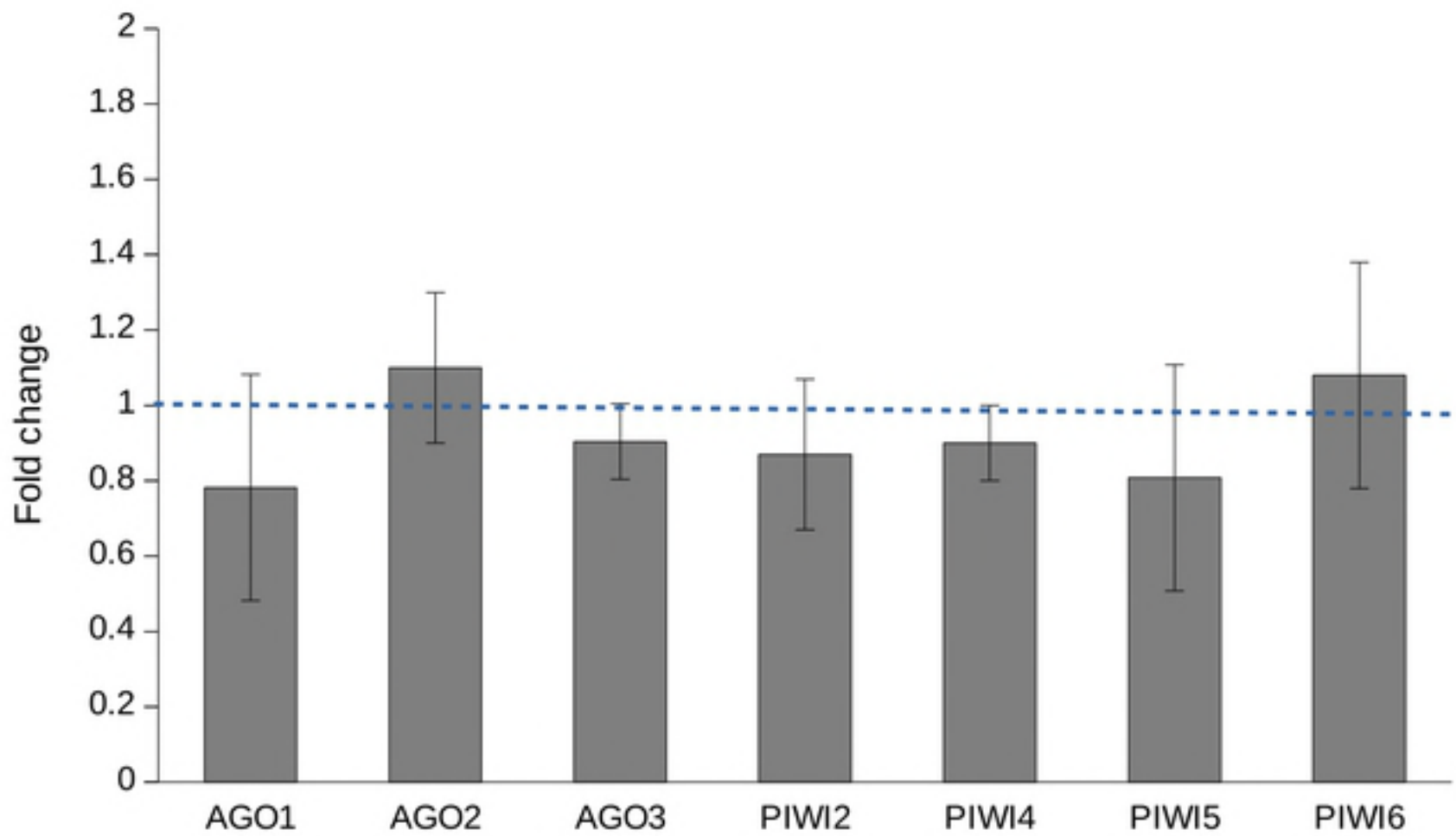

A
RNA-seq data process
RNA-seq reads
Read mapping (HISAT2)
De novo assembly (Stringtie)
Compare to reference annotation (Gffcompare)
Novel lncRNA prediction
Select transcripts with class code "i", "u", "x" (Gffcompare)
Remove transcripts with ORF (TransDecoder)
Coding potential assesment (CPAT)
Remove false positive prediction using BLASTX against Swissprot
Novel lncRNAs
B
SRP RNA 3
snoRNA 10
snRNA 66
pre-miRNA 111
rRNA 126
pseudogene 384
nontranslating CDS 635
tRNA 671
Known lncRNA 4155
Novel lncRNA 7221
Protein coding 28316
0 5000 10000 15000 20000 25000 30000
Number of transcripts

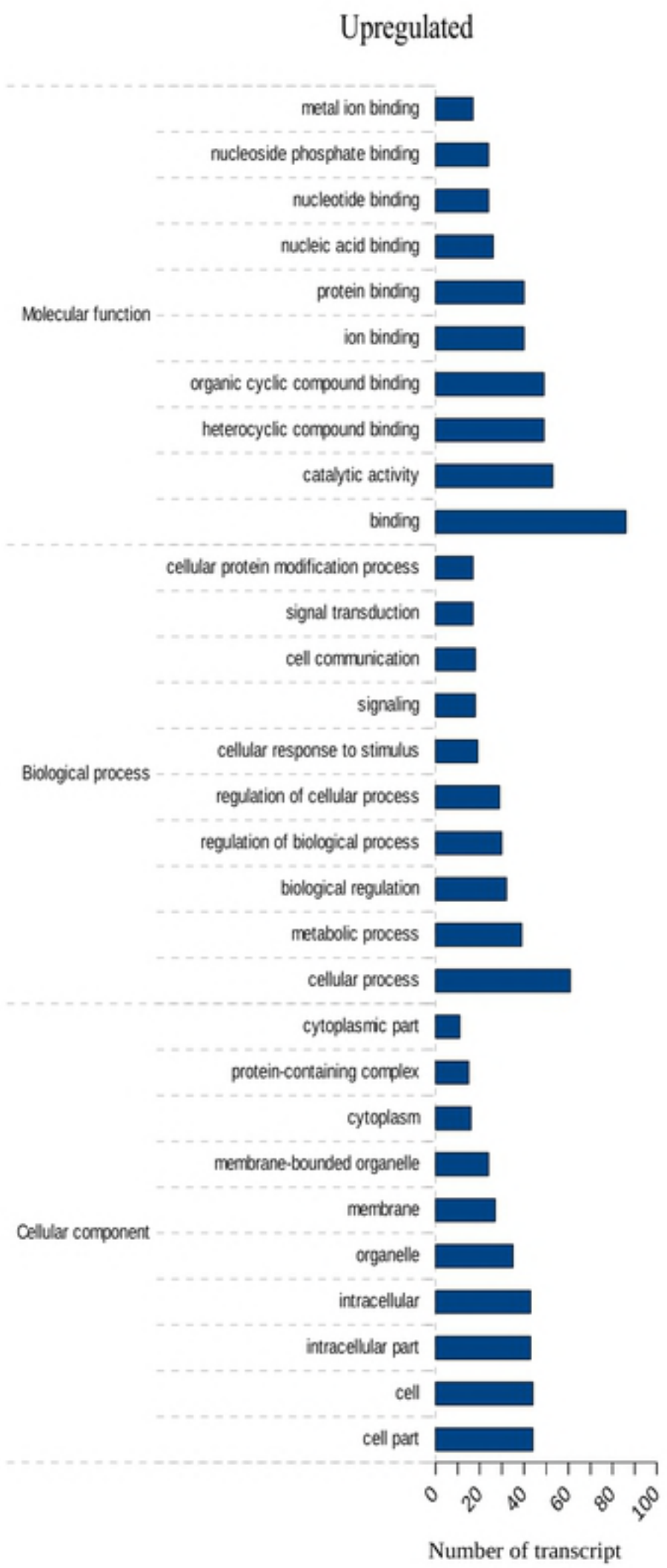

A
log2 (1+CPM)
10
5
0
Transcript
lncRNA
mRNA

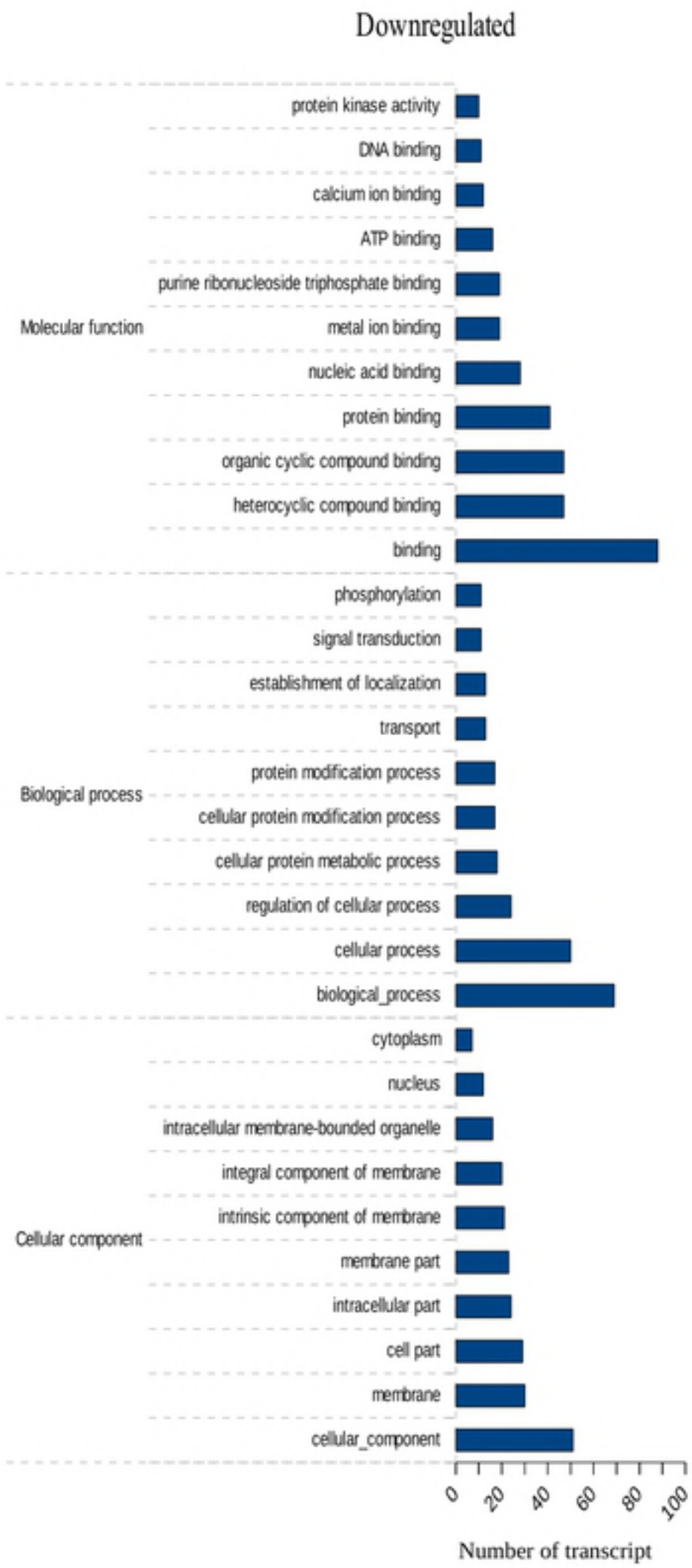

B
-log10 of P-value
15
10
5
0
-2
-1
0
1
2
3
4
log2 FC


C
log2 fold change
10
5
0
-5
-10
Transcript
lncRNA
mRNA


D
Density
0.00075
0.00050
0.00025
0.00000
-10000
-5000
0
5000
10000
Distance to neighboring genes


Fold change
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
AGO1
AGO2
AGO3
PIWI2
PIWI4
PIWI5
PIWI6


Upregulated
Molecular function
metal ion binding
nucleoside phosphate binding
nucleotide binding
nucleic acid binding
protein binding
ion binding
organic cyclic compound binding
heterocyclic compound binding
catalytic activity
binding
Biological process
cellular protein modification process
signal transduction
cell communication
signaling
cellular response to stimulus
regulation of cellular process
regulation of biological process
biological regulation
metabolic process
cellular process
Cellular component
cytoplasmic part
protein-containing complex
cytoplasm
membrane-bounded organelle
membrane
organelle
intracellular
intracellular part
cell
cell part
0
20
40
60
80
100
Number of transcript


Downregulated
Molecular function
protein kinase activity
DNA binding
calcium ion binding
ATP binding
purine ribonucleoside triphosphate binding
metal ion binding
nucleic acid binding
protein binding
organic cyclic compound binding
heterocyclic compound binding
binding
Biological process
phosphorylation
signal transduction
establishment of localization
transport
protein modification process
cellular protein modification process
cellular protein metabolic process
regulation of cellular process
cellular process
biological_process
Cellular component
cytoplasm
nucleus
intracellular membrane-bounded organelle
integral component of membrane
intrinsic component of membrane
membrane part
intracellular part
cell part
membrane
cellular_component
0
20
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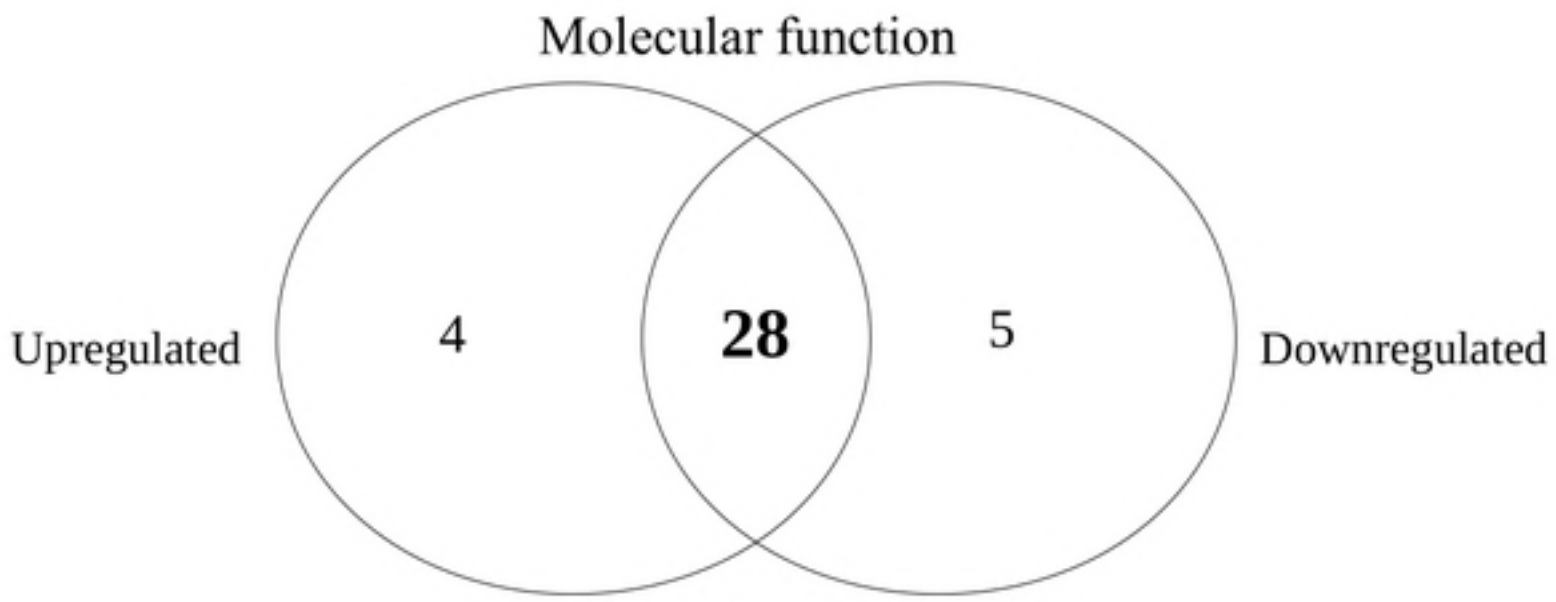
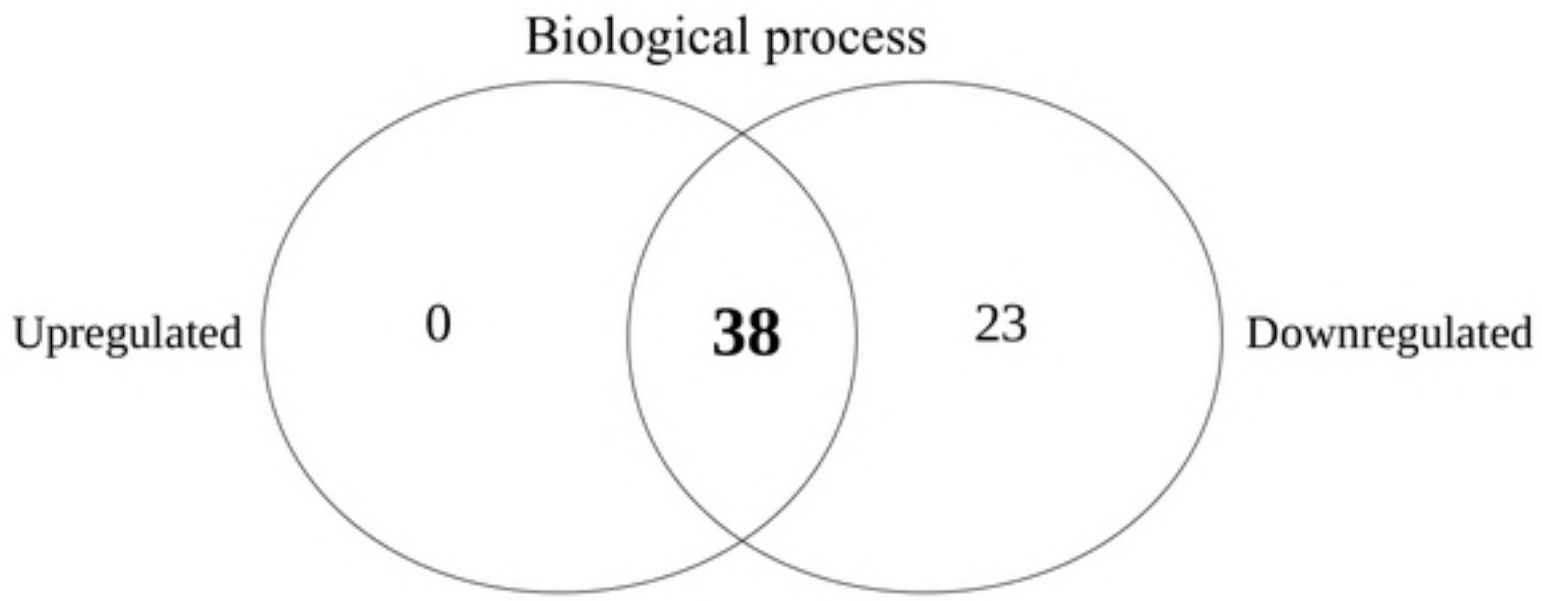
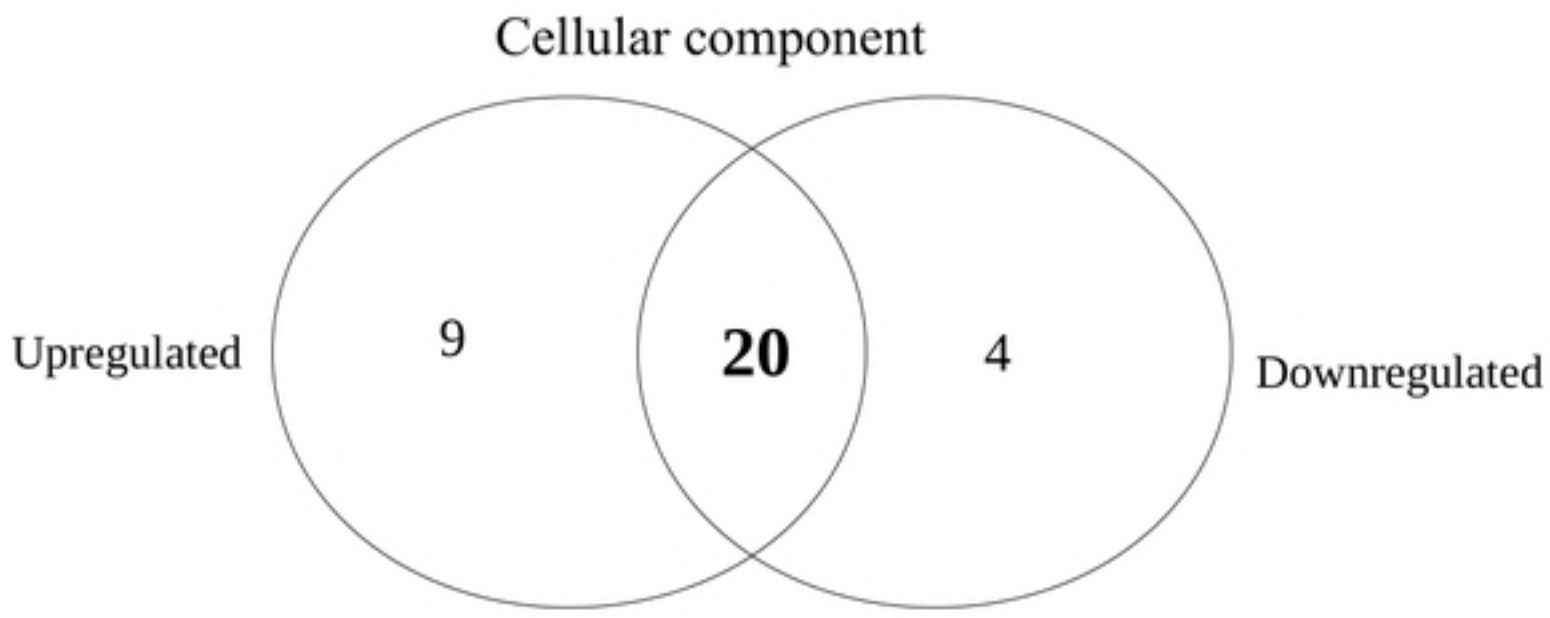
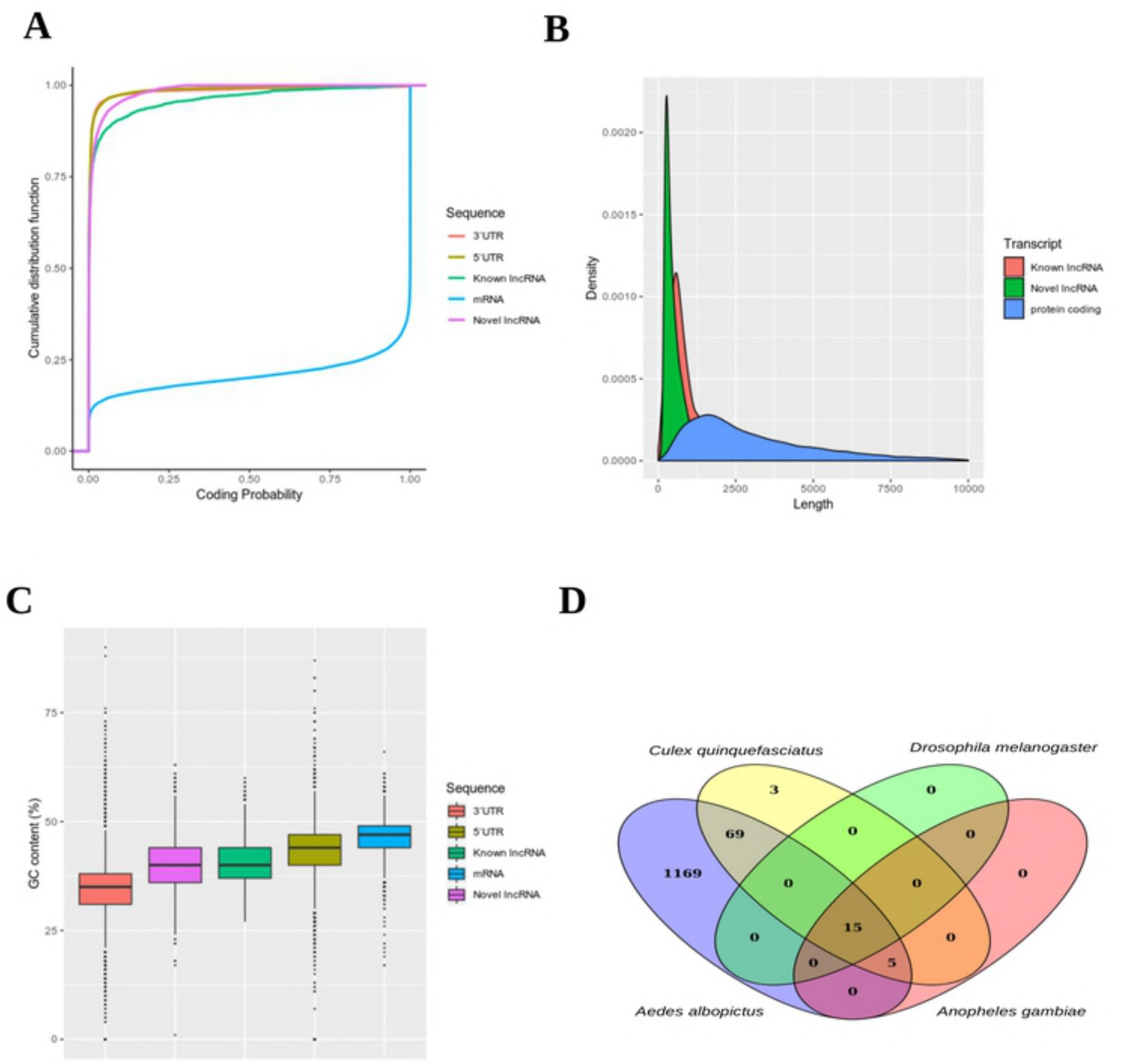
60
80
100
Number of transcript


Molecular function
Upregulated
4
28
5
Downregulated


Biological process
Upregulated
0
38
23
Downregulated


Cellular component
Upregulated
9
20
4
Downregulated


A
Cumulative distribution function
Coding Probability
Sequence
3'UTR
5'UTR
Known lncRNA
mRNA
Novel lncRNA
B
Density
Length
Transcript
Known lncRNA
Novel lncRNA
protein coding
C
GC content (%)
Sequence
3'UTR
5'UTR
Known lncRNA
mRNA
Novel lncRNA
D
Culex quinquefasciatus
Drosophila melanogaster
Aedes albopictus
Anopheles gambiae
3
0
69
0
0
1169
0
0
0
15
0
0
5
0