Original Article

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A two-component signal transduction system contributes to the virulence of *Riemerella anatipestifer*

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Similar to other studies of bacterial pathogens, current studies of the pathogenesis of *Riemerella anatipestifer* (RA) are focused mainly on *in vitro* culture conditions. To elucidate further the pathogenesis of RA *in vivo*, bacterial RNA was extracted from overnight tryptic soy broth cultures (*in vitro*) and from the blood of infected ducks (*in vivo*) for comparative RNA sequencing analysis. In total, 682 upregulated genes were identified *in vivo*. Among the upregulated genes, a signal transduction response regulator (*ArsR*) and a signal transduction histidine kinase (*SthK*) were predicted to be located on the same operon. A mutant was constructed by deletion of both of these genes. Duck infection tests showed that genes *ArsR* and *SthK* were related to the virulence of the pathogen *in vivo*. Differentially expressed genes identified by comparison of *in vitro* and *in vivo* conditions provided an insight into the physiological process of RA infection and provided an opportunity to identify additional virulence factors.

Keywords: *Riemerella anatipestifer*, comparative RNA sequencing, pathogenesis, virulence factors

Introduction

Riemerella anatipestifer (RA), once considered to be a member of the *Pasteurella* family, is now classified as a member of the *Flavobacteriaceae* family based on 16S ribosomal RNA (rRNA) gene analysis [29]. This Gram-negative bacterium causes septicemia and death in young ducks, geese, and other poultry worldwide [15,23].

Continuous culture of bacterial pathogens *in vitro* can result in decreased virulence. In most cases, virulence is recovered in infection of sensitive hosts indicating that this phenomenon is not due to changes in the bacterial DNA. Thus, it can be hypothesized that altered virulence can be caused by transcriptional and translational changes in virulence factors.

To date, only five potential virulence factors have been reported for RA; outer membrane protein A (OmpA) [14], VapD [34], CAMP cohemolysin [4], TbdR1 [21] and siderophore-interacting protein (Sip) [32]. Among these, OmpA is an immunogenic protein involved in RA adherence [14]. The RA *VapD* gene shows homology with virulence-associated genes of other bacteria [34], and CAMP cohemolysin promotes the dissolution of red blood cells [4]. Sip significantly influences iron uptake and RA pathogenicity [32], while TbdR1 is involved in hemin and iron acquisition [21]. However, the molecular mechanisms involved in RA infection *in vivo* remain incompletely described.

Two-component signal transduction systems (TCSTs) are signal transduction mechanisms that exist widely in pathogenic bacteria. TCSTs respond to various environmental conditions and are composed of a sensor histidine kinase (HK) and a response regulator protein (RR) [35].

Recent research on RA has been limited to the *in vitro* investigation of the bacteria, while the processes involved in infection of ducks *in vivo* remain to be elucidated. It is conceivable that focusing on the growth and metabolism of RA in the host will be important not only in identifying new potential virulence factors but also in clarifying the pathogenic mechanisms of infection.

In this study, we used RNA sequencing (RNA-seq) as a sensitive and rapid method to determine transcriptional patterns and the sequence of the entire transcriptome [6] by comparing the total RNA obtained from bacteria grown both *in vitro* and *in vivo*. These results will provide insights into the mechanism underlying the pathogenesis of RA under *in vivo* conditions.

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Materials and Methods

Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study are summarized in Table 1 [33]. The serotype 2 RA strain RAf_{153} was isolated from an infected domestic duck in Fujian Province, China. RA strains were cultured in tryptic soy broth (TSB; Difco Laboratories, USA) in a shaking incubator (180 rpm) at 37°C. *E. coli* strains X7213, X7213-pRE112 [10] and X7213-*LSR*-pRE112 were grown at 37°C in Luria Bertani broth (LB) or plates supplemented with 50 µg/mL diaminopimelic acid (DAP). When necessary, the medium was supplemented with an appropriate concentration of antibiotics.

In vitro culture preparation

Strain RAf_{153} was inoculated into 5 mL TSB and cultured overnight. Bacteria (500 µL) were then transferred to 50 mL TSB and cultured to the logarithmic phase (optical density at 600 nm [OD_{600}] 0.7–0.8). *In vitro*-cultured bacteria were harvested by centrifugation at 10,000 × g for 10 min at 4°C and washed once with phosphate buffered saline (PBS). Three biological replicates were prepared.

Establishment of duck RA infection model

For virulence recovery, RAf_{153} were streaked onto tryptic soy agar (TSA) containing 7% sheep blood and cultured at 37°C for 24 h. Single colonies of RAf_{153} were picked, inoculated into TSB, and cultured to the logarithmic phase (OD_{600} 0.7–0.8). The bacteria were then harvested and washed with PBS three times before use in the *in vivo* challenge. The Cherry Valley ducks used in this study were purchased from a local duck farm. Cherry Valley ducks (aged 10 days) were injected intramuscularly with RAf_{153} at a dose of 1.5×10^9 colony-forming unit (CFU). After the appearance of symptoms such as lethargy, diarrhea, and tremor, infected ducks were humanely killed and the pathogen was isolated from heart and brain tissue. Considering the clinical signs of septicemia, *in vivo* conditions may provide a favorable environment for bacterial growth. The isolated bacteria were cultured *in vitro* and used to inject Cherry Valley ducks (aged 10 days) intravenously at a dose of 10^8 CFU. Infections were repeated in this way using doses of 10^7 CFU and 10^6 CFU. Eventually, we isolated the recovered virulence strain, $vRAf_{153}$.

In this study, a model of acute RA infection was established in 2-week-old ducks challenged with a dose of 10^7 CFU of $vRAf_{153}$ by plantar injection. Infected ducks showed symptoms such as lethargy, diarrhea, and tremor within 24 h. The levels of pathogens in the blood increased with exacerbation of the symptoms, with the highest levels reached immediately before death.

Identification and enrichment of *in vivo* cultured bacteria

RA-naïve ducks, according to indirect enzyme-linked immunosorbent assay results, were challenged on day 14 by plantar injection with a 0.2 mL inoculum (5×10^7 CFU/mL) of strain $vRAf_{153}$ (OD_{600} 0.7–0.8) [36]. The challenged ducks were closely monitored for 48 h post-infection. Cardiac blood (25–30 mL with added 3.8% trisodium citrate) was obtained before death. Giemsa staining and polymerase chain reaction (PCR) were used for detection and verification of RA.

Cardiac blood (20 µL) was streaked on TSA containing 7% sheep blood and cultured at 37°C for 24 h. Single colonies were picked and inoculated into TSB for PCR identification. The primers were designed according to the 16S rRNA sequence named 16S rRNA-F and 16S rRNA-R (Table 2).

In order to evaluate the concentration of bacteria in blood, cardiac blood was 10-fold series diluted with PBS and cultured on TSB agar at 37°C for 24 h. The number of bacteria were

Table 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Descriptions	Sources or references
Strains		
RAf_{153}	A serotype 2 strain isolated from an infected domestic duck in Fujian Province of China	Fujian Province (China)
$vRAf_{153}$	A virulent strain with the same genome as RAf_{153}	This study
ΔArsR-SthK	*ArsR* and *SthK* deletion mutant of $vRAf_{153}$ strain, Spc^R	This study
E. coli x7213	The host of recombinant plasmids	[33]
E. coli x7213-pRE112	The host of plasmid pRE112, Cm^R	[33]
E. coli x7213-*LSR*-pRE112	*E. coli* X7213 with recombinant plasmid *LSR*-pRE112, Spc^R, Cm^R	This study
Plasmids		
pSET4s	Thermosensitive suicide vector; Spc^R	Stored in our laboratory
pRE112	Suicide vector; Cm^R	[33]
LSR-pRE112	Recombinant vector with the background of pRE112, Spc^R, Cm^R	This study

Spc^R, spectinomycin resistance; *E. coli*, *Escherichia coli*; Cm^R, chloramphenicol resistance.

Table 2. Primers used in this research

Primers	Sequences (5′−3′)	Cleavage site
16S rRNA-F	TGAGACACGGACCAGA	
16S rRNA-R	TAACACCTCACGGCAC	
ST-L1	ACGGGTACCATCAAAGGTAGCTCCTGC	*Kpn I*
ST-L2	TCTCTTGCCAGTCACGTTACATCAGGCTCATCATCTATTAG	
Spc-1	TAATAGATGATGAGCCTGATGTAACGTGACTGGCA	
Spc-2	ATAAAACTCGCTCCGTTTAAAAAAGTGTTTCCACC	
ST-R1	AAAATGGTGGAAACACTTTT TTAAACGGAGCGAGTTTT	
ST-R2	CGAGCTCAACTCCGTTTGCTACAGG	*Sac I*
inST-1	GTTAGCGGGCTACCAAGC	
inST-2	CGCTCAAAAACACGAGGA	
inSpc-1	AACGTGACTGGCAAGAGA	
inSpc-2	TCTAATGGAGAAGATTCAGCC	
up-1	CAGAGTTCCCTTGGTCTACATATC	
up-2	GCACTATTGGGCTTAGGCTATAA	
down-1	GAAGCTGCCGAAGGAGATTAT	
down-2	GCTTACTTTACCCTCTTCTACCTTTA	

Underlined sequences denote restriction sites. F, forward; R, reverse.

counted.

The bacteria in the collected blood were extracted by differential centrifugation according to the method described by Tan [30] with modifications. Briefly, three blood samples were centrifuged first at 200 × g for 10 min at 4°C; then, at 1,000 × g for 1 min and 1,500 × g for 30 sec to remove blood cells. The supernatant was centrifuged at 8,000 × g to collect bacteria. The purity of the RA extracted from blood was examined by Giemsa staining.

The animal experiments were approved by the Ethical Committee for Animal Experiments of Nanjing Agricultural University (Nanjing, China), and complied with the guidelines of the Animal Welfare Council of China.

RNA extraction

Total RNA was extracted by using the SV Total RNA Isolation System (Promega, USA) according to the manufacturer's protocols. RNA purity and integrity were assessed by measuring the $A_{260/280}$ ratio and by 2% agarose gel electrophoresis. The RNA concentration was determined by using a NanoDrop2000 (Thermo Scientific, USA).

Six bacterial RNA samples comprising three biological replicates of both the *in vivo* and *in vitro* conditions were analyzed by RNA-seq at Guangzhou Genedenovo Biotechnology (China).

cDNA library construction and sequencing

The complementary DNA (cDNA) library construction and sequencing were performed by Guangzhou Genedenovo Biotechnology using a previously described protocol [19]. Briefly, the rRNA was removed, and the mRNA was extracted and fragmented by adding a fragmentation buffer. First-strand cDNA was then generated by randomly primed reverse transcription and the second-strand cDNA was synthesized using dNTPs, RNase H, and DNA polymerase I. The QIAquick PCR extraction kit (Qiagen, Germany) was used to purify the cDNA fragments. EB buffer was added for end reparation, poly(A) addition, and addition of sequencing adapters. After agarose gel electrophoresis and isolation of cDNA, the cDNA library was constructed by performing PCR amplification with the cDNA fragments as templates. Finally, the library was sequenced by using Illumina HiSeq 2000 (Illumina, USA). All raw transcriptome data were deposited in the GenBank Short Read Archive (accession No. SRP099396; National Center for Biotechnology Information, USA).

RNA sequencing data processing

In terms of the information provided by Guangzhou Genedenovo Biotechnology, raw reads generated by the sequencer are stored in a fastq file format. Impurity data including adaptor reads, low quality reads, and reads that contain unknown bases account for more than 10%. Clean reads are obtained by removing the impurity data from raw reads, and comprised over 95% of the total number of reads. Processing included comparing the clean reads to the reference sequence and analyzing the distribution. Based on the results of the comparison, the corresponding reads of each transcript are then calculated and standardized. The RPKM method was used to calculate gene expression levels.

Functional annotation and enrichment analysis

Based on gene expression, the relationships between samples and genes were performed by performing hierarchical clustering, and heat maps were used to present the clustering results. The genes with similar expression patterns usually have functional correlation. The differentially expressed genes (DEGs) were used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses via the GO and KEGG databases, respectively. GO is an internationally standardized gene functional classification system that provides a dynamically updated controlled vocabulary to comprehensively describe the properties of genes and gene products in organisms. GO has three ontologies, describing molecular function, cellular components, and biological processes. *In vivo*, different genes coordinate and perform their biological functions, and analysis based on pathways will help to elucidate the biological function of genes. KEGG is the main public pathway database. Enrichment analysis of pathways is based on the KEGG pathway database, and the hypergeometric test is used to identify the pathway in which DEGs are significantly enriched.

Quantitative real-time PCR validation

RNA samples similar to those sent for RNA-seq analysis were reverse-transcribed into cDNA in a total reaction volume of 20 μL by using the Vazyme HiScript Q RT SuperMix (Vazyme, China) for qPCR (+gDNA wiper). The reactions were as follows: 25°C for 10 min, 50°C for 30 min, and 85°C for 5 min. Completed reaction products were collected and pooled before 20 μL aliquots were stored at −70°C. The cDNAs were diluted 1:50 before use in quantitative real-time PCR (qRT-PCR) analysis to confirm the accuracy of the RNA-seq data. Six DEGs identified by RNA-seq were selected as templates for the qRT-PCR assays. Using the cDNA from RAf_{153} cultured *in vivo* and *in vitro* as templates, three replicates were prepared for each reaction. Primers for qRT-PCR were designed by Primer Express software (ver. 3.0; Applied Biosystems, USA). The gene *gmk* (GI:874285954) was used as an internal control, and relative quantification was calculated based on the $2^{-\Delta\Delta Ct}$ method [20].

Construction of the mutant strain ΔArsR-SthK

Briefly, genomic DNA was isolated from $vRAf_{153}$ and vector pSET4s (containing the spectinomycin resistance cassette, spc^R cassette). Primers for the amplification of upstream (ST-L1, ST-L2) and downstream (ST-R1, ST-R2) fragments of the target gene and the spc^R cassette (Spc-1, Spc-2) are listed in Table 2. After amplification and purification, these three fragments were fused by overlap-extension PCR with the spc^R cassette located between the upstream and downstream fragments of the target gene.

E. coli X7213-pRE112 was cultured in LB medium containing 50 μg/mL DAP and 25 μg/mL chloramphenicol (Cm) and shaken overnight at 37°C. The pRE112 and fusion segment were digested with *Kpn I* and *Sac I* and ligated overnight at 16°C to generate the recombinant plasmid *LSR*-pRE112. Competent cell *E. coli* X7213 cells were transformed with *LSR*-pRE112 and cultured on LB plates supplemented with 50 μg/mL DAP at 37°C for more than 24 h. Single colonies were picked for sequencing and positive clones were identified as the donor strain, designated *E. coli* X7213-*LSR*-pRE112.

The recipient strain $vRAf_{153}$ was cultured in TSB and the donor strain was cultured in LB medium (supplemented with 50 μg/mL DAP, 50 μg/mL Spc, and 25 μg/mL Cm) until the culture reached an OD_{600} value of 0.6 to 0.8. Aliquots (5 mL) of the donor and receptor strains were centrifuged at 5,000 × g for 3 min and washed three times with 1 mL 10 mmol/L $MgSO_4$. After the third wash, the supernatant was removed and the pellets were combined. The mixed cultures were then incubated on a TSB plate containing 50 μg/mL DAP at 37°C for 10 h. The bacteria on the plate were washed with TSB and streaked onto a TSB plate containing Spc (50 μg/mL) to isolate the putative $vRAf_{153}$ mutants from the mixed-strains culture. Single colonies were selected from the plate and the candidate mutants were screened and identified.

Validation of gene-deleted strain ΔArsR-SthK

PCR amplification was performed with ΔArsR-SthK as a template and ST-L1 and ST-R2 as primers. The PCR products were extracted from 1% agarose gel and were sequenced by Guangzhou Genedenovo Biotechnology. The amplified fragments were blasted and aligned to the genome of RAf_{153} to make sure that the Spc^R cassette and genes *ArsR* and *Sthk* were replaced via allelic exchange.

Internal primers (inST-1 and inST-2 in Table2) of the deleted genes were designed to verify that the genes *ArsR* and *SthK* had been deleted. Primers inSpc-1 and inSpc-2 (Table 2) were used to confirm that the resistance gene has been inserted. Moreover, ΔArsR-SthK was further validated by primers 16S rRNA-F and 16S rRNA-R (Table 2).

In addition, according to the whole genome sequence published on GeneBank, the upstream and downstream genes of *ArsR* and *Sthk* were *CG09_1826* and *CG09_1830*. Internal detection primers (up-1, up-2 and down-1, down-2 listed in Table 2) were designed. The cDNA of ΔArsR-SthK and RAf_{153} were amplified to verify whether the deleted fragments affect the upstream and downstream genes.

Adhesion and invasion assays

Adhesion and invasion assays were performed to determine whether the deletion of *ArsR* and *SthK* could affect the adhesion and invasion abilities of RA. Briefly, Vero cells were seeded into 24-well plates and incubated to 95% confluence. Strains $vRAf_{153}$ and ΔArsR-SthK were cultured to mid-log phase (OD_{600} = 0.7–0.8) and Vero cells were infected at a multiplicity of infection of

50, centrifuged (800 × g for 10 min), and incubated at 37°C with 5 % CO_2 for 2 h. Cells were washed three times by PBS to remove non-adherent bacteria. For the adherence assay, cells were lysed by using sterile water. Cell lysates were 10-fold series diluted and spread on TSB agar plate to count the number of bacteria. For the invasion assay, extracellular bacteria were killed by adding DMEM containing 5 µg/mL penicillin and 100 µg/mL gentamicin and incubated for 1 h. Then, after three washes, cells were lysed and spread on TSB agar to determine the quantity of bacteria. Both assays mentioned above were performed in triplicate and repeated three times.

Determination of LD_{50} of $vRAf_{153}$ and ΔArsR-SthK

Five-day-old Cherry Valley ducklings were obtained from a local duck farm. After 2 days of acclimatization, the ducklings were divided into 11 groups (n = 6 per group). The $vRAf_{153}$ strain and the mutant strain ΔArsR-SthK were grown in TSB at 37°C in a shaker and harvested when the culture reached an OD_{600} value of 0.7 to 0.8. The cultures were then centrifuged at 6,000 × g for 2 min and resuspended in PBS at the appropriate densities. Ducks were injected intramuscularly with $vRAf_{153}$ or the deletion mutant strain at the doses listed in Table 3. The 50% lethal dose (LD_{50}) was calculated by using the Reed-Muench formula [24].

Statistical analysis

For RNA-seq results, the significance of differential gene expression was analyzed by using a threshold for the false discovery rate of ≤ 0.005 and an absolute value of the $\log_2$ ratio ≥ 1. For the qRT-PCR data analysis, a p value less than 0.05 was considered statistically significant.

Table 3. Results of LD_{50} evaluation

Strains	Dose of challenge (CFU)	Death/total	LD_{50} (CFU)
$vRAf_{153}$	1.48×10^7	6/6	2.14×10^4
	1.48×10^6	5/6	
	1.48×10^5	4/6	
	1.48×10^4	3/6	
	1.48×10^3	3/6	
ΔArsR-SthK	1.33×10^8	6/6	4.09×10^5
	1.33×10^7	5/6	
	1.33×10^6	5/6	
	1.33×10^5	3/6	
	1.33×10^4	2/6	

The 50% lethal dose (LD_{50}) was calculated by applying the Reed-Muench formula [24]. CFU, colony-forming unit.

Results

Bacterial extraction from *in vivo* cultures

Approximately 20 h after challenge with $vRAf_{153}$, blood was obtained by exsanguination from the heart immediately before death. Bacteria presence in the blood was determined by Giemsa staining and visualization via light microscopy (panel A in Fig. 1; left). RA pathogens *in vivo* were collected by differential centrifugation and high purity was confirmed by Giemsa staining (panel A in Fig. 1; middle). In addition, RA in blood was identified by performing PCR (panel B in Fig. 1), which confirmed the bacteria isolated from the blood were RA.

The blood from the heart was diluted prior to counting the bacteria as the number of colonies per milliliter of blood can reach 10^9 CFU. In general, there were 10^{10} CFU of bacteria extracted from the blood, and one-third of the bacteria sample was used for each RNA sample preparation.

GO and KEGG analyses of DEGs

By comparison with bacterial genes expressed under *in vitro* conditions, we identified 682 (682/2,101, 32.46%) upregulated genes (Supplementary Table 1) and 121 (121/2,101, 5.76%)

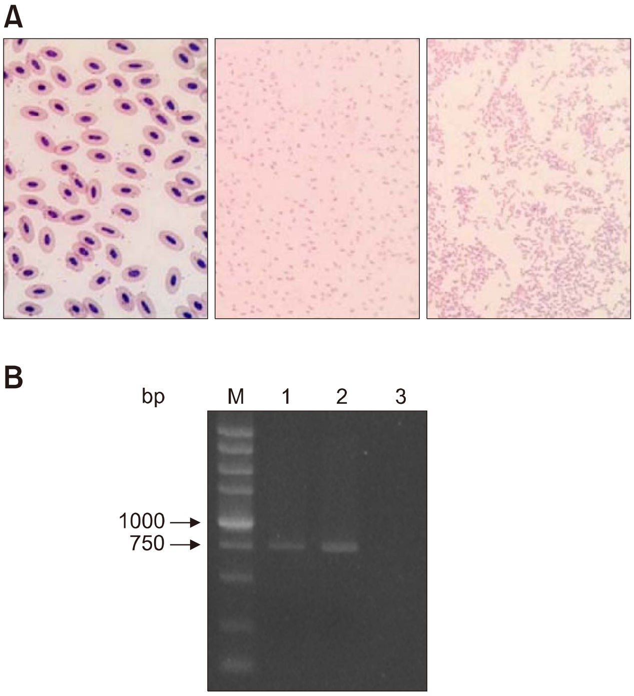

Fig. 1. (A) Giemsa staining of *Riemerella anatipestifer* (RA). RA in duck blood after challenge with $vRAf_{153}$ (left). Purified RA *in vivo* (middle). RA cultured *in vitro* conditions (right). 100× (left to right). (B) Polymerase chain reaction identification of RA isolated from blood. M, DL5,000 DNA marker; Lane 1, RAf_{153} isolated from blood; Lane 2, $vRAf_{153}$ cultured *in vitro*; Lane 3, negative control with distilled H_2O as template.

downregulated genes (Supplementary Table 2) under *in vivo* conditions (Fig. 2).

To gain additional insight into the expression of genes by RAf153 cultured *in vivo*, all DEGs were classified into three GO categories: biological process, cellular component, and molecular function (Fig. 3). As summarized in Supplementary Table 3, cellular and metabolic processes were the dominant subcategories in the biological process category, cell and cell part were the most abundant in the cellular component category, and catalytic activity and binding were highly represented in the molecular function category.

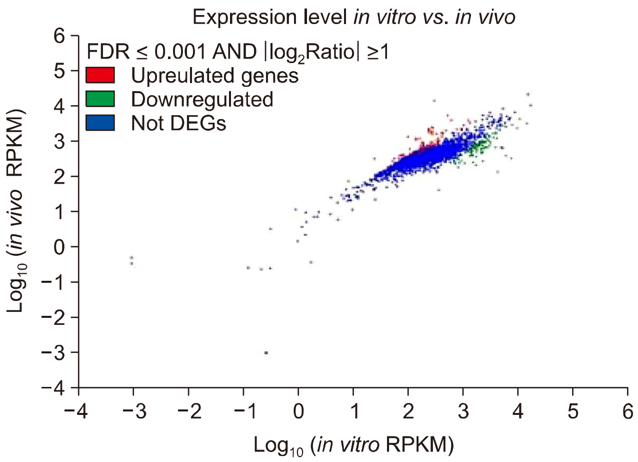

Fig. 2. Differentially expressed genes (DEGs) between RAf153 cultured *in vivo* and *in vitro*. Among the 2,157 genes of RAf153, a total of 2,101 genes were identified by RNA sequencing. Among those, 853 genes were differentially expressed (682 upregulated [red] and 121 downregulated [green]) in bacteria cultured under *in vivo* conditions. FDR, false discovery rate; RPKM, Reads Per Kilobase per Million mapped reads.

In KEGG pathway annotation, 313 DEGs were represented among the pathway annotations, with metabolic pathways, biosynthesis of secondary metabolites, and ribosomes (62, 29, and 19, respectively) accounting for the majority of annotations (Supplementary Table 4).

In addition, a heatmap of expression was constructed for those genes that showed significantly different expression between the *in vivo* and *in vitro* culture conditions (Fig. 4). In Fig. 4, each column represents an experimental sample, each row represents a gene, and the expression levels of the gene in different samples are shown in different colors; red indicates an upregulated expression level and green indicates a downregulated expression level.

qRT-PCR analysis

Six genes were selected as templates for validation of the transcriptomic data by qRT-PCR analysis, and the reactions

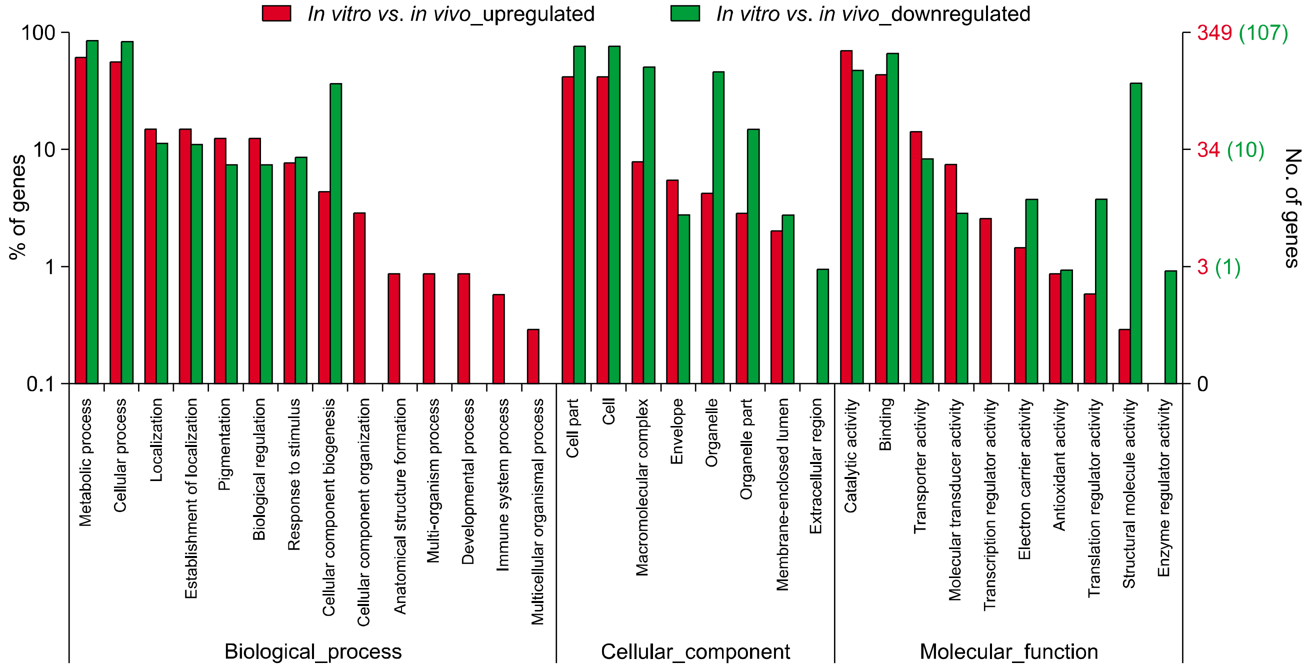

Fig. 3. Gene ontology classification of differentially expressed genes (DEGs). In the biological processes category, upregulated DEGs were identified in relation to anatomical structure formation, cellular component organization, developmental process, immune system process, multi-organism process, and multicellular organismal process. In the molecular function processes category, DEGs related to transcription regulator activity were also upregulated in *Riemerella anatipestifer* cultured under *in vivo* conditions. Upregulated DEGs are shown in red and downregulated DEGs are shown in green.

were performed in triplicate. The qRT-PCR results were largely consistent with those obtained from the RNA-seq analysis (Fig. 5), which confirmed the validity of the Illumina sequencing data.

Significant KEGG pathway enrichment

Pathway enrichment analysis reflects the metabolic changes of pathogenic bacteria during culture. Some significantly upregulated genes were identified in three pathways (Table 4) involved in virulence, protein secretion, and signal transduction.

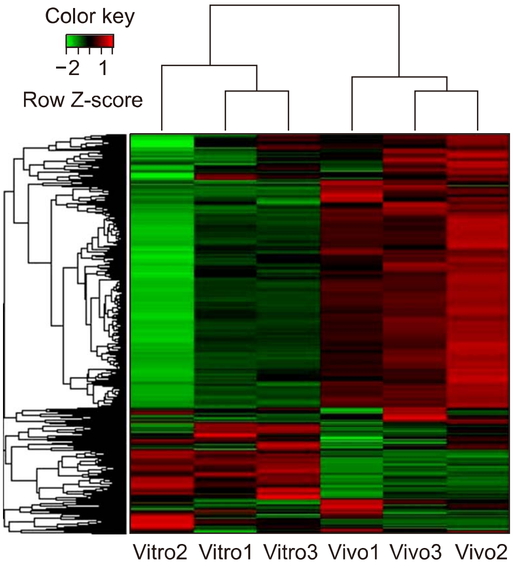

Fig. 4. Heatmap of differentially expressed genes (DEGs). The heatmap illustrates expression levels for all DEGs. Red indicates high expression and green indicates low expression. Good repeatability was observed for the results of the *in vivo* and *in vitro* culture conditions.

Characterization of ΔArsR-SthK

Primers ST-L1 and ST-R2 were used to amplify ΔArsR-SthK, and the target fragment was sequenced for BLAST analysis. Compared to the genes of RAf$_{153}$, the genes *ArsR* and *SthK* on strain ΔArsR-SthK were deleted (panel A in Fig. 6; upper). Moreover, panel A in Fig. 6 (lower) indicated that the *spc*^{R} cassette was integrated into the genome of ΔArsR-SthK. Thus, the *spc*^{R} cassette and the genes *ArsR* and *SthK* were allelically replaced, and the deleted strain ΔArsR-SthK was constructed. The mutant strain was further validated by primers inST-1 and inST-2, inSpc-1 and inSpc-2, 16S rRNA-F and 16S rRNA-R (panel B in Fig. 6).

In addition, the upstream and downstream fragments of deleted genes were amplified through qRT-PCR, and the results in panel C in Fig. 6 show that upstream and downstream genes of *ArsR* and *Sthk* underwent transcription normally and that a polar effect did not occur.

Adhesion and invasion ability of vRAf$_{153}$ and ΔArsR-SthK on Vero cells

The results suggested that deletion of genes *ArsR* and *SthK* decreased the capacity for adhesion and invasion significantly (Fig. 7), which suggested that genes *ArsR* and *SthK* may be involved in RA's infection of host cells.

Virulence of vRAf$_{153}$ and ΔArsR-SthK in ducklings

To evaluate the virulence of the mutant strain, the LD$_{50}$ of the vRAf$_{153}$ and mutant ΔArsR-SthK strains were determined in Cherry Valley ducklings. Observation of mortality of ducklings for 7 days post-challenge revealed that the LD$_{50}$ value for the mutant strain was more than 19 times higher than that for vRAf$_{153}$ (4.09 × 10^{5} CFU *vs*. 2.14 × 10^{4} CFU, respectively; Table 3). These data indicated that inactivation of *ArsR* and

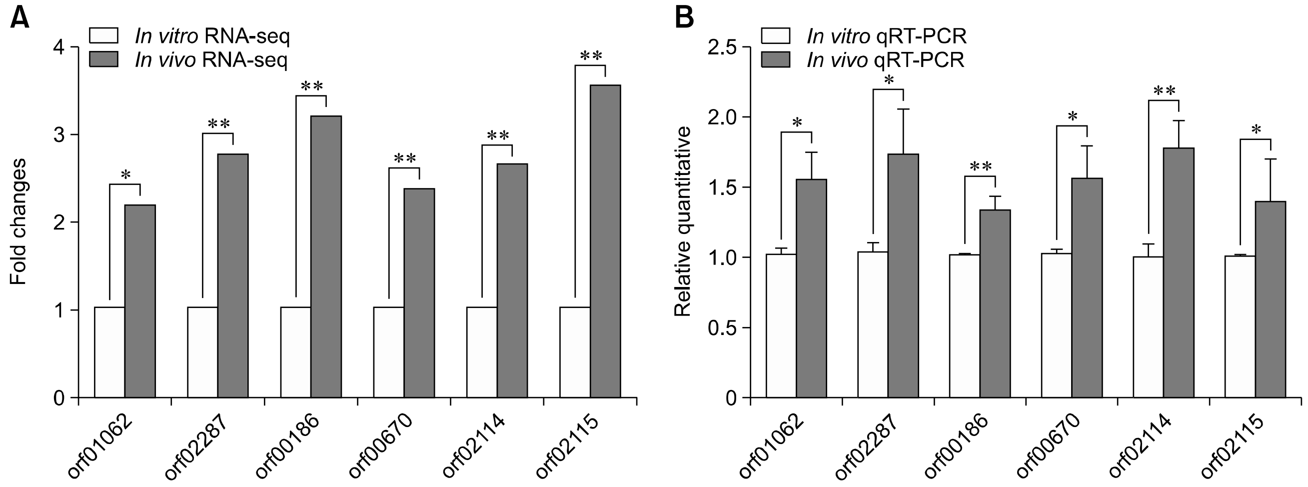

Fig. 5. Validation of differentially expressed genes (DEGs) selected from RNA sequencing (RNA-seq) by quantitative real-time polymerase chain reaction (qRT-PCR). Transcriptional changes of six selected DEGs based on RNA-seq results (A) were validated by qRT-PCR (B). *$p < 0.05$ and **$p < 0.01$ were considered significant differences.

Table 4. DEGs on three KEGG pathways

KEGG	ORFs	Product description	Log_2 ratio (*in vivo*/*in vitro*)
KO00270	orf00625	Methionine synthase II	1.64
	orf01062*	Transferase hexapeptide repeat-containing protein	1.12
	orf01383	Aminotransferase class i and ii	−1.16
	orf02088	Serine o-acetyltransferase	−2.64
	orf02089	Cysteine synthase	−1.45
	orf02287*	Methionine synthase I	1.46
	orf02288	Methionine synthase	1.38
	orf02289	Methionine synthase	1.69
KO02010	orf00016	Cell division protein ftsx	1.07
	orf00638	ATPase component	1.53
	orf02097	Multidrug ABC transporter ATPase	1.16
	orf00477	ABC transporter substrate-binding protein	1.35
	orf00539	ABC transporter	1.52
	orf01135	ABC transporter	1.36
	orf01136	Iron transporter	1.41
	orf01137	Periplasmic binding protein	1.09
	orf02031	Hypothetical protein Riean_1584	1.02
KO02020	orf00186*	Beta-lactamase class D	1.67
	orf00670*	Alkaline phosphatase	1.24
	orf02114*	Signal transduction response regulator, receiver region	1.39
	orf02115*	Signal transduction histidine kinase	1.82
	orf02325	Beta-lactamase class D	1.15
	orf02116	Signal transduction histidine kinase	1.58
	orf02222	Alkaline phosphatase	1.70
	orf02318	Beta-lactamase	1.23

DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; ORFs, open reading frames; ATPase, adenosine triphosphase; ABC, ATP-binding cassette. *Genes were selected for quantitative real-time polymerase chain reaction to confirm the RNA sequencing data.

SthK reduced bacterial virulence.

Discussion

In this study, we identified a panel of DEGs for RA cultured *in vivo* and *in vitro* by using RNA-seq. With regard to RAf_{153}, 2101 of 2157 genes were identified, with a coverage rate of 97.68%. BLAST searching showed two of the upregulated genes, *ArsR* and *SthK*, located on a single operon and revealed that these genes form a two-component system. The function of these genes in bacterial virulence was then investigated by generating a knockout mutant strain. Animal experiments showed decreased virulence of the mutant compared to that of the wild-type strain, thus implicating *ArsR* and *SthK* as potential RA virulence genes.

By comparison with bacterial genes expressed *in vitro*, we identified 682 genes that were upregulated under *in vivo* culture conditions. Some of these genes were enriched in one KEGG pathway, and some were reported to be related to virulence or to enabling the pathogen to survive and multiply *in vivo*.

The upregulated DEGs enriched on pathway KO00270 were related to methionine biosynthesis, which is critical for replication and infection and has been shown to be required for the full virulence of *Brucella melitensis* [17] and *Haemophilus parasuis* [12]. The methionine transport system is also critical for group B *Streptococcus* survival *in vivo* [28]. The DEGs enriched on pathway KO02010 related to ATP-binding cassette (ABC) transporters were upregulated under *in vivo* culture conditions. In bacteria, ABC transporters play a significant role in the uptake of essential nutrients, such as iron, heme, and vitamin B12, and are also important in the clearance of toxins and drugs [13,22]. Upregulated DEGs were also identified in pathway KO02020 related to two-component systems.

The urease gene cluster of RAf_{153}, containing the structural genes encoding urease alpha, beta, and gamma subunits as well as accessory genes (*ured*, *ureg*, and *uref*), are homologous to other bacterial urease genes. The urease beta and gamma subunits were significantly upregulated in bacteria cultured *in vivo*. Urease functions as a virulence factor in several pathogenic bacteria, such as *Helicobacter pylori*, and *Klebsiella aerogenes*

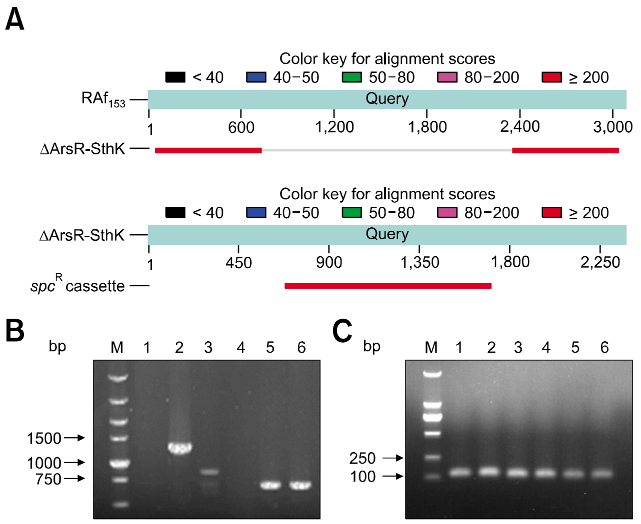

Fig. 6. Characterization of ΔArsR-SthK. (A) Sequence alignment of RAf$_{153}$ and ΔArsR-SthK (upper). Sequence alignment of ΔArsR-SthK and *spc*^{R} cassette (lower). (B) Construction of ΔArsR-SthK-deleted strain. M, DL5,000; Lane 1, ΔArsR-SthK-deleted strain was amplified by detected primers of deleted genes; Lane 2, vRAf$_{153}$ was amplified by detected primers of deleted genes; Lane 3, ΔArsR-SthK-deleted strain was amplified by detected primers of *spc*^{R} cassette; Lane 4, vRAf$_{153}$ was amplified by detected primers of *spc*^{R} cassette; Lane 5, 16S rRNA fragment was amplified from ΔArsR-SthK-deleted strain; Lane 6, 16S rRNA fragment was amplified from vRAf$_{153}$ strain. (C) Polar effect identification of ΔArsR-SthK. M, DL2,000 DNA marker; Lane 1, amplification fragment of *CG09_1826* with ΔArsR-SthK as template; Lane 2, vRAf$_{153}$ control; Lane 3, amplification fragment of *CG09_1830* with ΔArsR-SthK as template; Lane 4, vRAf$_{153}$ control; Lane 5, quantitative real-time polymerase chain reaction amplification of reference gene with ΔArsR-SthK as template; Lane 6, vRAf$_{153}$ control.

[26]. Although this function for urease has not been previously reported, the upregulation of urease gene cluster genes identified in this study implicates urease as a virulence factor in this strain. However, this speculation requires further investigation. Upregulation of transcriptional regulators is important in host acclimatization to various environmental and physiological stresses. In bacteria, the AraC family of transcriptional regulators are reported to be involved in carbon metabolism, stress responses, and virulence regulation [9]. Furthermore, mutation of the AraC family transcriptional regulator *Rv1931c* suggested its roles in regulating the expression level of genes involved in the virulence of *Mycobacterium tuberculosis* [8]. The expression of four genes, *orf02176*, *orf02155*, *orf01543*, and *orf00132*, annotated as CRP/FNR family transcriptional regulators, tended to increase *in vivo*. In *Enterococcus faecalis*, the transcriptional regulator Ers, which is a member of the CRP/FNR family, was reported play a role in oxidative stress responses as well as in bacterial virulence [25]. Furthermore, the CRP/FNR family protein Bcam1349 binds the second messenger cyclic diguanosine monophosphate (c-di-GMP) to regulate biofilm formation and virulence in the pathogenic strain *Burkholderia cenocepacia* [7]. *Thermusther mophilus* SdrP, which is also a member of the CRP/FNR family proteins, alters the expression of genes in response to a variety of signals, such as cAMP concentration and anaerobic conditions [1]. Previous studies have shown that CRISPR-Cas systems function in the defense of bacteria against foreign nucleic acids. The *Francisella novicida* Cas9 protein has a critical role in evading the host immune response and promoting bacterial virulence, and mutation of CRISPR-Cas proteins restores the ability of the host to mount an effective immune response, thus representing a strategy that may also applied to other pathogens [27]. According to our RNA-seq analysis, CRISPR clusters encoding Cas proteins 1, 2, and 9 were all upregulated under *in vivo* culture conditions, which indicates the involvement of the CRISPR-Cas systems as virulence factors in RA infection. Upregulated DEGs were identified in relation to biosynthesis of peptidoglycans, which are essential for maintaining shape in strains such as *Leptospira interrogans* in which peptidoglycans provide sufficient strength and flexibility to withstand osmotic pressure [2]. An intact cell wall is required for full bacterial virulence.

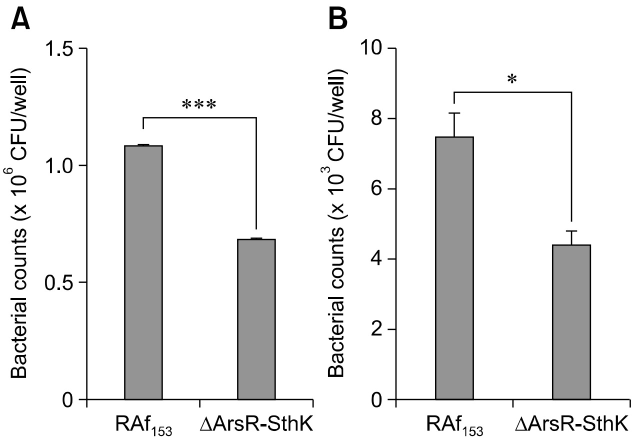

Fig. 7. Adherence and invasion assays of vRAf$_{153}$ and ΔArsR-SthK. (A) Adherence assay. (B) Invasion assay. CFU, colony-forming unit. $*p < 0.05$; $***p < 0.001$.

By comparison with bacterial genes expressed *in vitro*, we identified 121 genes that were significantly downregulated under *in vivo* culture conditions. These included genes involved in protein synthesis, which is consistent with the DEGs identified in *Actinobacillus pleuropneumoniae* cultured under *in vivo* conditions [5]. Nearly 40 downregulated genes were enriched on pathway KO03010 related to ribosomes, which are required for protein synthesis. Among these DEGs, 37 were 30S and 50S ribosomal proteins showing downregulation ranging from −1.02-fold to −3.15-fold. The specific regulation mechanism of this process remains to be fully elucidated. The expression of molecules involved in the protein folding process were also downregulated. Trigger factor chaperones are responsible for regulating the folding of small proteins, while complete folding of some larger proteins requires DnaK and DnaJ, and encapsulated proteins are further folded by the GroEL and GroES system [3,31]. Furthermore, *groel* is highly conserved among different RA strains, and Chaperonin GroEL has been identified as an immunogenic protein in different serotypes of RA [11]. Thus, genes encoding trigger factors, DnaK, GroEL, GroES, and GrpE, which are important in the DnaK chaperone system, were all downregulated in RA cultured under *in vivo* conditions.

TCSTs are widely used signal transduction mechanisms that respond to various environmental conditions in both Gram-positive and Gram-negative pathogenic bacteria. These systems are composed of a sensor HK protein located on the cell membrane and a RR protein located in the cytoplasm [16,35]. The HK monitors environmental changes, while the RR conveys the signals from HK to regulate gene expression in response to environmental changes. In this study, eight upregulated DEGs were annotated to pathway KO02020 related to TCSTs. These included genes encoding structural components, with *ArsR* coding signal transduction RR and *SthK* coding the protein HK. TCSTs play major roles in regulation of virulence and pathogenesis [18]. Evaluation of a mutant containing a deletion of the *ArsR* and *SthK* genes, which are located in the same operon, showed decreased virulence compared with that of the wild-type strain. This result indicates that the *ArsR* and *SthK* genes represent the signal transduction HK and signal transduction RR genes in RA, indicating their potential as virulence factors.

In this study, we established a duck model of acute RA infection and conducted comparative transcriptome analysis to provide new insights into the mechanism of RA pathogenesis *in vivo*. The *ArsR* and *SthK* knockout mutant revealed the essential role of these genes as an effective signal transduction system in bacterial virulence. Further analysis of DEGs is required to fully elucidate some of the adaptations that occur in RA during *in vivo* infection.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

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Supplementary Table 1. Significantly up-regulated genes

Gene ID	vitro_rpkm	vivo_rpkm	log2 Ratio (vivo/vitro)	*p* value
orf01688	293.265151	12398.21841	5.40178337	4.24965E-34
orf01967	0.896518173	10.84696555	3.596814866	0.000171804
orf01689	97.95715586	1170.459951	3.578780874	8.0332E-12
orf00369	24.03700843	285.5334319	3.570330415	1.77371E-11
orf01786	508.1687659	4760.500638	3.227733693	6.20311E-22
orf00238	232.5263332	1960.515006	3.075766671	2.28487E-11
orf00368	268.7102437	2243.560939	3.061667145	7.85385E-32
orf01780	8.24086967	67.58131247	3.035755869	3.67411E-07
orf01690	135.1617649	1069.75868	2.984526385	1.09837E-10
orf00221	238.7470015	1885.198361	2.981161813	6.32774E-21
orf02434	276.9105293	2090.779851	2.916549344	8.83808E-25
orf00079	236.066399	1771.925194	2.908053086	7.33734E-17
orf00239	139.5865601	964.094918	2.788015151	3.149E-12
orf01295	230.964026	1593.101922	2.786098503	3.13112E-15
orf01787	157.9753233	1059.971183	2.746253921	5.28782E-12
orf00405	1.41319097	9.060246707	2.680593899	0.002943384
orf00185	254.4159378	1540.656561	2.598284341	1.67604E-15
orf01346	273.0211387	1623.930502	2.572405331	3.96715E-13
orf02467	3.974599604	23.62855576	2.571650013	0.000386441
orf01785	598.3418285	3516.600463	2.555139606	6.35152E-10
orf00543	7.390604432	40.15932153	2.441970633	0.000101375
orf00805	54.79531954	293.0841166	2.419190212	2.41865E-05
orf00150	103.2261562	541.5698437	2.39133883	2.87057E-10
orf00137	68.25995661	355.8253499	2.382057893	6.46687E-06
orf01241	72.11185157	364.3455701	2.336999159	1.07722E-05
orf01783	274.2589962	1358.594997	2.308504597	6.69748E-08
orf00370	51.26646406	248.9057274	2.279512127	2.67737E-05
orf00055	76.20645617	354.0326272	2.215897191	1.38359E-07
orf00136	176.6322487	819.8771328	2.214658955	2.00065E-07
orf00804	81.52072651	368.4173754	2.17610229	0.000200991
orf01883	66.9064257	296.3137235	2.146908765	4.27353E-06
orf01781	323.4590967	1422.063329	2.136330522	2.2781E-10
orf01784	6.284918878	27.56504018	2.132873676	0.002620473
orf00545	10.05175301	43.95902696	2.128712325	0.000143641
orf00806	80.14310672	350.0612354	2.12695697	5.5953E-05
orf01788	187.0945592	782.3985524	2.064136096	3.8982E-10
orf00559	25.04354409	101.9096117	2.02477948	0.003212584
orf00371	42.92976781	171.1120477	1.994891066	3.12476E-05
orf02276	184.1417804	731.7030349	1.990441242	7.77109E-07
orf01701	11.49715097	45.09269458	1.971617322	0.00112429
orf00210	76.43602603	299.1951461	1.968762092	0.001398573
orf00209	57.12216399	222.2970796	1.960366455	0.005444862
orf00078	167.1330623	647.3011261	1.953439858	9.76611E-08
orf02400	55.25467747	213.780927	1.951964642	0.001384632
orf02261	11.83696332	45.71598705	1.949399752	0.002597197
orf02155	42.4738961	163.00609	1.940277508	0.001677054
orf00050	29.98641542	114.9113745	1.938140633	0.004081224
orf01737	47.09625538	180.4327764	1.937777174	0.002112035
orf00069	2.42846939	9.296216717	1.9365964	0.020553741
orf00081	16.12473286	61.58096551	1.933209228	0.007556491

orf02390	31.51082407	119.8561179	1.927384162	0.000329355
orf00685	104.1767081	395.6766285	1.925289096	1.13415E-05
orf01060	409.6754975	1553.546863	1.923012248	1.02045E-08
orf00035	30.4619633	115.4408084	1.922072475	0.001107058
orf00202	26.9898882	101.8789053	1.916364456	0.003114446
orf00362	34.66048371	130.7742858	1.915715197	0.001396405
orf00205	25.05890254	93.87715185	1.90545084	0.005304526
orf01965	28.4665837	105.5069329	1.889996532	0.015438745
orf00395	35.98992487	133.3444363	1.889492635	0.001196163
orf00298	48.20483843	178.0610126	1.8851218	0.002460236
orf02274	48.27994244	177.6559375	1.879590045	0.00185003
orf01342	479.9186177	1763.964831	1.877960111	2.17148E-10
orf02402	20.32560896	74.69172222	1.877649787	0.004961692
orf00207	79.71119211	292.625098	1.876199303	0.004219094
orf00198	86.99122972	318.9768584	1.874509897	0.000213564
orf00200	69.05770333	252.2025172	1.868708415	0.001884142
orf02398	43.9277691	159.7289192	1.862420401	0.000853472
orf01982	31.52643626	114.0515155	1.855051614	0.001607737
orf00276	27.21804533	98.22959974	1.851594358	0.001787169
orf02401	84.1684938	303.1048353	1.848464661	0.000186141
orf00206	48.10860529	172.4144721	1.841513996	0.020985424
orf02404	12.81099764	45.83632461	1.839108537	0.006084649
orf00383	69.74636893	248.4687666	1.832874496	0.004745686
orf01978	56.83375497	202.3018165	1.831689332	0.005089381
orf01341	423.3323453	1502.313156	1.827322944	3.64278E-11
orf02399	58.14245841	206.3125376	1.827167517	0.000784201
orf00130	7.350450776	26.0035879	1.822806063	0.011074118
orf02115	25.58344417	90.48043707	1.822395398	0.001591622
orf00606	55.40248471	195.6050045	1.819920696	0.001510095
orf01706	32.40649084	114.2717556	1.818114147	0.016452689
orf01746	30.74775951	108.0766721	1.813501962	0.004391052
orf02149	42.28557303	148.4529311	1.811768145	0.010376026
orf02039	188.000557	659.5544838	1.810754905	0.004455986
orf02152	38.7865878	136.0445764	1.810449676	0.00761966
orf00034	72.35949967	253.78205	1.810335693	0.000164107
orf02257	75.50213832	264.2225199	1.807164025	0.002653304
orf02255	78.13103875	273.396553	1.807027353	0.000270791
orf01987	120.4161954	420.8661956	1.805332195	4.79095E-05
orf01727	56.89138127	198.4127294	1.802222572	0.003436731
orf00361	14.73372056	51.38123582	1.802119805	0.000450003
orf01525	48.48641335	168.9157194	1.800651149	0.000712575
orf01739	30.64737595	106.3643638	1.795179414	0.011789923
orf00343	74.86838684	259.5982941	1.793852328	0.005545298
orf02347	28.71625239	99.56597528	1.793785331	0.001390041
orf00954	411.76996	1421.882301	1.787891558	9.00384E-09
orf02153	72.92669695	251.8007228	1.787763467	0.005615683
orf00598	79.25503582	272.9634007	1.784133014	0.005665763
orf00803	94.23588341	324.316241	1.783052849	0.000610587
orf00736	130.9590554	450.3397302	1.781897941	0.000395137
orf02225	64.01130782	219.9957181	1.781076754	0.001373959
orf01979	102.4199657	351.5656409	1.779297099	0.003843603
orf00584	37.30152934	127.9447758	1.778214555	0.001847693
orf00199	78.92181903	270.535551	1.777322077	0.000224219
orf02142	64.47463475	217.9676173	1.757310215	0.006201248

orf00459	122.896813	415.4360694	1.757178977	3.67779E-05
orf02143	55.75124032	188.3417512	1.756277043	0.009988911
orf02384	88.56896968	298.4925534	1.752821699	0.000198321
orf01735	27.64602806	93.01805363	1.750438531	0.016250405
orf01197	21.12641276	71.07145256	1.750222365	0.021232521
orf01643	170.5032002	573.1127915	1.749020279	2.76304E-06
orf01574	8.226139129	27.64451874	1.748706079	0.009012511
orf01757	21.85020855	73.20643796	1.744323479	0.008312253
orf02080	43.22495167	144.6536799	1.742666769	0.002003454
orf00479	48.54496503	162.2202185	1.740560067	0.00667115
orf01724	77.50229372	258.9142316	1.740163354	0.01312933
orf00215	61.09154855	204.0614051	1.73995863	0.001986313
orf00583	70.22011642	234.2233955	1.737928894	0.001118457
orf01520	17.15563918	57.20050496	1.737345004	0.004352947
orf01729	76.94026868	256.4478711	1.736854822	0.009856257
orf01747	48.23821129	159.8481406	1.728453646	0.01282285
orf01748	35.22825873	116.653472	1.727424175	0.013433814
orf02397	59.2730265	196.1819849	1.726744938	0.003509948
orf01734	26.08848811	86.30048266	1.725955289	0.020408207
orf02145	53.99344729	178.5437554	1.725421441	0.006996559
orf02147	70.8385316	234.2259442	1.725294674	0.001608133
orf02275	139.845014	462.3226779	1.725071314	0.002874344
orf02290	93.62696473	309.3243581	1.724124451	0.00300303
orf00396	42.21657296	139.4668799	1.724041183	0.002582078
orf02113	70.89631031	233.7882007	1.721419667	0.004464551
orf00297	23.73319057	78.22524154	1.720728146	0.001772828
orf02144	57.35943236	188.6297793	1.717454803	0.005610006
orf00232	82.68969219	271.7745944	1.716631194	0.009072076
orf01745	99.23390545	325.7428775	1.714828596	0.002648484
orf01199	36.540267	119.8037517	1.713114006	0.008429031
orf01524	44.60504156	145.9469715	1.710165587	0.018053339
orf01722	70.95843392	232.1609565	1.710079294	0.011140935
orf00390	97.50406068	319.000892	1.71002625	0.000138921
orf02079	5.229637919	17.04764218	1.704789249	0.006597736
orf00810	165.5832617	539.4237698	1.703862253	9.20803E-05
orf01752	83.14716585	270.7302597	1.703117154	0.006114765
orf00273	60.84401309	197.9458562	1.701918649	0.002845028
orf02200	105.4560037	342.7657271	1.700581631	0.002949257
orf00448	56.49852099	183.606503	1.70033215	0.002745881
orf02222	79.84762541	259.3742824	1.699714031	0.001195449
orf02306	125.6695134	406.7956604	1.694669585	0.00025633
orf00692	52.24738693	168.9789589	1.693412825	0.003299399
orf01980	77.12569819	249.0391205	1.691088837	0.006321468
orf02259	90.36101728	291.7720772	1.691069405	0.005632588
orf00684	98.76566192	318.7075347	1.690151678	0.006023029
orf02223	63.42148996	204.4485317	1.688694026	0.004271496
orf02289	72.11989164	232.1825265	1.686790268	0.001560759
orf02008	156.6358141	503.979521	1.685950996	0.005363004
orf02281	8.138139159	26.18408337	1.685919246	0.008553016
orf01710	132.4096522	425.9845686	1.685792876	0.005917222
orf00607	114.0424123	366.8810438	1.685741903	0.007152319
orf01725	78.88140408	253.1049186	1.681978407	0.004251305
orf02148	51.4725396	165.0564438	1.681084592	0.007355395
orf02362	49.27621561	157.9635687	1.6806285	0.001298875

orf01705	75.83072778	243.0251886	1.680251377	0.018567618
orf00419	139.760894	447.7952045	1.679878337	0.00186356
orf01743	34.9944284	112.0374663	1.678784115	0.01679345
orf00597	47.68467794	152.6497137	1.678627206	0.003184855
orf01056	34.86302752	111.5762421	1.678260103	0.005350772
orf02273	102.7763947	328.8837095	1.6780686	0.001120398
orf00740	123.1267983	393.957224	1.677894194	0.003115596
orf00039	42.87080282	137.0732044	1.676879235	0.003929689
orf02160	85.76358534	274.0143318	1.675814229	0.001859105
orf01716	80.20878248	256.0850538	1.674790935	0.003501056
orf00060	70.41955896	224.6380087	1.673553956	0.017828363
orf00186	66.45230649	211.8709198	1.672794405	0.001085076
orf02161	21.22886515	67.58249563	1.670622376	0.001009518
orf00683	93.38696411	297.1962071	1.670122621	0.001605493
orf02226	92.28340704	292.9422223	1.666472973	0.009391797
orf00808	121.1122626	384.3993627	1.666260999	0.000446653
orf02448	184.5744558	585.4725471	1.665398619	0.000592652
orf02044	99.08408476	314.2877453	1.665360769	0.002402769
orf00562	65.21916761	206.8520009	1.66523098	0.000258746
orf02366	55.37558823	175.5929609	1.664912988	0.001162342
orf00363	253.5889695	804.0559733	1.664803943	2.1649E-07
orf01759	29.34791698	93.04509621	1.664672008	0.014955912
orf01754	135.2513277	427.9228731	1.661708038	0.006678878
orf02387	60.48515923	190.8577658	1.657844781	0.002057741
orf00249	38.15335362	120.2960626	1.656707645	0.003026656
orf02150	54.93834578	172.8134015	1.653329725	0.009089197
orf01723	89.55514075	281.6866578	1.653243076	0.004397451
orf02385	76.07137659	238.8476389	1.650664998	6.00611E-05
orf00498	86.58301882	270.8942274	1.645573643	0.008768764
orf00862	99.15440839	310.2001005	1.645450333	0.003293864
orf00861	116.8761821	365.5986593	1.645279823	0.000230299
orf00107	124.6352988	389.470986	1.643803136	0.001298294
orf01521	55.77410157	174.1821023	1.642929116	0.017077898
orf01981	131.9511613	411.8401394	1.642080399	0.000553455
orf02042	75.24304969	234.514919	1.640049475	0.004217409
orf00625	343.6709378	1070.846195	1.63965152	3.47634E-08
orf01708	107.3819473	334.2183635	1.638039531	0.006547414
orf00624	92.9525968	288.9464443	1.636235042	0.004273549
orf00135	42.38045817	131.310815	1.631514655	0.014053856
orf00739	114.799941	355.5427786	1.630901253	0.006666933
orf00894	29.80217711	92.20173866	1.629376229	0.001016496
orf02388	271.0432493	838.2656786	1.628884487	9.89825E-06
orf02049	48.58879826	150.1202722	1.627423155	0.007675043
orf00031	170.2292988	525.8136347	1.627072186	4.61356E-07
orf00381	25.28933806	77.98351552	1.624639918	0.003460031
orf00637	96.66177531	297.9383781	1.623996575	0.006859231
orf01721	122.7785683	378.2203583	1.623168269	0.00157101
orf01677	144.8263852	445.9336249	1.622504524	0.000100433
orf00402	113.8500346	350.3783423	1.621778872	0.000202677
orf02297	96.35634675	296.3660461	1.62092857	0.003559224
orf01726	103.5202288	316.8149116	1.61372753	0.016652873
orf01522	44.49605345	136.0380725	1.612261182	0.023180148
orf00234	74.3029147	227.1021746	1.611850811	0.000323334
orf02375	218.8657189	667.6964357	1.609146337	0.000390055

orf01534	45.27255412	137.9441656	1.607375833	0.016364704
orf01960	49.44422798	150.5425277	1.606297081	0.005850748
orf00781	50.29245238	153.0384345	1.605480211	0.003161984
orf01347	166.2091346	505.4909934	1.604685715	0.011033195
orf01894	97.97299327	297.9191315	1.604464749	0.00555256
orf01977	37.28304164	113.2997387	1.603553065	0.013707572
orf02224	36.69716987	111.4246289	1.602327445	0.007904277
orf01892	79.83733157	242.3567285	1.60199673	0.004086649
orf00134	17.14666807	52.01024255	1.600867506	0.003847897
orf01713	64.07660737	194.3519908	1.600802216	0.005048406
orf00809	107.8782238	327.1087063	1.600366485	0.00316654
orf00531	110.4776639	334.8134077	1.599602583	0.000374437
orf01719	105.8938584	320.920271	1.599596001	0.004413893
orf01052	98.75089926	299.192593	1.599208669	0.011290756
orf02136	108.6875687	328.3410791	1.595008319	0.000100976
orf00980	191.6977082	578.562133	1.593638812	1.97008E-06
orf02451	76.56217066	230.7914974	1.591886436	0.00058326
orf01550	108.6542026	327.2307484	1.590564341	0.00213239
orf01740	17.86678544	53.80333792	1.590415589	0.023043754
orf01961	97.64673877	293.860898	1.589489636	0.001566023
orf00726	33.19102637	99.45542309	1.583256797	0.011647329
orf01984	135.947742	406.7597876	1.581124871	0.000582131
orf02452	62.41009165	186.5723308	1.579883811	0.001720373
orf02116	80.78460665	241.3933825	1.579233805	0.004726775
orf01767	17.55515497	52.43307054	1.578582302	0.014332738
orf00567	88.27184212	262.8547243	1.574240454	0.001141849
orf02166	106.6098071	317.3102466	1.573553951	1.13143E-05
orf01736	56.20653242	167.2473958	1.573174029	0.006094469
orf01058	225.3097333	669.671724	1.571544415	1.90201E-06
orf00416	96.20809724	285.9375775	1.571470002	0.001013172
orf02003	171.6521461	509.187975	1.568710454	0.004950278
orf02346	49.02957144	145.3646358	1.567952277	0.010811152
orf02162	46.30321679	137.1166805	1.566219759	0.001167992
orf00475	32.53042194	96.29354736	1.565649591	0.013079458
orf01963	74.24614042	219.2515811	1.56219931	0.000895863
orf01744	47.67637904	140.6095674	1.560348188	0.014708145
orf02052	174.1495273	512.769948	1.557985155	1.51609E-05
orf01718	79.08014426	232.8255178	1.557861779	0.014709053
orf02183	73.1501609	215.3113867	1.557491675	0.006525276
orf02135	50.41175116	148.3695013	1.557362588	0.006850441
orf00244	405.3555555	1192.243135	1.556418655	6.60533E-08
orf00489	105.2868726	309.6121882	1.556136697	0.005043886
orf00979	75.3642396	221.125636	1.552914262	0.002203776
orf00091	83.32577041	243.6977143	1.548258063	0.002468319
orf02156	102.596606	299.3114145	1.544664293	0.000286417
orf02131	99.99264599	291.0432769	1.541339791	0.00067978
orf00636	69.56388998	202.1278608	1.538857678	0.005674461
orf01156	106.8999842	310.4529876	1.538113175	0.012643152
orf02403	11.76589322	34.14958263	1.537257098	0.019800676
orf00800	66.85524527	193.9789815	1.536787679	0.007173396
orf01195	60.97510337	176.8851142	1.536520439	0.0153144
orf00578	95.96508043	277.9089858	1.534031041	0.006039865
orf01720	101.9556592	295.0956746	1.533240917	0.003238526
orf02450	136.6142286	395.0536773	1.531940939	0.003929849

orf01860	79.30494461	229.3129406	1.531835046	0.00395377
orf02101	94.81575074	274.1561399	1.53179914	0.003862667
orf00934	104.0961489	300.8484828	1.531120385	0.008246923
orf01707	134.554735	388.242979	1.528766675	0.007167911
orf01804	64.71550944	186.7227455	1.528714271	0.002914117
orf00601	1211.537865	3494.36207	1.528189607	7.43247E-06
orf02045	174.6552818	503.5358707	1.527584284	0.000793865
orf00638	110.0745415	317.0680225	1.526311548	0.004208672
orf00539	145.6841676	419.3777968	1.525406383	0.001416681
orf01773	63.96060333	184.0369307	1.524739848	0.010746949
orf00169	54.22355955	155.7168558	1.521933392	0.001724441
orf00724	98.60283747	283.1621358	1.521927294	0.002164832
orf02311	73.15591865	209.9272001	1.520842612	0.004334707
orf01245	111.1316661	318.5668201	1.519326052	0.007469477
orf00054	70.51853146	202.1266271	1.519185051	0.000628556
orf02111	96.75411339	277.3062942	1.519085461	0.002067577
orf00216	67.66927706	193.9225348	1.518907581	0.000986501
orf01133	85.69173398	244.7347113	1.513990786	0.012865926
orf00359	105.6572503	301.4596613	1.512573196	0.00123357
orf00982	33.20975028	94.74379106	1.512424526	0.008972165
orf00179	82.84771654	236.0801925	1.510743163	0.002059323
orf02158	91.68088369	260.7074348	1.507738871	0.010892676
orf00151	62.2539589	176.9305424	1.506945622	0.001354145
orf01157	206.8902993	587.5708534	1.505896831	0.000694129
orf00178	46.5669204	131.9911141	1.503063427	0.003776568
orf02102	77.02407651	218.1158382	1.501713148	0.002279941
orf01002	58.38493765	164.9542605	1.49839791	0.006690178
orf00374	329.4358125	930.3179257	1.497726428	1.3455E-05
orf00399	88.67001712	250.3399648	1.497370365	0.000635463
orf01969	63.29611622	178.5338991	1.496009146	0.002649604
orf01693	114.2775898	321.4712567	1.492147235	0.003419736
orf01692	74.85790873	210.4930898	1.491546222	0.006545277
orf00599	123.5139449	346.8805138	1.489764866	0.004716283
orf02321	50.96984132	142.9770872	1.488068199	0.001708887
orf01717	87.17006382	244.2082175	1.486207076	0.008793981
orf01571	112.5996032	315.1496414	1.484835279	0.004112624
orf00943	130.29029	364.6463615	1.484768429	0.007380212
orf01715	104.3643427	291.2742198	1.480749133	0.008696547
orf00605	75.64198056	210.9799154	1.479846622	0.003187621
orf02100	70.46469336	196.3651964	1.478566775	0.004011783
orf02458	101.7885987	283.6302388	1.478435376	0.005965154
orf00414	70.93466365	197.6350412	1.478276056	0.000726156
orf02405	70.18140278	195.3145673	1.476638866	0.007835226
orf00910	87.5930662	243.5348988	1.47523995	0.007439981
orf00038	55.58024258	154.3636954	1.47368945	0.003269012
orf00155	54.81304276	151.7169699	1.468791335	0.003010858
orf02309	67.11746774	185.5970743	1.467413777	0.002370924
orf02406	80.60463523	222.7212631	1.466304588	0.001703563
orf01899	86.05976736	237.7869435	1.466258656	0.007346333
orf02287	132.3988496	365.2001455	1.463796754	0.00701487
orf00110	114.1419874	314.6963375	1.463130801	8.25355E-05
orf00418	19.61415828	53.93982911	1.459455526	0.003626694
orf02333	88.40042905	243.0407097	1.459072711	0.000919291
orf01959	38.09523357	104.7206029	1.458862903	0.00938244

orf00550	67.55089609	185.3098312	1.455892609	0.001570337
orf02016	101.5268365	278.4451928	1.455532261	0.003321126
orf01840	162.0957833	444.5541328	1.455512544	0.000963987
orf00214	53.75610073	147.3686003	1.454928764	0.007242081
orf01192	90.86574359	248.9534242	1.454067453	0.012170119
orf01849	255.1149558	698.1061629	1.45229897	1.60115E-05
orf01132	197.7039322	540.113724	1.449921642	0.003792099
orf02085	154.4352533	421.4578909	1.448386377	0.004987366
orf01968	70.77185347	192.935699	1.446872502	0.005015387
orf02159	93.53247706	254.5461344	1.444387856	0.005725352
orf02328	38.35928626	104.2599309	1.442537027	0.003986524
orf01791	145.6818409	395.8514988	1.442138256	0.001029709
orf01523	85.18756497	231.459412	1.442044472	0.016148633
orf01769	157.1531985	426.5139418	1.440421266	0.000961098
orf00628	79.43185851	215.3833374	1.439116979	0.001679753
orf01515	102.6921741	277.9743269	1.436625403	0.004251424
orf02015	82.08278186	222.1613629	1.436456402	0.002913777
orf00408	91.7640337	248.1570933	1.435252978	0.000762446
orf02096	161.2967633	435.8270692	1.434038317	0.000195507
orf01490	83.46263305	225.4581972	1.433657624	0.006886975
orf00058	59.96753122	161.6382806	1.430515425	0.003570961
orf01755	142.9500904	385.0940847	1.42969943	0.001067321
orf00123	64.95088256	174.8695955	1.428858436	0.008423048
orf02466	69.55645583	186.6857937	1.424355818	0.003055244
orf00983	79.67332518	213.7209421	1.423559589	0.001203513
orf02410	114.3682044	306.7650441	1.423448074	0.0013181
orf01983	72.08892409	193.2896031	1.422914516	0.004148196
orf01732	111.1216669	297.2904103	1.419732781	0.005157266
orf01762	131.7849743	352.3184005	1.418693936	0.008426742
orf00618	49.24587699	131.4826413	1.416797496	0.008111345
orf01641	47.03080516	125.4598327	1.415547607	0.019720763
orf01136	187.9061767	500.265745	1.412682178	0.012967184
orf01865	108.5041777	288.3063539	1.409852041	0.009038677
orf02163	149.6468902	397.6078496	1.40978394	5.2181E-06
orf01867	72.72852466	193.21402	1.409606567	0.019450088
orf01939	146.6574253	389.2666029	1.408308457	0.010999786
orf01772	204.6305416	542.4779904	1.406543118	0.000819735
orf01244	123.2288736	326.3233234	1.404961774	0.009468249
orf01586	110.6493476	292.6049348	1.402959156	0.000459746
orf01025	174.4517133	460.9443373	1.401764778	0.005106581
orf00967	197.1094308	520.7505343	1.40159561	0.002546299
orf00259	69.91854231	184.6979545	1.401420877	0.000610934
orf01544	41.28471793	108.8923672	1.399223078	0.021744873
orf02474	96.27442284	253.3948967	1.396162995	0.001954409
orf02114	104.6711008	275.3962592	1.395645787	0.005392355
orf00963	147.2187759	387.2976464	1.395481053	0.004516315
orf01661	238.0213293	625.83812	1.394698676	0.000345927
orf01868	99.0350271	260.3774495	1.394593728	0.007881635
orf01531	86.08994617	225.9036633	1.391790995	0.007170114
orf01760	121.3158906	318.0850481	1.390644023	0.006723056
orf01850	216.0826558	565.4702548	1.38786786	0.005386664
orf01325	69.19947225	181.0548583	1.387593949	0.014996143
orf01015	141.1579966	368.3327485	1.383698814	0.003639974
orf02288	126.2096442	328.5105793	1.380117675	0.002037153

orf00387	240.5836792	625.8843237	1.379357266	7.71127E-05
orf01857	133.4174962	347.0197194	1.379069774	0.001883009
orf01711	106.6287384	277.1581394	1.378113053	0.001371726
orf01487	177.8915203	461.9156881	1.376631804	0.003380624
orf00912	157.5217557	408.7058685	1.375511863	0.005267932
orf01945	147.5678351	382.6854713	1.374780834	0.003124856
orf00580	27.6969163	71.79760963	1.374210453	0.015901015
orf00019	51.67457696	133.9278642	1.373929572	0.009661706
orf00987	158.0850554	409.4444222	1.372966646	0.000551313
orf02050	119.57046	309.3539472	1.371397427	0.002789248
orf01576	195.802687	506.3259494	1.370665861	0.000275274
orf01152	183.3671217	474.0965698	1.370445972	0.01081115
orf01858	87.50551134	226.1625273	1.36991412	0.00118321
orf00523	132.78583	343.0053174	1.369129742	0.004399311
orf01995	89.58302148	231.3584544	1.368832588	0.002054493
orf00758	150.3346776	388.0693205	1.36813655	0.006409334
orf02051	20.1565324	52.01369496	1.36764406	0.020000924
orf01676	123.7105139	319.1847539	1.367423625	0.011140295
orf02420	160.555106	414.0378391	1.366694076	0.001644874
orf01188	87.73042173	226.0138035	1.365261777	0.008399696
orf01193	75.122524	193.3811798	1.364129955	0.012967326
orf01135	122.6186025	315.547706	1.363680268	0.02027516
orf00600	150.4744452	387.1419793	1.363344255	0.003651484
orf01031	75.92405111	195.1309717	1.361813907	0.009635788
orf02455	131.2632692	337.2814632	1.361489761	0.000789851
orf01348	116.0203237	297.5472067	1.358741025	0.003196232
orf02308	993.1410666	2543.529272	1.356761138	1.06769E-06
orf01889	111.7946845	286.2456937	1.356402395	0.012223654
orf01068	92.52606838	236.3573119	1.353037702	0.008507486
orf01970	104.288063	266.1636554	1.351739551	0.001880897
orf00451	42.7959707	108.9850078	1.348582812	0.006755573
orf02190	95.7453996	243.7905708	1.348367253	0.005734229
orf02383	65.42533718	166.4836676	1.347459294	0.010436838
orf00477	118.5510815	301.6642056	1.347434697	0.003201574
orf02179	98.75726458	251.2809864	1.34734273	0.004156372
orf00251	120.3661368	306.0978905	1.346563533	0.001157866
orf01340	750.3732962	1904.469232	1.343708588	4.71535E-06
orf00864	109.4304221	277.5646884	1.342810172	0.013865556
orf00779	156.2207904	395.6733091	1.340723283	0.004663223
orf02447	148.0934169	374.9521592	1.340199021	0.000826058
orf02236	83.54386698	211.4638492	1.339805221	0.007325802
orf00985	137.900943	348.8500051	1.338974532	0.000271919
orf00516	97.95575966	247.7871748	1.338899288	0.010934862
orf00187	109.3303258	276.1075396	1.336536656	0.002438118
orf02081	52.87211316	133.4567005	1.335792847	0.004519242
orf00627	147.3157078	371.3358706	1.333813416	0.003699471
orf00741	259.5146238	653.0722911	1.331426861	3.50777E-05
orf00183	74.81449982	188.2644508	1.331370796	0.000969607
orf02087	152.3853419	383.1708039	1.330263503	0.004467134
orf00520	98.39224644	247.3972538	1.330212949	0.004359976
orf01144	360.1515148	904.967626	1.32926221	2.61181E-07
orf01498	340.4203358	855.2426731	1.329016617	0.001686489
orf01845	123.5353381	310.3427444	1.328938623	0.004008079
orf01492	111.270288	279.4135608	1.328333634	0.016307017

orf00874	196.282118	492.7179	1.32783314	0.003652908
orf02293	152.2756832	381.9371428	1.326649649	0.0126658
orf00057	112.0998462	280.7835049	1.324673886	0.001135097
orf00595	128.2222459	319.9380712	1.319146094	0.00336257
orf01554	112.8386881	280.9007356	1.315798604	0.012116769
orf02240	110.4649912	274.7957936	1.3147707	0.005033699
orf01190	173.5485879	431.39911	1.313683574	0.0046886
orf01038	110.0540883	273.4104524	1.312855658	0.017293723
orf01003	187.7110224	465.9122082	1.311544766	2.00321E-05
orf01679	618.7297256	1535.290497	1.311130406	0.000400854
orf00981	181.9233105	450.1057827	1.306933687	0.000120421
orf01028	130.9429664	323.8939258	1.306582846	0.000280415
orf01211	92.84643666	229.4322685	1.305149866	0.019888873
orf01496	143.6909327	354.5192278	1.302894849	0.007175084
orf02199	40.9537675	100.9483796	1.301549671	0.006577881
orf02164	142.805434	351.4722364	1.299359855	0.000124289
orf02256	127.092708	312.68363	1.298822434	0.00480452
orf00645	134.2768118	330.1765058	1.298027279	0.009428942
orf02298	70.24573708	172.6375103	1.297263382	0.00648731
orf00077	213.8732541	525.3361811	1.296484872	0.000179051
orf00527	95.18110281	233.5789384	1.295163118	0.01333758
orf01473	240.467199	590.0214496	1.294927287	0.006776032
orf01790	116.9717364	286.8876306	1.294325789	0.012400894
orf01418	241.024161	590.9989077	1.29397769	0.000442571
orf00417	41.71821926	102.2805783	1.29378274	0.007620676
orf02218	216.4975669	530.658804	1.293433743	0.001204898
orf01477	111.2888681	272.7077409	1.293046361	0.007077471
orf00041	82.08549659	200.9902996	1.291926629	0.000617902
orf00376	284.8090472	697.2743572	1.291731451	0.001176524
orf00334	46.03909388	112.5282789	1.289356258	0.006010688
orf00696	108.1673748	264.3752989	1.289321966	0.002400924
orf01007	129.2318934	315.7828656	1.288970733	0.004501959
orf00274	56.44152219	137.8838446	1.288624629	0.005587685
orf00307	160.0870006	389.7457561	1.283677151	0.002649244
orf01789	129.4871507	315.2399927	1.28364163	0.000550338
orf02221	73.17247586	178.1362016	1.283607757	0.003041409
orf01485	246.6043999	599.8718527	1.282455798	0.001031762
orf00460	103.7660582	252.1472887	1.280932096	0.003968293
orf02227	84.41015753	204.7379726	1.278290181	0.005302085
orf01872	217.7217063	527.9244071	1.277846119	0.00320694
orf02348	109.8626323	266.2897304	1.277296028	0.005620579
orf01019	98.41086948	238.4612318	1.276865162	0.010027302
orf00635	46.17967333	111.8363176	1.276058891	0.011196877
orf00708	169.7968038	410.8855747	1.274927379	0.022004788
orf00132	64.40668444	155.7820213	1.274246411	0.017307763
orf02176	116.604573	282.0024975	1.27408357	0.005858007
orf00407	103.029927	249.0692484	1.273483451	0.005185145
orf00868	121.7698322	293.6973258	1.270173371	0.004867238
orf01288	136.7775298	329.1858024	1.267070875	0.010489134
orf00866	121.3159016	291.6513015	1.265475847	0.015137883
orf01158	147.2185986	353.9047029	1.265400991	0.005918615
orf01856	161.2812882	387.6859573	1.265309412	0.000703696
orf02252	195.6322278	470.0649369	1.264716016	0.000535502
orf01024	186.5716538	447.8249389	1.263205061	0.002138471

orf02071	101.7642978	244.2535504	1.263148027	0.005232007
orf00620	119.6350916	286.8704602	1.261758792	0.000340256
orf01208	186.7866432	447.5435814	1.260636883	0.005991023
orf00168	120.9142229	289.6793768	1.260473023	0.006556985
orf01494	70.39643932	168.4461583	1.258713162	0.013051997
orf02353	7.965055594	19.04909579	1.257966182	0.01989686
orf00908	174.8436139	417.998684	1.257433297	0.013587923
orf02480	116.538593	278.5622039	1.257191725	0.005395841
orf01343	194.4672813	464.6206226	1.256525745	0.003953715
orf02465	71.03795955	169.6608106	1.255991312	0.006318797
orf00629	115.5261778	275.7525834	1.255154604	0.001045829
orf00544	89.97261595	214.7499811	1.25510013	0.008063968
orf00898	124.0316986	295.8719594	1.254264099	0.002418072
orf01913	107.5967977	256.6505644	1.254170293	0.001189839
orf00006	194.9231449	464.5376672	1.252890178	0.001316073
orf01805	153.2993871	364.786922	1.250702079	0.000814056
orf02127	135.540306	322.4457306	1.250334434	0.003962082
orf02294	102.8461049	244.4980821	1.249335992	0.005206677
orf01064	116.5587091	276.8838739	1.248224227	0.015661128
orf02094	201.4502377	476.9968693	1.24355629	0.001897731
orf00043	73.06802291	172.7932216	1.241734548	0.007313081
orf02277	117.8008512	278.392751	1.240771682	0.001078677
orf00970	290.0925484	685.179415	1.239968571	0.001317703
orf00941	194.6463485	458.906725	1.237345668	0.008767729
orf00670	332.9945671	785.0735372	1.237329157	3.74949E-05
orf02157	101.841412	240.0931015	1.237269625	0.010406359
orf01937	91.00109482	214.3948224	1.236314258	0.006393298
orf00122	132.7937793	312.8011299	1.236058159	0.005199081
orf02263	126.5048313	297.6029903	1.234196539	0.003318541
orf02215	61.64721849	144.7629632	1.231584837	0.017628324
orf02238	90.47460623	212.3474641	1.230842051	0.007163855
orf02318	87.23523591	204.5564767	1.229516328	0.006480884
orf00693	104.0591011	243.7974064	1.228279629	0.002238958
orf00053	165.8816622	387.9085665	1.225562228	7.29548E-05
orf00610	168.369135	393.2212553	1.223713617	0.000434146
orf02047	223.1142473	520.9199849	1.223279144	9.79351E-05
orf02475	143.0665064	333.6531252	1.221663058	0.008096966
orf02203	172.5525261	402.4060032	1.221616233	0.000260255
orf00301	76.71419632	178.6762036	1.219782022	0.004131606
orf01774	106.5577183	248.1088396	1.219338041	0.014257494
orf00547	65.5420692	152.4897164	1.218218828	0.014150785
orf00121	130.0478634	302.4619947	1.217711177	0.001793112
orf02457	110.9285354	257.5279649	1.215098569	0.002819385
orf01870	1284.589362	2980.814739	1.214399458	6.30286E-05
orf01863	44.76047168	103.8594662	1.214335571	0.01234239
orf02304	78.58977578	182.2159317	1.213235563	0.012780255
orf02210	136.7307565	316.8117241	1.212287923	0.002552232
orf00796	39.36408321	91.15263634	1.211404505	0.013832945
orf01938	304.802647	702.6180465	1.2048652	4.82388E-05
orf01775	82.39911742	189.8373058	1.204062741	0.011301667
orf02478	108.6797913	250.33149	1.203756084	0.001595438
orf01481	138.1744615	318.1577492	1.203251271	0.008947604
orf00250	74.37329914	171.089041	1.201890677	0.003808011
orf02242	338.9883028	778.8081524	1.200032494	1.92195E-05

orf02371	175.4108321	401.9810308	1.196389582	0.003822034
orf02482	142.6019198	326.5442419	1.195285064	0.003176105
orf00522	129.2998962	295.9641737	1.194701432	0.002808637
orf00101	136.8926248	313.2560897	1.194297834	0.000562608
orf00248	110.5616098	252.9351158	1.193916818	0.003009809
orf01674	138.3453849	316.458399	1.19374134	0.012432971
orf00102	108.0708981	247.1506064	1.193412367	0.003138241
orf02241	74.0101452	169.2433386	1.193304098	0.012680738
orf01039	82.43680931	188.4812916	1.193060759	0.014507978
orf01730	82.24361672	188.0173713	1.192890347	0.014104137
orf01921	115.1314866	263.0764273	1.192199542	0.004826546
orf02270	232.2354345	530.4637425	1.19166603	0.001244591
orf02095	113.8280658	258.7288559	1.184584649	0.006784213
orf00094	156.7069956	356.1359139	1.184358344	0.007649862
orf02278	79.9243056	181.51282	1.183365238	0.007899326
orf01026	122.4302802	278.0159549	1.183207261	0.003309144
orf00219	76.12889166	172.6651495	1.181460941	0.001052189
orf00306	35.37493035	80.21161412	1.181083837	0.014458339
orf00830	130.6246085	296.0431603	1.18038081	0.007495621
orf01036	102.3264269	231.2457113	1.176247825	0.017144892
orf01224	189.9088947	429.0263974	1.175758939	0.013996907
orf00745	221.8792088	500.6678897	1.174079445	2.31667E-05
orf02247	470.136979	1060.614754	1.173747656	0.000549133
orf01852	84.2001371	189.8894724	1.173265436	0.015217932
orf02070	98.48791044	222.1022279	1.173205317	0.009804577
orf00113	141.4534028	318.8232656	1.172430029	0.007110619
orf02026	145.0466785	326.4357665	1.170281879	0.010162496
orf00501	129.4533831	291.192539	1.169540723	0.008691049
orf01640	157.5541271	354.2848343	1.169062164	0.016602135
orf01838	138.2934616	310.5836078	1.16724874	0.0118024
orf01206	256.1038545	574.6617255	1.165983998	0.000934693
orf02097	204.6446049	458.9271517	1.165144531	0.00399002
orf02181	122.5927971	274.4473588	1.162655239	0.001701445
orf01514	162.0720712	362.6868556	1.162088958	0.004452408
orf01666	107.0373722	239.3149582	1.160795972	0.015779564
orf00535	167.7583666	373.9530776	1.156472537	0.001976682
orf01013	141.6377612	315.4864854	1.15537226	0.006561684
orf00022	163.0181487	362.5906606	1.153309178	0.001516905
orf01213	83.45538959	185.5286792	1.15256509	0.023673402
orf02248	272.9114133	606.4109832	1.151863153	0.000519782
orf00780	664.3078316	1476.042631	1.151810562	0.000582209
orf02325	166.9884626	370.9426663	1.151447789	0.001664178
orf01869	100.3608915	222.801516	1.150561858	0.007219378
orf01153	182.2755117	403.2644925	1.14560563	0.018479295
orf00532	135.9572293	300.2555406	1.143038002	0.005367884
orf00413	93.13505662	205.4956182	1.141711417	0.020359203
orf01792	86.93665586	191.6513925	1.140447974	0.016923652
orf00391	209.6236297	462.0887676	1.140368668	0.001169764
orf00052	2207.539363	4865.125865	1.140037966	0.000226636
orf00138	111.6310935	245.9535486	1.139646942	0.008537028
orf00272	176.9530447	389.1336214	1.13689905	8.31601E-05
orf00707	109.0564793	239.5650124	1.135341736	0.002318161
orf01843	138.3795947	303.9130614	1.13502746	0.007958921
orf00621	96.02759678	210.8504107	1.134698854	0.017713374

orf02254	254.2142085	557.0136348	1.131667975	4.79051E-05
orf00114	146.3599064	320.5329148	1.130952113	0.007164635
orf00640	910.833636	1994.129549	1.130499663	6.30063E-06
orf00738	167.5687122	366.1706352	1.1277633	0.008526813
orf01600	114.9953499	251.170925	1.127093947	0.01414898
orf01282	185.052619	403.5381496	1.124769516	0.018857106
orf02423	124.2551944	270.9288878	1.124608066	0.008338436
orf01561	173.287329	376.4758999	1.119391347	0.007626254
orf02073	104.0750436	225.7188392	1.116902672	0.003563108
orf01023	146.9059221	318.4351877	1.11610721	0.007047702
orf01062	137.1593482	297.253503	1.115840858	0.021586788
orf02110	102.6270344	222.1703102	1.114255214	0.008721146
orf00024	68.04780967	147.2550339	1.113696323	0.012279713
orf00746	149.5619593	323.0547437	1.111035383	0.006367728
orf00517	118.8267884	256.4126996	1.109607603	0.002703961
orf00404	151.2683054	326.2807323	1.109004057	0.003196754
orf02040	262.7155615	566.3267338	1.108132976	0.004669662
orf00612	106.4818588	229.1170325	1.105477051	0.010291185
orf02229	129.6574206	278.5151372	1.103050962	0.017248439
orf00180	119.6443377	256.7484826	1.101603628	0.003708816
orf00497	130.9375131	280.8271386	1.100803878	0.008274174
orf01602	124.8468954	267.5244626	1.099510873	0.012793006
orf01137	164.7030543	351.6605298	1.094316107	0.011933865
orf00392	335.724367	716.55659	1.093803394	0.000434053
orf02132	920.9097666	1962.610785	1.091642384	3.99854E-05
orf00430	180.4035769	384.2392889	1.0907771	0.001547561
orf02018	153.0703315	325.89816	1.090226524	0.002907496
orf01538	122.4458967	260.5588731	1.089464957	0.009502703
orf02382	128.1002125	272.4537612	1.088738538	0.012141952
orf01599	101.7560001	216.0196842	1.086048914	0.008448517
orf00829	157.9764018	335.0842041	1.084814612	0.003640631
orf01807	166.9601	354.0610835	1.084494909	0.012635374
orf01625	166.6831235	353.1605549	1.083216175	0.003550725
orf02035	230.5484371	486.9504328	1.078705041	0.014480513
orf01668	177.9757714	374.8560795	1.074655946	0.011205598
orf00016	240.6700328	503.9602426	1.066253409	0.001059868
orf00905	149.0521031	312.0636286	1.066023488	0.010084832
orf00457	172.4952199	360.9037366	1.065057697	0.001600107
orf02424	110.4493428	231.0677507	1.064931087	0.014062755
orf00869	176.8366625	369.7898824	1.064288342	0.005101866
orf01051	181.724789	379.6396626	1.062875494	0.004933635
orf01800	243.1065079	507.5085975	1.061843748	0.002305011
orf02361	264.9692478	552.72997	1.060749909	0.012005978
orf02272	125.6649414	262.1329672	1.06071659	0.010191552
orf02357	83.13169201	173.2859529	1.059684229	0.022407519
orf00018	179.3081045	372.6648601	1.055438091	0.002191824
orf00775	95.63675873	198.6108483	1.054307285	0.020198486
orf00488	158.3432857	328.7129221	1.053772478	0.009416889
orf02479	124.5418701	258.3955459	1.052950354	0.000273804
orf00990	189.8163253	393.7934358	1.052834985	0.008075389
orf02038	183.1826959	379.8842219	1.052276565	0.004915613
orf01758	158.3620068	328.1076012	1.050942762	0.008259325
orf00222	181.6066719	376.0708717	1.050187362	0.006883787
orf02239	447.8124611	927.2955788	1.050134602	0.000171731

orf00858	277.6521166	574.8302892	1.049857689	0.004160055
orf01488	164.0193211	339.5603055	1.049802047	0.013978299
orf01826	150.8444662	311.3661355	1.045550273	0.015515697
orf01828	241.0877287	496.3388453	1.04176715	0.006915728
orf00842	185.1307655	380.9417821	1.041025867	0.015818557
orf01030	125.3631549	257.9478988	1.040966302	0.005047228
orf00393	353.659201	727.0480719	1.039690961	1.86038E-05
orf01054	99.85368187	205.2299209	1.03935355	0.022152922
orf00524	160.3459277	329.070506	1.037209012	0.016819375
orf01339	107.2458599	219.9564794	1.036296146	0.013076265
orf00846	180.6435411	370.228563	1.035270531	0.01819124
orf01964	124.1501998	254.2072118	1.033918376	0.01217402
orf01764	201.492403	412.4640366	1.033542889	0.007863402
orf00506	114.9019961	235.1914113	1.033431515	0.010056999
orf00956	140.841523	287.9302251	1.031646509	0.016975843
orf01842	179.0839831	365.8474549	1.030605911	0.00390383
orf02173	190.7252368	389.3196059	1.029459246	0.022781764
orf01925	176.6889104	360.6537346	1.029402869	0.0166098
orf02245	207.7444418	424.0176276	1.029314365	0.002718748
orf02167	144.118114	293.6678237	1.026933525	0.010193728
orf01999	133.8736982	272.7214531	1.026555645	0.00499887
orf01543	114.2689879	232.635935	1.02564005	0.022664681
orf00667	332.9571	675.7478066	1.021148619	0.004890223
orf01936	275.4726246	557.7113146	1.017609583	0.002307803
orf02031	129.0040663	261.0934239	1.01714958	0.005640026
orf01801	140.1214166	283.5696263	1.017025532	0.009203016
orf00770	275.4163785	556.4067446	1.014525552	0.004485515
orf02084	141.1630294	284.5924863	1.011535277	0.014888937
orf00051	1618.359393	3258.164444	1.009527394	0.005641889
orf02194	136.3279137	274.3750265	1.009068184	0.020659661
orf00023	124.0637567	249.5184201	1.008064607	0.014094559
orf01559	143.3268268	288.1520262	1.007521497	0.022278661
orf00686	219.703335	441.50419	1.00687036	0.01421974
orf01592	192.0370586	385.5366182	1.00548315	0.018797891
orf00737	160.7754554	322.4883679	1.004199947	0.014371112
orf00594	104.0041298	208.5768119	1.003937962	0.02163435
orf01235	128.8455039	258.3615165	1.003748998	0.016941653
orf02283	233.8873258	468.2434651	1.001445175	0.004985486

Supplementary Table 2. Significantly down-regulated genes

Gene ID	vitro_rpkm	vivo_rpkm	log2 Ratio (vivo/vitro)	*p* value
orf00552	4073.833552	121.8713431	−5.062956201	2.88526E-29
orf00555	7065.805948	298.9772825	−4.562746356	3.83846E-49
orf00553	5724.54799	349.4545696	−4.033984965	2.21838E-41
orf01049	352.7366738	37.99487144	−3.214714978	2.72049E-09
orf00564	2177.682467	244.78002	−3.153235898	1.14192E-22
orf00569	4911.827951	552.6270852	−3.15188185	2.87785E-23
orf00340	5035.018159	571.080387	−3.140231238	1.36527E-28
orf00160	1654.213583	190.5680889	−3.117767058	1.31985E-21
orf00828	4632.753922	607.2910651	−2.931410004	2.95486E-18
orf00454	1469.214985	227.945521	−2.68828455	3.58331E-12
orf02088	3727.655586	598.674191	−2.638425588	2.78114E-19
orf00964	1676.315383	329.4752361	−2.347051665	2.18179E-14
orf01467	2132.045264	420.4319602	−2.342293819	1.97465E-13
orf02231	13844.43329	2818.693207	−2.296207634	1.55447E-09
orf01468	2186.316117	447.8610411	−2.287378936	6.30364E-15
orf01779	2039.485349	418.3610761	−2.285384607	2.06847E-11
orf01432	1028.334679	214.3082179	−2.262550798	4.06606E-10
orf00424	790.2548832	165.133625	−2.258684129	7.97709E-11
orf01465	2731.913945	587.5361108	−2.21716261	4.35042E-15
orf01926	9047.135438	2077.231835	−2.122798828	6.76554E-15
orf01778	2894.044801	686.1867354	−2.076414113	7.27009E-15
orf01116	2306.139081	555.8676505	−2.052666193	1.04588E-12
orf01466	2473.80617	598.6799179	−2.046875683	5.26847E-08
orf01929	1444.920176	350.6508083	−2.042882835	8.05477E-09
orf01931	1444.248835	356.855715	−2.016906548	1.4963E-09
orf01100	2041.756724	508.6766036	−2.004990334	1.64841E-09
orf01464	2403.730064	609.8895772	−1.978654926	5.09483E-10
orf02407	1411.459549	365.0891389	−1.950867128	1.23516E-10
orf01114	1698.763759	446.3739178	−1.9281606	4.90433E-09
orf00255	829.9425466	220.4070821	−1.912840887	5.00178E-06
orf01115	2089.311847	556.2570377	−1.909204253	4.29597E-13
orf02169	1285.365919	343.4605156	−1.903962965	1.68922E-07
orf00975	1354.302869	363.1381995	−1.898959807	6.57076E-11
orf01415	3012.850989	820.546463	−1.876472388	2.21564E-10
orf01112	3905.566394	1069.830805	−1.868149135	6.79866E-09
orf01098	910.0245994	252.0896018	−1.851968933	2.65394E-05
orf02057	694.5790466	205.7064048	−1.755552176	1.08993E-06
orf01101	2188.527767	664.381257	−1.719877409	5.12194E-08
orf01930	1728.611983	533.4003863	−1.696323293	3.31404E-09
orf01895	1687.298051	522.7178007	−1.690610644	5.04246E-09
orf00565	696.148879	218.5324994	−1.671548027	4.15432E-05
orf01097	1339.855946	422.7830585	−1.664088426	6.91919E-07
orf00511	1397.259676	446.3718259	−1.64628229	3.07258E-09
orf01041	2814.261814	915.9956568	−1.619343888	2.4554E-08
orf01113	3392.543569	1112.912828	−1.60802675	3.8295E-08
orf01897	2408.650792	803.5025183	−1.583850795	1.26808E-07
orf01457	801.7232455	270.8257998	−1.565739123	8.31987E-07
orf00447	1399.819043	491.6354035	−1.509579624	7.32563E-07
orf02186	2096.213107	738.1704432	−1.505759516	2.99692E-09

orf01107	1789.93113	634.2295136	−1.49682716	2.34339E-05
orf00453	1920.149847	682.7336994	−1.491824033	7.85831E-07
orf00049	7389.933103	2639.227068	−1.485445825	3.93451E-07
orf01111	2462.693689	882.9170015	−1.479887467	7.47226E-07
orf01655	327.7848582	117.754826	−1.476963023	0.004012393
orf01102	1536.796524	555.0892537	−1.469134492	7.73675E-05
orf02089	2257.454759	828.6618746	−1.445841623	4.0021E-06
orf01942	1896.632894	696.963395	−1.44428567	2.71979E-08
orf01896	1910.505252	705.7884503	−1.436646497	1.26557E-05
orf01683	1219.577379	454.8362099	−1.422962279	3.56694E-07
orf01932	658.6474763	245.9013337	−1.421426947	0.000181117
orf02064	10092.50716	3774.25042	−1.419022557	5.81296E-07
orf01109	1609.03579	602.3395163	−1.417547602	1.06898E-05
orf01230	2214.95433	831.9711731	−1.412671506	1.76279E-06
orf01105	1761.427994	668.5222338	−1.397698052	1.85922E-05
orf01104	3761.205369	1433.773634	−1.391377815	8.92823E-06
orf01431	762.8692414	292.3924835	−1.38352957	3.25852E-07
orf01096	2637.303371	1015.617632	−1.376706188	1.83005E-06
orf01941	1504.549389	579.4151293	−1.376662205	4.85993E-08
orf01181	2201.601465	849.3098622	−1.374190427	1.27171E-05
orf01448	1369.59566	531.9614366	−1.364356465	3.11888E-05
orf01439	1926.731528	749.7288415	−1.361714752	3.23996E-08
orf01110	1697.999999	662.956925	−1.356849417	9.25428E-07
orf01301	5776.297285	2261.948384	−1.352578986	5.26348E-06
orf01251	1325.13717	519.033455	−1.352242269	1.62906E-06
orf01482	569.7594514	226.1189013	−1.333271359	0.007458881
orf00382	1719.973347	684.5303753	−1.329199743	1.90663E-07
orf01229	1756.953341	700.2270494	−1.32718118	1.50234E-07
orf01090	1049.365672	418.8098128	−1.325150351	3.58402E-05
orf00965	1129.555238	452.6298027	−1.319351339	5.94883E-07
orf01091	1742.701774	699.4265273	−1.317081285	2.95035E-06
orf00509	758.6869142	304.6187981	−1.316499681	2.29655E-05
orf00356	878.0718349	357.7438516	−1.295411999	6.37605E-06
orf01048	1234.075681	506.8415951	−1.28382404	6.90614E-06
orf02307	2860.738894	1183.863624	−1.272884928	2.86405E-06
orf00358	317.5403667	132.1166879	−1.265127296	0.001629486
orf01358	3775.381827	1573.745767	−1.262420062	4.54113E-07
orf00735	876.2457855	367.0669633	−1.255292325	0.000464471
orf01084	2864.361774	1202.563131	−1.252101086	1.03455E-06
orf02056	1481.746886	622.6404308	−1.25082786	2.74322E-06
orf01793	886.6452675	374.7170502	−1.242555397	4.05514E-05
orf01099	4377.202607	1850.788905	−1.241868808	0.000187829
orf01106	1962.266562	844.6193971	−1.216147752	0.000128084
orf01322	1886.425708	819.2324771	−1.20331047	3.8187E-06
orf01777	1313.809098	571.9765393	−1.199727782	0.000273114
orf02065	2099.699226	922.6868791	−1.186269635	4.38876E-06
orf01796	777.7629206	341.9420435	−1.185578636	0.00012012
orf00305	654.2519799	287.885106	−1.184353233	5.29047E-06
orf01449	3337.173037	1471.561643	−1.181278516	5.06577E-05
orf00357	691.1937851	306.0057009	−1.175531715	8.61038E-05
orf00751	832.1301522	370.7017383	−1.166550315	0.000220447
orf00379	1653.672991	737.284616	−1.165380415	2.28782E-06
orf01908	1484.039895	663.0679998	−1.16230114	2.34193E-05
orf01383	1898.583823	851.6101349	−1.156658671	2.40942E-06

orf01108	691.3710237	313.9955447	−1.138716051	0.005552332
orf02189	2805.567042	1282.141626	−1.129736756	3.04183E-05
orf00768	2400.31534	1097.275346	−1.129298357	6.91911E-06
orf01414	1391.992197	644.1209362	−1.111747634	0.004155494
orf00352	344.5397977	159.7602331	−1.108762295	0.000822661
orf00315	1073.538463	500.8763899	−1.099847368	0.000111189
orf00727	5242.721068	2451.611374	−1.096585488	2.27513E-05
orf00817	1116.599621	525.0337747	−1.088629834	0.003869284
orf01547	1598.137856	755.2763251	−1.081315391	7.93279E-05
orf01776	1075.361207	508.6915295	−1.079958357	0.004751798
orf01944	1143.830486	542.2409575	−1.076867268	8.89618E-06
orf01016	1478.467856	706.8199767	−1.064688157	2.86809E-05
orf00263	1596.505343	772.9164037	−1.046533091	3.33188E-05
orf00566	347.0500675	168.3075208	−1.044044165	0.019553606
orf01578	549.9224872	269.9475883	−1.026548954	0.009809118
orf01092	2438.712443	1199.936486	−1.02316161	0.000430389
orf01333	1418.849667	703.2831801	−1.012544119	4.58131E-05
orf00972	463.5778182	230.1987963	−1.009931247	0.016223162

Supplementary Table 3. Gene Ontology analysis of the differentially expressed genes

Ontology	Class	No. of vitro-VS-vivo up	No. of vitro-VS-vivo down	Genes of vitro-VS-vivo up	Genes of vitro-VS-vivo down
biological_process	anatomical structure formation	3	0	orf02451;orf02135;orf01152	-
biological_process	biological regulation	43	8	orf02114;orf01235;orf02115;orf02132;orf02116;orf00102;orf00417;orf00524;orf01643;orf00297;orf02451;orf02135;orf02176;orf00023;orf00418;orf00488;orf02410;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01340;orf01003;orf01341;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf01666;orf01282;orf00043;orf02218;orf00584;orf00956	orf00552;orf00553;orf01930;orf02064;orf01944;orf00972;orf00263;orf00768
biological_process	cellular component biogenesis	15	39	orf02451;orf02135;orf00667;orf00781;orf01152;orf01719;orf01496;orf01282;orf01668;orf00043;orf00186;orf02325;orf00605;orf02458;orf00780	orf00340;orf01110;orf01931;orf01930;orf01109;orf01896;orf01932;orf00565;orf01897;orf01114;orf01929;orf01100;orf01092;orf01098;orf00255;orf01779;orf01101;orf01102;orf01112;orf01777;orf01482;orf00564;orf01104;orf01776;orf01108;orf01097;orf01111;orf01895;orf01414;orf01113;orf01116;orf01099;orf01106;orf01105;orf01090;orf00566;orf01415;orf01107;orf01115
biological_process	cellular component organization	10	0	orf02451;orf02135;orf00488;orf02410;orf01666;orf01282;orf00043;orf01152;orf00979;orf00980	-
biological_process	cellular process	198	90	orf02114;orf01235;orf02115;orf02132;orf02116;orf00102;orf00457;orf00943;orf00941;orf00606;orf00628;orf02194;orf02475;orf02306;orf01661;orf00796;orf00078;orf00368;orf00393;orf01792;orf01913;orf02147;orf01133;orf00686;orf0013	orf01383;orf01084;orf00735;orf01230;orf01229;orf00315;orf01358;orf00727;orf02307;orf00965;orf00964;orf00509;orf00511;orf01908;orf00263;orf00768;orf01016;orf01942;orf01091;orf01941;orf00305;orf01778;orf01926;orf01457;orf0037

8;orf01132;orf00187;orf00459;orf01339;orf01485;orf00488;orf00094;orf00134;orf02097;orf01945;orf01158;orf02096;orf00869;orf00638;orf02143;orf00612;orf01135;orf00232;orf00539;orf00413;orf00307;orf00645;orf01925;orf00497;orf00383;orf00963;orf01487;orf00524;orf01025;orf00726;orf00637;orf01031;orf01625;orf00610;orf01039;orf01995;orf00737;orf01805;orf01872;orf01641;orf00775;orf00198;orf01534;orf00387;orf01722;orf02050;orf02451;orf02135;orf00417;orf01643;orf00297;orf02176;orf00023;orf00418;orf00667;orf00781;orf00956;orf00780;orf02410;orf00343;orf00402;orf00707;orf00809;orf00550;orf02283;orf01062;orf01600;orf00180;orf00179;orf02016;orf00178;orf00391;orf00970;orf00077;orf00392;orf00222;orf00595;orf00272;orf00532;orf00298;orf01058;orf02293;orf00724;orf00259;orf02179;orf02100;orf01559;orf01852;orf00016;orf01282;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01340;orf01003;orf01341;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf00183;orf01666;orf00043;orf01152;orf02038;orf01865;orf00758;orf02289;orf02287;orf02290;orf00625;orf02288;orf00527;orf02294;orf02297;orf00908;orf01019;orf00523;orf00522;orf02159;orf00598;orf02127;orf01719;orf01496;orf01668;orf00186;orf02325;orf02382;orf00905;orf00858;orf01754;orf01807;orf02102;orf00621;orf02347;orf02346;orf00132;orf02044;orf01801;orf00594;orf02222;orf00670;orf02252;orf00708;orf02095;orf00605;orf02458;orf00600;orf02218;orf00607;orf02015;o

9;orf00382;orf01415;orf00552;orf00553;orf00340;orf01110;orf01931;orf01109;orf01896;orf01932;orf00565;orf01897;orf01114;orf01929;orf01100;orf01092;orf01098;orf00255;orf01779;orf01101;orf01102;orf01112;orf01777;orf01482;orf00564;orf01104;orf01776;orf01108;orf01097;orf01111;orf01895;orf01414;orf01113;orf01116;orf01099;orf01106;orf01105;orf01090;orf00566;orf01107;orf01115;orf01930;orf00828;orf00569;orf00454;orf02088;orf02089;orf00817;orf00049;orf01096;orf00447;orf00356;orf00424;orf01683;orf01793;orf02064;orf01944;orf00972;orf02189;orf00357;orf01796;orf00453;orf02186;orf00751;orf00352

				rf00979;orf00980;orf00898;orf00696;orf01838;orf00584;orf00693;orf00489;orf00517;orf00024;orf00567;orf00578	
biological_process	developmental process	3	0	orf01666;orf01282;orf00043	-
biological_process	establishment of localization	52	12	orf01485;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01136;orf00535;orf01340;orf01867;orf01003;orf01341;orf00138;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf00489;orf01693;orf01692;orf00567;orf01158;orf02229;orf01559;orf01852;orf00809;orf01058;orf00232;orf00150;orf01024;orf00018;orf01498;orf02096;orf02450;orf02448;orf01913;orf02147;orf00829;orf00830;orf00024;orf00578;orf02240;orf00638;orf00517	orf01096;orf01251;orf02064;orf01944;orf00972;orf02065;orf00735;orf00511;orf02186;orf01908;orf00509;orf00352
biological_process	immune system process	2	0	orf00979;orf00980	-
biological_process	localization	52	12	orf01485;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01136;orf00535;orf01340;orf01867;orf01003;orf01341;orf00138;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf00489;orf01693;orf01692;orf00567;orf01158;orf02229;orf01559;orf01852;orf00809;orf01058;orf00232;orf00150;orf01024;orf00018;orf01498;orf02096;orf02450;orf02448;orf01913;orf02147;orf00829;orf00830;orf00024;orf00578;orf02240;orf00638;orf00517	orf01096;orf01251;orf02064;orf01944;orf00972;orf02065;orf00735;orf00511;orf02186;orf01908;orf00509;orf00352
biological_process	metabolic process	217	92	orf00457;orf00943;orf00941;orf00606;orf02311;orf01867;orf01845;orf02309;orf00628;orf02194;orf02475;orf02306;orf00796;orf00169;orf01856;orf01002;orf00121;orf01661;orf00078;orf00368	orf01383;orf01084;orf00735;orf00315;orf01230;orf01229;orf01358;orf00727;orf02307;orf00965;orf00964;orf00509;orf00511;orf01908;orf00263;orf00768;orf01016;orf01942;orf01091;orf01941

;orf00393;orf01792;orf01913;orf02147;orf01133;orf00686;orf00138;orf01132;orf00187;orf00459;orf01339;orf01485;orf00488;orf00094;orf00134;orf02097;orf01945;orf01158;orf02096;orf00869;orf00638;orf02143;orf00612;orf01135;orf00232;orf00539;orf00413;orf00307;orf00645;orf01925;orf00497;orf00383;orf00963;orf01487;orf00524;orf01025;orf00726;orf00637;orf01031;orf01625;orf00610;orf01039;orf01995;orf00737;orf01805;orf01872;orf01641;orf00775;orf00198;orf01534;orf00387;orf01722;orf02050;orf02451;orf02135;orf00417;orf02114;orf01643;orf02132;orf00297;orf02176;orf00023;orf00418;orf00667;orf00781;orf00956;orf00780;orf02410;orf00343;orf00402;orf00707;orf00809;orf01343;orf01481;orf00746;orf01157;orf01959;orf01721;orf02480;orf00404;orf00221;orf00031;orf00238;orf02203;orf01473;orf00110;orf00745;orf01599;orf00239;orf00101;orf00550;orf02283;orf01062;orf01600;orf00180;orf00179;orf02016;orf00178;orf00391;orf00970;orf00077;orf00392;orf01496;orf02222;orf01282;orf00222;orf00595;orf00272;orf00532;orf00298;orf01058;orf02293;orf00724;orf00259;orf02179;orf02100;orf01488;orf01538;orf01968;orf00244;orf01052;orf00599;orf01640;orf02423;orf00183;orf02038;orf01865;orf01224;orf01857;orf00621;orf00758;orf02289;orf02287;orf02290;orf00625;orf02288;orf00527;orf02294;orf02297;orf00908;orf01019;orf00523;orf00522;orf02159;orf00598;orf02127;orf01719;orf01668;orf00043;orf02382;orf00905;orf01754;orf01807;orf02102;orf02347;orf02346;orf00132;orf02044;orf01801;orf00594;orf00670;or

;orf00305;orf01778;orf01926;orf01457;orf00379;orf00382;orf01415;orf00552;orf00553;orf00340;orf01110;orf01931;orf01109;orf01896;orf01932;orf00565;orf01897;orf01114;orf01929;orf01100;orf01092;orf01098;orf00255;orf01779;orf01101;orf01102;orf01112;orf01777;orf01482;orf00564;orf01104;orf01776;orf01108;orf01097;orf01111;orf01895;orf01414;orf01113;orf01116;orf01099;orf01106;orf01105;orf01090;orf00566;orf01107;orf01115;orf01930;orf00828;orf00569;orf00454;orf02407;orf01439;orf02088;orf02089;orf00817;orf00049;orf00447;orf00356;orf00424;orf01683;orf01793;orf01333;orf02189;orf00357;orf01796;orf01048;orf01049;orf00453;orf02186;orf00751;orf01465;orf01464

				f02325;orf02252;orf00708;orf02095;orf00605;orf02458;orf00600;orf02218;orf02015;orf01762;orf01679;orf00448;orf01860;orf00416;orf02035;orf00979;orf00980;orf00898;orf00696;orf01838;orf00584;orf00693;orf00866;orf01828;orf02160;orf00301;orf00738;orf00399;orf00501;orf00535;orf02110;orf02190;orf01576;orf01038;orf00079;orf00990	
biological_process	multi-organism process	3	0	orf01023;orf00979;orf00980	-
biological_process	multicellular organismal process	1	0	orf00584	-
biological_process	pigmentation	43	8	orf02114;orf01235;orf02115;orf02132;orf02116;orf00102;orf00417;orf00524;orf01643;orf00297;orf02451;orf02135;orf02176;orf00023;orf00418;orf00488;orf02410;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01340;orf01003;orf01341;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf01666;orf01282;orf00043;orf02218;orf00584;orf00956	orf00552;orf00553;orf01930;orf02064;orf01944;orf00972;orf00263;orf00768
biological_process	response to stimulus	27	9	orf00524;orf01025;orf00726;orf00963;orf00497;orf00637;orf01031;orf01625;orf00610;orf01039;orf01995;orf00737;orf01805;orf01158;orf00430;orf00866;orf00301;orf00078;orf00077;orf01133;orf01132;orf00724;orf00259;orf00607;orf02321;orf00979;orf00980	orf01415;orf00453;orf00828;orf00569;orf00454;orf01048;orf01049;orf00964;orf00049
cellular_component	cell	143	82	orf02289;orf00489;orf02287;orf01995;orf00737;orf02288;orf01031;orf00610;orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418;orf01487;orf02294;orf01133;orf00343;orf00934;orf00180;orf01485;orf00905;orf00667;orf0072	orf01084;orf00305;orf00379;orf00382;orf01942;orf01091;orf01941;orf02407;orf00453;orf01793;orf00552;orf00315;orf00828;orf01048;orf01049;orf01358;orf00768;orf01683;orf00454;orf00553;orf00340;orf01931;orf01109;orf00751;orf0189

				6;orf00094;orf00956;orf00134;orf00527;orf02156;orf02102;orf00963;orf02100;orf00497;orf02038;orf02458;orf01805;orf01132;orf00402;orf00708;orf00606;orf01062;orf01807;orf00645;orf02290;orf00222;orf00595;orf00780;orf00488;orf02410;orf00781;orf01925;orf02242;orf02254;orf00829;orf01677;orf00684;orf00638;orf01136;orf02031;orf01340;orf01003;orf00517;orf01341;orf01244;orf01970;orf00830;orf02166;orf02226;orf00051;orf01559;orf02073;orf01282;orf01894;orf00043;orf01028;orf02455;orf02225;orf02457;orf01852;orf01913;orf01668;orf00359;orf00248;orf00770;orf01746;orf00457;orf01872;orf01693;orf02113;orf01056;orf01152;orf00370;orf02311;orf00150;orf01054;orf00018;orf01868;orf01869;orf02115;orf01692;orf02116;orf01498;orf01845;orf00024;orf00567;orf00578;orf02309;orf02203;orf00102;orf00053;orf02240;orf00052;orf01921;orf00707;orf02478;orf01058;orf00016;orf01235;orf02229;orf01158;orf00151;orf02450;orf02096;orf01496;orf01666;orf01514;orf01867;orf02448;orf00531;orf02008;orf02239;orf00122;orf02142;orf01052;orf01068;orf00234;orf02278;orf01586;orf00629;orf00809;orf02159;orf02147	6;orf01932;orf01778;orf01041;orf00565;orf01897;orf01114;orf01929;orf01100;orf00255;orf01779;orf01101;orf01102;orf01482;orf00564;orf01104;orf01108;orf01926;orf01414;orf01116;orf01105;orf01090;orf00566;orf01415;orf01107;orf01096;orf01115;orf00509;orf02064;orf00263;orf01944;orf00511;orf02186;orf01016;orf00160;orf01230;orf02089;orf01229;orf01930;orf01098;orf01097;orf01111;orf01113;orf01106;orf01110;orf01092;orf01112;orf01777;orf01776;orf01895;orf01099;orf00972;orf00352;orf02065;orf00357;orf01908;orf00964;orf00965
cellular_component	cell part	143	82	orf02289;orf00489;orf02287;orf01995;orf00737;orf02288;orf01031;orf00610;orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418;orf01487;orf02294;orf01133;orf00343;orf00934;orf00180;orf01485;orf00905;orf00667;orf00726;orf00094;orf00956;orf00134;orf00527;orf02156;orf02102;orf00963;orf02100;orf00497;orf02038;orf02458;orf01805;orf01132;orf00402;orf0	orf01084;orf00305;orf00379;orf00382;orf01942;orf01091;orf01941;orf02407;orf00453;orf01793;orf00552;orf00315;orf00828;orf01048;orf01049;orf01358;orf00768;orf01683;orf00454;orf00553;orf00340;orf01931;orf01109;orf00751;orf01896;orf01932;orf01778;orf01041;orf00565;orf01897;orf01114;orf01929;orf01100;orf00255;orf01779;orf01101;orf01102;orf01482;orf00564;orf0

				0708;orf00606;orf01062;orf01807;orf00645;orf02290;orf00222;orf00595;orf00780;orf00488;orf02410;orf00781;orf01925;orf02242;orf02254;orf00829;orf01677;orf00684;orf00638;orf01136;orf02031;orf01340;orf01003;orf00517;orf01341;orf01244;orf01970;orf00830;orf02166;orf02226;orf00051;orf01559;orf02073;orf01282;orf01894;orf00043;orf01028;orf02455;orf02225;orf02457;orf01852;orf01913;orf01668;orf00359;orf00248;orf00770;orf01746;orf00457;orf01872;orf01693;orf02113;orf01056;orf01152;orf00370;orf02311;orf00150;orf01054;orf00018;orf01868;orf01869;orf02115;orf01692;orf02116;orf01498;orf01845;orf00024;orf00567;orf00578;orf02309;orf02203;orf00102;orf00053;orf02240;orf00052;orf01921;orf00707;orf02478;orf01058;orf00016;orf01235;orf02229;orf01158;orf00151;orf02450;orf02096;orf01496;orf01666;orf01514;orf01867;orf02448;orf00531;orf02008;orf02239;orf00122;orf02142;orf01052;orf01068;orf00234;orf02278;orf01586;orf00629;orf00809;orf02159;orf02147	1104;orf01108;orf01926;orf01414;orf01116;orf01105;orf01090;orf00566;orf01415;orf01107;orf01096;orf01115;orf00509;orf02064;orf00263;orf01944;orf00511;orf02186;orf01016;orf00160;orf01230;orf02089;orf01229;orf01930;orf01098;orf01097;orf01111;orf01113;orf01106;orf01110;orf01092;orf01112;orf01777;orf01776;orf01895;orf01099;orf00972;orf00352;orf02065;orf00357;orf01908;orf00964;orf00965
cellular_component	envelope	19	3	orf02242;orf02254;orf01677;orf00684;orf00359;orf01340;orf01003;orf01341;orf01244;orf01970;orf02166;orf02226;orf00051;orf00248;orf01894;orf01028;orf02455;orf02225;orf00150	orf02064;orf01944;orf00160
cellular_component	extracellular region	0	1	-	orf01084
cellular_component	macromolecular complex	27	55	orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418;orf00222;orf00595;orf00780;orf00488;orf02410;orf00781;orf01913;orf00489;orf00550;orf00457;orf01872;orf01235;orf02115;orf02116;orf00102;orf01031;orf01805;orf0185	orf01084;orf00340;orf01931;orf01109;orf00751;orf01896;orf01932;orf01778;orf01041;orf00565;orf01897;orf01114;orf01929;orf01100;orf00255;orf01779;orf01101;orf01102;orf01482;orf00564;orf01104;orf01108;orf01926;orf01414;orf0111

				2;orf00963;orf02147	6;orf01105;orf01090;orf00566;orf01415;orf01107;orf01096;orf01115;orf01016;orf01230;orf02089;orf01229;orf01930;orf01098;orf01097;orf01111;orf01113;orf01106;orf01110;orf01092;orf01112;orf01777;orf01776;orf01895;orf01099;orf00509;orf00511;orf02186;orf01908;orf00964;orf00965
cellular_component	membrane-enclosed lumen	7	3	orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418	orf01942;orf01091;orf01941
cellular_component	organelle	15	49	orf01031;orf00610;orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418;orf01487;orf00780;orf00488;orf02410;orf00781;orf00809	orf00379;orf00382;orf01942;orf01091;orf01941;orf00340;orf01931;orf01109;orf00751;orf01896;orf01932;orf01778;orf01041;orf00565;orf01897;orf01114;orf01929;orf01100;orf00255;orf01779;orf01101;orf01102;orf01482;orf00564;orf01104;orf01108;orf01926;orf01414;orf01116;orf01105;orf01090;orf00566;orf01415;orf01107;orf01096;orf01115;orf01930;orf01098;orf01097;orf01111;orf01113;orf01106;orf01110;orf01092;orf01112;orf01777;orf01776;orf01895;orf01099
cellular_component	organelle part	10	16	orf01031;orf00610;orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418;orf00809	orf01942;orf01091;orf01941;orf01930;orf01098;orf01097;orf01111;orf01113;orf01106;orf01110;orf01092;orf01112;orf01777;orf01776;orf01895;orf01099
molecular_function	antioxidant activity	3	1	orf00866;orf00301;orf01038	orf00049
molecular_function	binding	151	71	orf00457;orf01828;orf01800;orf00417;orf00898;orf00077;orf00696;orf00874;orf01838;orf00595;orf00941;orf00202;orf01792;orf00522;orf01640;orf00418;orf02294;orf01025;orf00094;orf00963;orf01872;orf00621;orf00606;orf00187;orf00775;orf01899;orf01754;orf00610;orf02114;orf02218;orf01039;orf00607;orf01643;orf01995;orf00584;orf00198;orf02257;orf00637;orf01031;orf0	orf01930;orf01109;orf01779;orf01777;orf01104;orf01776;orf01114;orf01084;orf00828;orf00555;orf00552;orf01942;orf01091;orf01415;orf00553;orf01941;orf00340;orf01048;orf00263;orf01049;orf00768;orf01112;orf01110;orf00751;orf01778;orf01041;orf01926;orf00379;orf00382;orf01796;orf01457;orf02407;orf01439;orf00453;orf00509;orf01358;orf01229;orf00569;orf00454;orf0

				0737;orf01805;orf02451;orf02135;orf02176;orf01534;orf02382;orf00387;orf00023;orf02050;orf00726;orf00497;orf00383;orf00524;orf00297;orf02095;orf02475;orf00956;orf00628;orf02194;orf02306;orf02087;orf02423;orf01487;orf00866;orf02148;orf00905;orf00301;orf01024;orf02149;orf02147;orf02097;orf01945;orf01235;orf01158;orf02096;orf00869;orf02115;orf00638;orf01600;orf02116;orf00251;orf01913;orf02143;orf02102;orf00612;orf00307;orf01625;orf01135;orf02052;orf02179;orf02420;orf00232;orf00102;orf00532;orf00539;orf01807;orf00645;orf01485;orf00488;orf00134;orf00298;orf02289;orf00413;orf00489;orf00746;orf02287;orf02127;orf00404;orf01339;orf00745;orf00625;orf02288;orf01852;orf00186;orf02325;orf01668;orf02160;orf02410;orf01133;orf00368;orf00180;orf02293;orf02297;orf00121;orf01894;orf01979;orf00781;orf01752;orf01525;orf01722;orf00407;orf00979;orf00829;orf00724;orf00535;orf02156;orf00830;orf02038;orf00244;orf01488;orf00259;orf00980;orf00738;orf00501;orf00079;orf00990;orf00179;orf02016;orf00178;orf02100;orf00022	0511;orf02186;orf00305;orf00565;orf01929;orf01100;orf01092;orf01101;orf01102;orf00564;orf01097;orf01111;orf01113;orf01116;orf01099;orf01106;orf01105;orf00566;orf01107;orf01115;orf01908;orf00049;orf01467;orf01383;orf00358;orf01793;orf02307;orf00817;orf00727;orf01301;orf01464;orf00964
molecular_function	catalytic activity	242	50	orf01339;orf01235;orf02115;orf02116;orf00102;orf01002;orf00898;orf01968;orf02132;orf00244;orf01838;orf01052;orf00599;orf01640;orf00272;orf01865;orf00550;orf00077;orf00943;orf00941;orf00625;orf00963;orf01039;orf00737;orf00524;orf01487;orf01485;orf00488;orf00094;orf00134;orf00501;orf00078;orf00368;orf00724;orf00259;orf00183;orf00079;orf00610;orf01913;orf02297;orf01058;orf02100;orf00413;orf00645;orf02147;orf00866;orf00301;orf01807;orf02294;or	orf00964;orf00768;orf00735;orf01333;orf00817;orf01942;orf01091;orf01941;orf00379;orf00382;orf00305;orf01778;orf01926;orf01230;orf01229;orf01383;orf00049;orf02089;orf01908;orf00972;orf02407;orf01793;orf00727;orf00263;orf01358;orf01084;orf00357;orf01016;orf00447;orf00315;orf01683;orf00555;orf00965;orf01439;orf00356;orf00424;orf02088;orf01465;orf01464;orf01113;orf02065;orf00358;orf01547;orf01322;orf02189;orf01457;orf01048;orf01049;orf00509;or

f01925;orf01762;orf01343;orf00746;orf00858;orf00031;orf00219;orf02203;orf00408;orf00745;orf00234;orf01561;orf00043;orf00404;orf01481;orf01157;orf00221;orf00238;orf00239;orf00686;orf00222;orf00595;orf00990;orf01600;orf01661;orf01800;orf02420;orf00796;orf00970;orf02290;orf00532;orf02447;orf00979;orf02221;orf01995;orf02382;orf00980;orf00956;orf01899;orf01856;orf02102;orf01792;orf00179;orf02016;orf00178;orf00459;orf00180;orf02283;orf00531;orf00402;orf00606;orf02179;orf00598;orf01722;orf02050;orf02306;orf00457;orf00307;orf00775;orf01679;orf00448;orf01860;orf00416;orf02035;orf02222;orf00187;orf00726;orf00605;orf00600;orf02218;orf00628;orf02194;orf00707;orf01959;orf01721;orf00110;orf02480;orf01599;orf01999;orf01473;orf00523;orf00522;orf00391;orf00392;orf00693;orf02252;orf01158;orf02127;orf02289;orf02287;orf02288;orf02293;orf01719;orf02325;orf02015;orf01668;orf01025;orf00908;orf00527;orf00621;orf01496;orf01282;orf00393;orf01062;orf00101;orf01872;orf01133;orf01132;orf01031;orf01805;orf02095;orf02475;orf00232;orf02096;orf01892;orf00132;orf02044;orf02176;orf01801;orf00343;orf02160;orf00535;orf02110;orf01576;orf01038;orf02190;orf00298;orf00399;orf00846;orf01840;orf00905;orf00910;orf00215;orf01224;orf01842;orf01490;orf02263;orf00250;orf00130;orf01676;orf01843;orf00912;orf01538;orf02423;orf01056;orf01054;orf01068;orf00419;orf01857;orf00168;orf01019;orf00107;orf01418;orf02424;orf02482;orf02347;orf02173;orf01213;orf02318;orf02200;orf01036;orf00	f00511

				618;orf01211;orf02346;orf02457;orf02227;orf00809;orf02094;orf00708;orf00594;orf00670;orf02248;orf00169;orf01488;orf00758;orf02144;orf00696;orf02097;orf01945;orf00869;orf00638;orf02143;orf00612;orf01135;orf00539;orf01641;orf00497;orf02052;orf00738;orf00121;orf01828;orf00138;orf02458	
molecular_function	electron carrier activity	5	4	orf00866;orf02148;orf00301;orf00535;orf02149	orf01908;orf00964;orf01467;orf01464
molecular_function	enzyme regulator activity	0	1	-	orf00828
molecular_function	molecular transducer activity	26	3	orf01235;orf02115;orf02116;orf00102;orf02114;orf02132;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01340;orf01003;orf01341;orf01244;orf01970;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225	orf02064;orf01944;orf00972
molecular_function	structural molecule activity	1	39	orf00780	orf00340;orf01110;orf01931;orf01930;orf01109;orf01896;orf01932;orf00565;orf01897;orf01114;orf01929;orf01100;orf01092;orf01098;orf00255;orf01779;orf01101;orf01102;orf01112;orf01777;orf01482;orf00564;orf01104;orf01776;orf01108;orf01097;orf01111;orf01895;orf01414;orf01113;orf01116;orf01099;orf01106;orf01105;orf01090;orf00566;orf01415;orf01107;orf01115
molecular_function	transcription regulator activity	9	0	orf02114;orf02132;orf00417;orf00524;orf00297;orf02451;orf02135;orf00023;orf00418	-
molecular_function	translation regulator activity	2	4	orf00488;orf02410	orf00751;orf01778;orf01041;orf01926
molecular_function	transporter activity	48	9	orf01913;orf02147;orf02113;orf02242;orf02254;orf01677;orf00684;orf01868;orf01869;orf01136;orf01340;orf01003;orf00517;orf01341;orf01244;orf01970	orf01908;orf01251;orf02064;orf01944;orf00972;orf02065;orf00509;orf00511;orf02186

;orf02166;orf02226;orf00051;orf01894;orf00053;orf01028;orf02455;orf02225;orf00567;orf00489;orf01693;orf01692;orf02450;orf02448;orf01852;orf01158;orf00150;orf02073;orf00018;orf01058;orf01498;orf02478;orf02239;orf02229;orf00829;orf00830;orf00232;orf02096;orf02240;orf00638;orf00024;orf00578

Supplementary Table 4. Result of pathway analysis for unigenes by Kyoto Encyclopedia of Genes and Genomes annotation

No.	Pathway	DEGs with pathway annotation (313)	All genes with pathway annotation (989)	Pathway ID
1	Ribosome	19 (6.07%)	22 (2.22%)	ko03010
2	RNA polymerase	3 (0.96%)	3 (0.3%)	ko03020
3	Penicillin and cephalosporin biosynthesis	3 (0.96%)	3 (0.3%)	ko00311
4	Sulfur metabolism	4 (1.28%)	5 (0.51%)	ko00920
5	RNA degradation	5 (1.6%)	8 (0.81%)	ko03018
6	Selenocompound metabolism	5 (1.6%)	9 (0.91%)	ko00450
7	Pantothenate and CoA biosynthesis	3 (0.96%)	5 (0.51%)	ko00770
8	Cysteine and methionine metabolism	7 (2.24%)	16 (1.62%)	ko00270
9	Cyanoamino acid metabolism	2 (0.64%)	3 (0.3%)	ko00460
10	Ubiquinone and other terpenoid-quinone biosynthesis	2 (0.64%)	3 (0.3%)	ko00130
11	Glycerolipid metabolism	2 (0.64%)	3 (0.3%)	ko00561
12	Pyrimidine metabolism	9 (2.88%)	23 (2.33%)	ko00240
13	Glutathione metabolism	3 (0.96%)	6 (0.61%)	ko00480
14	African trypanosomiasis	1 (0.32%)	1 (0.1%)	ko05143
15	Taurine and hypotaurine metabolism	1 (0.32%)	1 (0.1%)	ko00430
16	Chagas disease (American trypanosomiasis)	1 (0.32%)	1 (0.1%)	ko05142
17	Inositol phosphate metabolism	1 (0.32%)	1 (0.1%)	ko00562
18	alpha-Linolenic acid metabolism	1 (0.32%)	1 (0.1%)	ko00592
19	Propanoate metabolism	2 (0.64%)	4 (0.4%)	ko00640
20	DNA replication	2 (0.64%)	4 (0.4%)	ko03030
21	Thiamine metabolism	2 (0.64%)	4 (0.4%)	ko00730
22	Homologous recombination	2 (0.64%)	4 (0.4%)	ko03440
23	Glycerophospholipid metabolism	2 (0.64%)	4 (0.4%)	ko00564
24	Photosynthesis	2 (0.64%)	4 (0.4%)	ko00195
25	Base excision repair	3 (0.96%)	7 (0.71%)	ko03410
26	Oxidative phosphorylation	3 (0.96%)	8 (0.81%)	ko00190
27	Arachidonic acid metabolism	1 (0.32%)	2 (0.2%)	ko00590
28	Butirosin and neomycin biosynthesis	1 (0.32%)	2 (0.2%)	ko00524
29	beta-Alanine metabolism	1 (0.32%)	2 (0.2%)	ko00410
30	Lipoic acid metabolism	1 (0.32%)	2 (0.2%)	ko00785
31	Vitamin B6 metabolism	1 (0.32%)	2 (0.2%)	ko00750
32	N-Glycan biosynthesis	1 (0.32%)	2 (0.2%)	ko00510
33	Biotin metabolism	2 (0.64%)	6 (0.61%)	ko00780
34	Glycolysis / Gluconeogenesis	6 (1.92%)	20 (2.02%)	ko00010
35	Mismatch repair	1 (0.32%)	3 (0.3%)	ko03430
36	One carbon pool by folate	4 (1.28%)	14 (1.42%)	ko00670
37	Tyrosine metabolism	2 (0.64%)	7 (0.71%)	ko00350
38	Arginine and proline metabolism	2 (0.64%)	8 (0.81%)	ko00330
39	Glyoxylate and dicarboxylate metabolism	2 (0.64%)	8 (0.81%)	ko00630
40	Valine, leucine and isoleucine degradation	2 (0.64%)	8 (0.81%)	ko00280
41	Phenylalanine metabolism	1 (0.32%)	4 (0.4%)	ko00360
42	Valine, leucine and isoleucine biosynthesis	1 (0.32%)	4 (0.4%)	ko00290
43	Pyruvate metabolism	3 (0.96%)	12 (1.21%)	ko00620
44	Folate biosynthesis	3 (0.96%)	12 (1.21%)	ko00790
45	Porphyrin and chlorophyll metabolism	3 (0.96%)	13 (1.31%)	ko00860
46	Galactose metabolism	1 (0.32%)	5 (0.51%)	ko00052
47	Starch and sucrose metabolism	1 (0.32%)	5 (0.51%)	ko00500
48	Terpenoid backbone biosynthesis	1 (0.32%)	5 (0.51%)	ko00900
49	Purine metabolism	6 (1.92%)	25 (2.53%)	ko00230
50	Glycine, serine and threonine metabolism	4 (1.28%)	18 (1.82%)	ko00260

51	Peroxisome	2 (0.64%)	10 (1.01%)	ko04146
52	Fatty acid metabolism	1 (0.32%)	6 (0.61%)	ko00071
53	Nicotinate and nicotinamide metabolism	1 (0.32%)	6 (0.61%)	ko00760
54	Protein export	1 (0.32%)	6 (0.61%)	ko03060
55	Fructose and mannose metabolism	1 (0.32%)	7 (0.71%)	ko00051
56	Lysine biosynthesis	1 (0.32%)	7 (0.71%)	ko00300
57	Tryptophan metabolism	1 (0.32%)	8 (0.81%)	ko00380
58	Metabolic pathways	62 (19.81%)	229 (23.15%)	ko01100
59	Citrate cycle (TCA cycle)	2 (0.64%)	14 (1.42%)	ko00020
60	Biosynthesis of secondary metabolites	29 (9.27%)	117 (11.83%)	ko01110
61	Phenylalanine, tyrosine and tryptophan biosynthesis	1 (0.32%)	9 (0.91%)	ko00400
62	Nitrogen metabolism	1 (0.32%)	10 (1.01%)	ko00910
63	Fatty acid biosynthesis	1 (0.32%)	10 (1.01%)	ko00061
64	Amino sugar and nucleotide sugar metabolism	1 (0.32%)	10 (1.01%)	ko00520
65	Pentose phosphate pathway	1 (0.32%)	12 (1.21%)	ko00030
66	Alanine, aspartate and glutamate metabolism	1 (0.32%)	13 (1.31%)	ko00250
67	Aminoacyl-tRNA biosynthesis	1 (0.32%)	23 (2.33%)	ko00970

DEG, differentially expressed gene.