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Source: Journal of Insect Science, 6(6) : 1-26

Published By: Entomological Society of America

URL: [https://doi.org/10.1673/1536-2442\(2006\)6\[1:ATAOTA\]2.0.CO;2](https://doi.org/10.1673/1536-2442(2006)6[1:ATAOTA]2.0.CO;2)

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## A transcriptome analysis of the *Aedes aegypti* vitellogenic fat body

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### Abstract

*Aedes (Stegomyia) aegypti* is an important dengue vector in tropical and subtropical zones throughout the world. A transcriptome of *Ae. aegypti* vitellogenic fat bodies is described here. The fat body is a dynamic tissue that participates in multiple biochemical functions of intermediate metabolism. A total of 589 randomly selected cDNAs were assembled into 262 clusters based on their primary sequence similarities. The putative translated proteins were classified into categories based on their function in accordance with significant similarity using the BlastX at NCBI FTP site and Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases. The characterization of transcripts expressed in the fat body of *Ae. aegypti* at 24 hours post blood meal provides a basic tool for understanding the processes occurring in this organ and could identify putative new genes whose promoters can be used to specifically express transgenes in the fat bodies of *Ae. aegypti*.

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Received: 4.5.2005 | Accepted: 8.11.2005 | Published: 6.8.2006

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ISSN: 1536-2442 | Volume 6, Number 6

Cite this paper as:

Feitosa FM, Calvo E, Merino EF, Durham AM, James AA, de Bianchi AG, Marinotti O, Capurro ML. A transcriptome analysis of the *Aedes aegypti* vitellogenic fat body. 26pp. *Journal of Insect Science* 6:06, available online: [insectscience.org/6.06](http://insectscience.org/6.06)

## Introduction

Insect vectors are responsible for transmitting human pathogens such as dengue viruses and malaria parasites that account for millions of disease cases every year with associated high mortality and morbidity (TDR, 2004). While research devoted to alleviate the burden of these diseases on human populations focuses on the development of vaccines, preventive and therapeutic drugs, pioneering avenues for the control of diseases may also be opened from the studies of vector biology. The *Anopheles gambiae* genome project has provided the scientific community with a powerful tool for the analyses of gene expression in this malaria vector species (Holt et al. 2002). The 280-Mb *An. gambiae* genome and the tens of thousands of ESTs generated from RNA extracted from specific tissues such as midgut, fat body, salivary gland, hemocytes and antennae have made it possible to better understand mosquito biology and its interactions with pathogenic organisms. As expected, taking advantage of all the sequence information, and gene search and annotation, new types of experiments are possible that likely will result in novel observations and discoveries (Marinotti et al. 2005; Tabachnick 2003; Toure et al. 2004).

The genome of the mosquito, *Aedes aegypti*, the major vector of dengue viruses, is currently being sequenced (Severson et al. 2004), and once completed it will be a fundamental tool for studies on vector biology. The characterization of the transcriptomes of mosquitoes at various physiological stages as well as gene expression patterns in isolated tissues are fundamental additions to the complete genome description. The focus here is on the description of the transcriptome of *Ae. aegypti* vitellogenic fat bodies, which are dynamic tissues that participate in multiple biochemical functions of intermediate metabolism, including protein, amino acid, lipid and carbohydrate synthesis and storage, xenobiotic detoxification, and immune response (Bartholomay et al. 2004). Because dengue fever is the most prevalent mosquito-borne viral disease among human populations, causing 50 million infections, 500,000 cases of dengue hemorrhagic fever and least 12,000 deaths per year (Gubler 2002), the transcriptome of *Ae. aegypti* could prove useful in control of dengue.

A total of 800 randomly-picked cDNAs were sequenced and after quality validation, 589 cDNAs were assembled in 262 clusters, annotated and assigned gene ontology terminology.

## Materials and Methods

### Animals

*Aedes aegypti* (Rockefeller strain) were reared in a local facility at the Institute of Biomedical Sciences, University of São Paulo, Brazil. Temperature was maintained at 26°C, humidity at 80% and a 12/12 photoperiod. Larvae were fed on powdered rat food. Adult mosquitoes were given continuous access to a 10% sucrose solution and five-day old females were fed on anesthetized mice when required.

### Fat body cDNA library construction

Fat body of adult female mosquitoes, not including the body-wall integument, were dissected at 24 hrs post blood meal (PBM). Adult females were anesthetized on ice and dissected with a stereoscopic microscope in 0.15 M sodium chloride. Fat bodies were transferred to 500 µl of the TRIZOL reagent (Invitrogen) and mRNA extracted using the Micro-FastTrack mRNA isolation kit (Invitrogen). A PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech). Four hundred nanograms of fat body mRNA were reverse-transcribed to cDNA using Superscript II RNase H-reverse transcriptase (Invitrogen) and the CDS III/3' PCR primer (Clontech) for 1 h at 42°C. Second-strand synthesis was performed through a PCR-based protocol using the SMART III primer (Clontech) as the sense primer and the CDS III/3' primer as antisense primer. These two primers create *Sfi* IA and B sites at the ends of nascent cDNA. Double-strand cDNA synthesis was carried out on a MJ Research Thermal cycler using the Platinum *Pfx* DNA polymerase. Amplification conditions were the following: 94°C for 2 min; 19 cycles of 94°C for 10 s and 68°C for 6 min. Double-stranded cDNA was treated immediately with proteinase K (0.8 µg/µl) for 20 min at 45°C. The double-stranded cDNA was digested with *Sfi* I for 2 h at 50°C. The cDNA then was fractionated using columns provide by the manufacturer (Clontech). Fractions containing cDNA of more than 400 base pairs (bp) in length were pooled and concentrated to a volume of 7 µl. The concentrated cDNA was ligated into a Lambda TriplEx2 vector

(Clontech), and the resulting ligation reaction packaged using the Gigapack Gold III from Stratagene/BioLabs. The library was plated by infecting log-phase XL1-Blue cells (Clontech).

### Sequencing and analysis of the *Ae. aegypti* cDNA library

Randomly-picked cDNA clones from the cDNA library were sequenced, assembled and analyzed (Durham *et al.* 2005; Calvo *et al.* 2004), using the CAP 3 program (Huang and Madan 1999). BLASTX searches were done locally from programs obtained at the NCBI FTP site (<ftp://ftp.ncbi.nih.gov/blast/executables/>) (Altschul *et al.* 1997). All the ESTs were deposited in dbEST at NCBI. Accession numbers for sequences originating from the *Ae. aegypti* cDNA library are given as DT366744 to DT367332 corresponds to the referenced gene product.

## Results and Discussion

### Organization of the transcriptome information

A total of 589 cDNA inserts were assembled into 262 clusters. Thirty-one clusters (237 cDNAs) were identified as corresponding to nuclear or mitochondrial rRNAs and were not investigated further. The 351 remaining cDNAs were grouped in 231 clusters and classified in 21 categories, according to function or putative assigned functions (Tables 1 and 2, and a supplementary table where cDNA is classified in categories: <http://lineu.icb.usp.br/~mcapurro/aafatbody.zip>).

The known or putative functional assignments were based on similarity of the deduced protein sequences to previously-described molecules deposited in the NR protein database at NCBI. Translated sequences also were screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman *et al.* 2000) and SMART (Schultz *et al.* 2000) databases (also known as the Conserved Domains Database [CDD]) (Table 1). The sequences were compared to those available through the *Ae. aegypti* genome-sequencing project, at the TIGR web site (09/22/2004).

The vast majority of identified ESTs were represented only once in the database, indicating the high complexity of the fat body tissue. Fifty-seven contigs correspond to cDNAs that occur more than once in the database, may be indicative

of a higher level of gene expression. Transcripts encoding proteins of the unknown group (68 ESTs), which did not show significant similarity with known proteins, could represent novel proteins unique to *Ae. aegypti* fat bodies and require further investigation.

### Description of the transcriptome

All of the cDNAs reported here were divided into 21 groups according to their predicted function by gene ontology (Ashburner *et al.* 2000), (Figure 1 and Table 2). In total, 142 sequences distributed in 75 clusters, representing 40.46% of the total of fat body ESTs database, correspond to genes whose products are associated with protein synthesis. This is consistent with the synthetic capability of the fat bodies. The majority of these cDNAs encode ribosomal proteins (67 clusters). The presence of transcripts encoding an elongation factor and a translation initiation factor supports further the conclusion of an abundant protein synthetic activity of this organ.

Six transcripts encoding molecules associated with post-translational modification of proteins and protein transport pathways were found. Furthermore, transcripts of mitochondrial components are among the most represented in the database and this is consistent with the energy requirements for protein synthesis during vitellogenesis. Sequences related to energy metabolism (28 sequences distributed in 24 clusters) correspond largely to cytochrome c oxidase, ATP synthase and NADH dehydrogenase. Two transcripts involved in oxidative metabolism were identified as members of the cytochrome P450 family.

Transcripts involved with a wide variety of functions such as carbohydrate, lipid and nucleotide metabolism, transport, storage, signal transduction, lysosomal and proteasome activities and, nuclear regulation, were found among the sequenced cDNAs. All of these were represented only once or twice in the database, except for a transferrin and an odorant binding protein-like transcript, which were represented three and four times, respectively. The function of *Ae. aegypti* transferrin has not been determined, but these molecules may be involved in iron transport, oogenesis and innate immunity against parasites and pathogens (Harizanova *et al.* 2005). Insect odorant binding proteins and pheromone binding

**Table 1.** *Aedes aegypti* vitellogenic fat body cDNA cluster encoding proteins

| GenBank Accession number | ID sequence | Number of sequences | Best match of AEGY-TIGR to NR protein database | E value | % identity | Best match of AEGY-TIGR to PFAM database | E value | Best match of AEGY-TIGR to GO database | E value | Comments                                     | class     |
|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|----------------------------------------|---------|----------------------------------------------|-----------|
| DT366820                 | F990        | 7                   | vitellogenin-C [Aedes aegypti]                 | 1E-161  | 99         | Podocalyxin                              | 2E-006  | nucleus                                | 0.000   | vitellogenin-C [Aedes aegypti]               | oogenesis |
| DT366849                 | F715        | 1                   | vitellogenin-C [Aedes aegypti]                 | 0.0     | 98         | Vitellogenin_N                           | 3E-069  | nucleocytoplasmic transport            | 0.0     | vitellogenin-C [Aedes aegypti]               | oogenesis |
| DT366800                 | F782        | 9                   | vitellogenin-B [Aedes aegypti]                 | 0.0     | 96         | Candida_ALS                              | 5E-026  | extracellular space                    | 0.0     | vitellogenin-B [Aedes aegypti] Partial clone | oogenesis |
| DT366845                 | F1436-D02   | 2                   | Vitellogenin A1 precursor (VG)                 | 0.0     | 99         | Vitellogenin_N                           | 5E-040  | nucleocytoplasmic transport            | 0.0     | vitellogenin-B                               | oogenesis |
| DT366844                 | F1404-A06   | 1                   | Vitellogenin A1 precursor (VG)                 | 0.0     | 99         | Vitellogenin_N                           | 5E-040  | nucleocytoplasmic transport            | 0.0     | vitellogenin-B                               | oogenesis |
| DT366847                 | f28         | 1                   | vitellogenin - yellow fever mosquito           | 0.0     | 99         | Candida_ALS                              | 9E-011  | cell wall (sensu Fungi)                | 0.0     | Vitellogenin A1 precursor                    | oogenesis |
| DT366836                 | F1513-B07   | 4                   | vitellogenin - yellow fever mosquito           | 0.0     | 99         | Candida_ALS                              | 9E-011  | cell wall (sensu Fungi)                | 0.0     | vitellogenin                                 | oogenesis |
| DT366811                 | F665        | 9                   | vitellogenic cathepsin-B like protease         | 0.0     | 99         | Peptidase_C1                             | 5E-064  | cathepsin B activity - lysosome        | 0.0     | vitellogenic cathepsin-B like protease       | oogenesis |
| DT366842                 | F1125       | 1                   | vitellogenic cathepsin-B like protease         | 0.0     | 99         | Peptidase_C1                             | 5E-064  | cathepsin B activity - lysosome        | 0.0     | vitellogenic cathepsin-B like protease       | oogenesis |
| DT366838                 | F1204-D10   | 1                   | vitellogenic cathepsin-B like protease         | 0.0     | 99         | Peptidase_C1                             | 5E-064  | cathepsin B activity-lysosome          | 0.0     | vitellogenic cathepsin-B like protease       | oogenesis |
| DT366852                 | F1213-E07   | 1                   | vitellogenic cathepsin-B like protease         | 0.0     | 99         | Peptidase_C1                             | 5E-064  | cathepsin B activity - lysosome        | 0.0     | vitellogenic cathepsin-B like protease       | oogenesis |
| DT366830                 | F269        | 3                   | Vitellogenic carboxypeptidase pre              | 0.0     | 99         | serine_carbpept                          | 1E-104  | mitochondrion - protein binding        | 0.0     | Vitellogenic carboxypeptidase pre            | oogenesis |
| DT366809                 | F690        | 2                   | Vitellogenic carboxypeptidase pre              | 0.0     | 99         | serine_carbpept                          | 1E-104  | mitochondrion - protein binding        | 0.0     | vitellogenic carboxypeptidase                | oogenesis |
| DT366839                 | F273        | 1                   | Vitellogenic carboxypeptidase pre              | 0.0     | 99         | serine_carbpept                          | 1E-104  | mitochondrion - protein binding        | 0.0     | vitellogenic carboxypeptidase                | oogenesis |
| DT366843                 | f17         | 1                   | ENSANGP00000014169 Anopheles gambia            | 1E-168  | 67         | serine_carbpept                          | 1E-098  | mitochondrion - protein binding        | 0.0     | Vitellogenic carboxypeptidase                | oogenesis |
| DT366827                 | F666        | 3                   | cathepsin B-like thiol protease                | 0.0     | 99         | Peptidase_C1                             | 5E-066  | cathepsin B activity - lysosome        | 0.0     | cathepsin B-like thiol protease              | oogenesis |
| DT366837                 | F278        | 1                   | cathepsin B-like thiol protease                | 0.0     | 99         | Peptidase_C1                             | 5E-066  | cathepsin B activity - lysosome        | 0.0     | cathepsin B-like thiol protease              | oogenesis |
| DT366840                 | F528        | 2                   | cathepsin B-like thiol protease                | 1E-122  | 98         | Peptidase_C1                             | 1E-045  | cathepsin B activity - lysosome        | 0.0     | cathepsin B-like thiol protease              | oogenesis |
| DT366850                 | F535        | 1                   | cathepsin B-like thiol protease                | 0.0     | 99         | Peptidase_C1                             | 5E-066  | cathepsin B activity - lysosome        | 0.0     | cathepsin B-like thiol protease              | oogenesis |

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|--------------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|----------------------------------------|---------|------------------------------------------------|-------------------------|
| DT366851                       | F559        | 1                   | cathepsin B-like thiol protease                | 1E-149  | 100        | Peptidase_C1                             | 2E-062  | cathepsin B activity - lysosome        | 0.0     | cathepsin B-like thiol protease                | oogenesis               |
| DT366853                       | F1033       | 1                   | ENSANGP00000021680<br>Anopheles gambia         | 3E-037  | 37         |                                          |         |                                        |         | similar to 27k hemolymph protein from Galleria | oogenesis?              |
| DT366793                       | F640        | 1                   | similar to Paramecium tetraurelia              | 3E-009  | 23         | Candida_ALS                              | 1E-004  | molecular_function unknown             | 0.000   | possible Cyclophilin-RNA interacting protein   | nuclear regulation      |
|                                |             | 54                  |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| <b>protein export</b>          |             |                     |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| DT366858                       | F825        | 1                   | ENSANGP00000020919<br>Anopheles gambia         | 1E-118  | 94         | SNF7                                     | 2E-047  | biological_process unknown             | 0.0     | Vacuolar assembly/sorting protein DID4         | protein export          |
| DT366856                       | F1246-H04   | 1                   | ENSANGP00000012186<br>Anopheles gambia         | 1E-121  | 78         | TRAP_alpha                               | 7E-086  | signal sequence binding                | 0.0     | Translocon-associated complex TRAP,            | protein export          |
| DT366857                       | F623        | 1                   | ENSANGP00000019007<br>Anopheles gambia         | 1E-030  | 95         | SecE                                     | 2E-013  | protein transporter activity           | 0.0     | Protein transport protein SEC61 gamma sub      | protein export          |
|                                |             | 3                   |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| <b>carbohydrate metabolism</b> |             |                     |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| DT366745                       | F694        | 1                   | ENSANGP00000018537<br>Anopheles gambia         | 6E-069  | 59         | UDPGT                                    | 3E-033  | xenobiotic metabolism                  | 0.0     | UDP-glucuronosyltransferase 8 - extracellular  | carbohydrate metabolism |
| DT366744                       | F1128       | 1                   | ENSANGP00000018537<br>Anopheles gambia         | 6E-069  | 59         | UDPGT                                    | 3E-033  | xenobiotic metabolism                  | 0.0     | UDP-glucuronosyltransferase 2B15 precursor     | carbohydrate metabolism |
|                                |             | 2                   |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| <b>energy metabolism</b>       |             |                     |                                                |         |            |                                          |         |                                        |         |                                                |                         |
| DT366779                       | F1450-E04   | 1                   | similar to Drosophila melanogaster             | 9E-010  | 50         |                                          |         | mitochondrion                          | 0.000   | ubiquinol-cytochrome c reductase               | energy metabolism       |
| DT366778                       | F438        | 1                   | ENSANGP00000018542<br>Anopheles gambia         | 5E-091  | 90         | mito_carr                                | 2E-021  | carrier activity - calcium ion binding | 0.0     | possible mitochondrial transporter             | energy metabolism       |
| DT366777                       | F354        | 1                   | LD14731p [Drosophila melanogaster              | 8E-014  | 52         | COX7C                                    | 2E-012  | integral to membrane - mitochondrion   | 0.0     | cytochrome c oxidase, subunit VIIc             | energy metabolism       |
| DT366766                       | F603        | 1                   | ENSANGP00000010310<br>Anopheles gambia         | 4E-048  | 80         | COX6A                                    | 5E-032  | energy pathways                        | 0.0     | Cytochrome c oxidase polypeptide VIa-liver     | energy metabolism       |
| DT366772                       | F993        | 2                   | Cytochrome c oxidase polypeptide               | 7E-074  | 92         | COX2                                     | 3E-061  | cytochrome-c oxidase activity          | 0.0     | Cytochrome c oxidase polypeptide               | energy metabolism       |
| DT366759                       | F1079       | 1                   | Cytochrome c oxidase polypeptide               | 1E-114  | 91         | COX2                                     | 4E-061  | cytochrome-c oxidase activity          | 0.0     | Cytochrome c oxidase polypeptide               | energy metabolism       |

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| GenBank Accession number | ID sequence | Number of sequences | Best match of AEGY-TIGR to NR protein database | E value | % identity | Best match of AEGY-TIGR to PFAM database | E value | Best match of AEGY-TIGR to GO database | E value | Comments                                  | class             |
|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|----------------------------------------|---------|-------------------------------------------|-------------------|
| DT366781                 | F709        | 1                   | Cytochrome c oxidase polypeptide               | 7E-074  | 92         | COX2                                     | 3E-061  | cytochrome-c oxidase activity          | 0.0     | mitochondrial product                     | energy metabolism |
| DT366776                 | F1373-F11   | 1                   | cytochrome oxidase subunit 3 An. g             | 1E-116  | 82         | COX3                                     | 1E-116  | cytochrome-c oxidase activity          | 0.0     | cytochrome c oxidase subun                | energy metabolism |
| DT366774                 | f19         | 1                   | ENSANGP00000020278 Anopheles g                 | 1E-166  | 88         | mito_carr                                | 2E-022  | molecular_function unknown             | 0       | ADP/ATP translocase [Apis mellifera       | energy metabolism |
| DT366760                 | F1313-A11   | 3                   | ENSANGP00000020278 Anopheles g                 | 1E-166  | 88         | mito_carr                                | 2E-022  | molecular_function unknown             | 0       | ADP/ATP translocase [Anopheles gambiae]   | energy metabolism |
| DT366769                 | F1064       | 1                   | cytochrome c oxidase subunit I Aedes           | 0.0     | 91         | COX1                                     | 0.0     | mitochondrion                          | 0       | cytochrome c oxidase subunit I [Aedes     | energy metabolism |
| DT366763                 | F314        | 1                   | cytochrome c oxidase subunit I Aedes           | 0.0     | 91         | COX1                                     | 0.0     | mitochondrion                          | 0       | cytochrome c oxidase subunit I [Aedes     | energy metabolism |
| DT366767                 | F249        | 2                   | cytochrome c oxidase subunit I Aedes           | 0.0     | 91         | COX1                                     | 0.0     | mitochondrion                          | 0       | cytochrome oxidase subunit I [Aedes c     | energy metabolism |
| DT366780                 | F637        | 1                   | ENSANGP00000012587 Anopheles                   | 6E-098  | 95         | NAD_kinase                               | 3E-033  | NAD+ kinase activity phosphorylation   | 0.0     | putative inorganic polyphosphate/ATP-NAD  | energy metabolism |
| DT366758                 | F364        | 1                   | ENSANGP00000015816 Anopheles                   | 2E-079  | 80         | COX4                                     | 7E-049  | growth - reproduction                  | 0.0     | Cytochrome c oxidase, subunit IV/COX5b    | energy metabolism |
| DT366784                 | F507        | 1                   | ENSANGP00000019912 Anopheles                   | 1E-030  | 70         | COX7a                                    | 3E-005  | cytochrome-c oxidase activity          | 0.000   | Probable cytochrome c oxidase polypeptide | energy metabolism |
| DT366771                 | F3          | 1                   | ENSANGP00000011122 Anopheles                   | 1E-062  | 87         | COX5B                                    | 2E-017  | determination of adult life span       | 0.0     | Cytochrome c oxidase, subunit Vb/COX4     | energy metabolism |
| DT366764                 | F1094       | 1                   | adenosine triphosphatase subunit 6             | 1E-104  | 85         | ATP-synt_A                               | 2E-047  | ATP synthesis coupled proton transport | 0.0     | ATP synthase F0 subunit 6                 | energy metabolism |
| DT366770                 | F460        | 1                   | adenosine triphosphatase subunit 6             | 1E-104  | 85         | ATP-synt_A                               | 2E-047  | ATP synthesis coupled proton transport | 0.0     | ATP synthase F0 subunit 6                 | energy metabolism |
| DT366783                 | F1289-F05   | 1                   | ENSANGP00000022850 Anopheles                   | 1E-033  | 73         | TT_ORF1                                  | 0.008   | mitochondrion - NADH dehydrogenase     | 0.0     | NADH dehydrogenase                        | energy metabolism |
| DT366749                 | F517        | 1                   | ENSANGP00000019428 Anopheles                   | 1E-105  | 97         | oxidored_q6                              | 4E-038  | mitochondrial electron transport, NADH | 0.0     | NADH-ubiquinone oxidoreductase 20 kDa sub | energy metabolism |
| DT366748                 | F1217-E11   | 1                   | ENSANGP00000019428 Anopheles                   | 1E-105  | 97         | oxidored_q6                              | 4E-038  | mitochondrial electron transport       | 0.0     | NADH-ubiquinone oxidoreductase 20 kDa sub | energy metabolism |
| DT366765                 | F619        | 1                   | NADH dehydrogenase subunit 6                   | 1E-039  | 59         | oxidored_q3                              | 0.001   | mitochondrial electron transport       | 0.015   | NADH dehydrogenase subunit 6              | energy metabolism |
| DT366775                 | F589        | 1                   |                                                |         |            |                                          |         |                                        |         | NADH-ubiquinone oxidoreductase c          | energy metabolism |
|                          |             | 28                  |                                                |         |            |                                          |         |                                        |         |                                           |                   |
| <b>cytoskeletal</b>      |             |                     |                                                |         |            |                                          |         |                                        |         |                                           |                   |
| DT366747                 | F1024       | 1                   | ENSANGP00000021085 Anopheles                   | 3E-089  | 93         | TCTP                                     | 5E-057  | positive regulation of growth rate     | 0.0     | translationally controlled tumor protein  | cytoskeletal      |

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|--------------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|--------------------------------------------------------------------------------------------------------------------------------------|---------|---------------------------------------------------------------|-------------------------|
| DT366746                       | F376        | 1                   | ENSANGP00000021085<br>Anopheles                | 3E-089  | 93         | TCTP                                     | 5E-057  | positive regulation of growth rate                                                                                                   | 0.0     | translationally controlled tumor protein                      | cytoskeletal            |
|                                |             | 2                   |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| <b>transcription machinery</b> |             |                     |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| DT367256                       | F1203       | 1                   | ENSANGP00000017886<br>Anopheles                | 1E-112  | 57         | Keratin_B2                               | 0.035   | DNA binding - transcription from Pol II                                                                                              | 0.0     | similar to small nuclear RNA                                  | transcription machinery |
|                                |             | 1                   |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| <b>protease</b>                |             |                     |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| DT366865                       | F916        | 1                   | ENSANGP00000024673<br>Anopheles                | 1E-101  | 51         | trypsin                                  | 6E-046  | serine-type endopeptidase activity                                                                                                   | 0.0     | serine protease                                               | protease                |
| DT366864                       | F810        | 1                   | ENSANGP00000016930<br>Anopheles                | 1E-095  | 72         | trypsin                                  | 7E-036  | chymotrypsin activity                                                                                                                | 0.0     | Testisin precursor - membrane fraction                        | protease                |
| DT366863                       | F592        | 1                   | ENSANGP00000019999<br>Anopheles                | 2E-089  | 45         | trypsin                                  | 3E-039  | proteolysis and peptidolysis                                                                                                         | 0.0     | similar to A. gambiae serine protease 14D                     | protease                |
|                                |             | 3                   |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| <b>protein synthesis</b>       |             |                     |                                                |         |            |                                          |         |                                                                                                                                      |         |                                                               |                         |
| DT367296                       | F995        | 1                   | ENSANGP00000012554<br>Anopheles                | 1E-124  | 68         | Tymo_45kd_70kd                           | 3E-016  | positive regulation of growth rate                                                                                                   | 0.0     | unknown                                                       | unknown                 |
| DT367289                       | F1334-C08   | 1                   | 60S acidic ribosomal protein p1                | 6E-033  | 69         | 60s_ribosomal                            | 7E-026  | RNA binding                                                                                                                          | 0.0     | unknown                                                       | unknown                 |
| DT367224                       | F578        | 1                   | ENSANGP00000022149<br>Anopheles                | 7E-064  | 76         | Ribosomal_L35Ae                          | 7E-031  | structural constituent of ribosome                                                                                                   | 0.0     | similar to ribosomal protein L35A                             | protein synthesis       |
| DT367200                       | F590        | 1                   | similar to D. melanogaster R protein           | 5E-080  | 68         | Ribosomal_L13e                           | 1E-076  | structural constituent of ribosome                                                                                                   | 0.0     | possible 60S Ribosomal protein L13                            | protein synthesis       |
| DT367223                       | F686        | 1                   | ENSANGP00000021108<br>Anopheles                | 6E-066  | 95         | Ribosomal_S8                             | 2E-037  | embryonic development (sensu Animalia)                                                                                               | 0.0     | possible 40S ribosomal protein S15A                           | protein synthesis       |
| DT366886                       | F349        | 1                   | elongation factor 1 beta Aedes aegypti         | 1E-125  | 100        | EF1BD                                    | 4E-035  | cytosol                                                                                                                              | 0       | elongation factor 1 beta                                      | protein synthesis       |
| DT367201                       | F197        | 1                   | ENSANGP00000011566<br>Anopheles                | 4E-077  | 63         | Mpp10                                    | 4E-005  | translation initiation factor activity - regulation of translational initiation - eukaryotic translation initiation factor 3 complex | 0.0     | Adam-PA similar to eukaryotic translation initiation factor 3 | protein synthesis       |
| DT367058                       | F546        | 1                   | ENSANGP00000025329<br>Anopheles                | 1E-129  | 84         | Ribosomal_L7Ae                           | 4E-020  | hermaphrodite genital morphogenesis - physiological process - reproduction                                                           | 0.0     | 60S ribosomal protein L7a                                     | protein synthesis       |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|---------------------------------------------------|--------------------------------|
| DT366877                 | F551        | 6                   | ENSANGP00000017987<br>Anopheles                | 8E-070  | 81         | L15                                      | 8E-006  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)                                                                                                | 0.0     | 60S ribosomal protein L27a                        | protein synthesis              |
| DT367214                 | F764        | 1                   | ENSANGP00000012554<br>Anopheles                | 1E-124  | 68         | Tymo_45kd_70kd                           | 3E-016  | positive regulation of growth rate                                                                                                                                                                                               | 0.0     | 60s ribosomal protein L23                         | protein synthesis              |
| DT367222                 | F526        | 1                   | ENSANGP00000016619<br>Anopheles                | 3E-091  | 94         | Ribosomal_L18ae                          | 7E-085  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)                                                                                                | 0.0     | 60S ribosomal protein L18a                        | protein synthesis              |
| DT367221                 | F695        | 1                   | ENSANGP00000022049<br>Anopheles                | 7E-096  | 94         | Ribosomal_L5_C                           | 4E-040  | protein binding                                                                                                                                                                                                                  | 0.0     | 60S ribosomal protein L11                         | protein synthesis              |
| DT366799                 | F410        | 1                   | ENSANGP00000013781<br>Anopheles                | 1E-086  | 56         | PBP_GOBP                                 | 0.005   | extracellular space                                                                                                                                                                                                              | 0.086   | 5'UTR of D7 family member                         | odorant binding protein family |
| DT367219                 | F729        | 1                   | ENSANGP00000013302<br>Anopheles                | 1E-138  | 92         | Ribosomal_S4e                            | 8E-024  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)                                                                                                | 0       | 40S ribosomal protein S4                          | protein synthesis              |
| DT367209                 | F468        | 1                   | 40S ribosomal protein S16<br>Aedes             | 5E-078  | 100        | Ribosomal_S9                             | 8E-044  | larval development (sensu Nematoda) - growth<br>- physiological process<br>- reproduction                                                                                                                                        | 0.0     | 40S ribosomal protein S16                         | protein synthesis              |
| DT367091                 | F544        | 2                   | ENSANGP00000020171<br>Anopheles                | 1E-125  | 77         | Ribosomal_S2                             | 4E-058  | laminin receptor activity<br>- regulation of translation - cell adhesion - cell surface receptor linked signal transduction - cytosolic small ribosomal subunit (sensu Eukarya) - integrin complex - integral to plasma membrane | 0.0     | 40S ribosomal protein SA (P40)/Laminin receptor 1 | protein synthesis              |
| DT367231                 | F1088       | 1                   | ENSANGP00000023607<br>Anopheles                | 7E-035  | 96         | Ribosomal_S4                             | 4E-015  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)                                                                                                | 0.0     | S9e ribosomal protein                             | protein synthesis              |
| DT367220                 | F747        | 1                   | ENSANGP00000014951<br>Anopheles                | 1E-107  | 91         | Ribosomal_S8e                            | 6E-054  | larval development (sensu Nematoda) - growth<br>- physiological process<br>- reproduction                                                                                                                                        | 0.0     | ribosomal protein S8                              | protein synthesis              |

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|--------------------------|-------------|---------------------|-------------------------------------------------|---------|------------|------------------------------------------|---------|--------------------------------------------------------------------------------------------------------------------------------------------|---------|------------------------------------|-------------------|
| DT367215                 | F612        | 1                   | ribosomal protein S6 <i>Aedes aegypti</i>       | 0.0     | 99         | Ribosomal_S6e                            | 7E-054  | protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)<br>- structural constituent of ribosome - cytoplasm            | 0.0     | ribosomal protein S6               | protein synthesis |
| DT367198                 | F1418-B08   | 1                   | ENSANGP00000025326 <i>Anopheles</i>             | 1E-108  | 88         | Ribosomal_S7                             | 1E-047  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)          | 0.0     | ribosomal protein S5               | protein synthesis |
| DT367063                 | F378        | 1                   | ribosomal protein S24 <i>Aedes aegypti</i>      | 1E-069  | 100        | Ribosomal_S24e                           | 1E-026  | larval development (sensu Nematoda) - growth<br>- physiological process<br>- reproduction                                                  | 0.0     | ribosomal protein S24              | protein synthesis |
| DT366883                 | F231        | 1                   | ENSANGP00000015322 <i>Anopheles</i>             | 1E-145  | 93         | Ribosomal_S5                             | 4E-021  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)          | 0       | ribosomal protein S2               | protein synthesis |
| DT367194                 | F752        | 2                   | ENSANGP00000012543 <i>Anopheles</i>             | 5E-073  | 76         | Ribosomal_S19e                           | 2E-058  | intracellular - ribosome<br>- protein biosynthesis                                                                                         | 0.0     | ribosomal protein S19              | protein synthesis |
| DT367093                 | F263        | 2                   | putative ribosomal protein S13 <i>Anopheles</i> | 1E-078  | 98         | Ribosomal_S15                            | 1E-017  | protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)<br>- structural constituent of ribosome                        | 0.0     | RIBOSOMAL PROTEIN S13              | protein synthesis |
| DT367177                 | F584        | 1                   | ENSANGP00000017313 <i>Anopheles</i>             | 2E-039  | 70         | Ribosomal_S17                            | 3E-006  | embryonic development (sensu Animalia)<br>- physiological process<br>- reproduction                                                        | 0.000   | ribosomal protein S11              | protein synthesis |
| DT367216                 | F658        | 3                   | RE33114p <i>Drosophila melanogaster</i>         | 1E-140  | 81         | Ribosomal_L18p                           | 3E-033  | structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya) - RNA binding                | 0       | ribosomal protein L5               | protein synthesis |
| DT366943                 | F1061       | 1                   | ENSANGP00000011028 <i>Anopheles</i>             | 0.0     | 93         | Ribosomal_L3                             | 1E-057  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis - nucleolus - cytosolic large ribosomal subunit (sensu Eukarya) | 0       | ribosomal protein L3 - RNA binding | protein synthesis |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|------------------------|-------------------|
| DT366935                 | F431        | 2                   | ENSANGP00000022122<br>Anopheles                | 6E-077  | 92         | KOW                                      | 2E-005  | intracellular - ribosome<br>- cytosolic large ribosomal subunit (sensu Eukarya)<br>- RNA binding - protein biosynthesis                                                                    | 0.0     | ribosomal protein L26  | protein synthesis |
| DT367061                 | F1449-E03   | 2                   | ENSANGP00000014421<br>Anopheles                | 1E-107  | 77         | Ribosomal_L13                            | 4E-029  | structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis                                                                              | 0.0     | ribosomal protein L13A | protein synthesis |
| DT367106                 | F434        | 18                  | 60S ribosomal protein                          | 2E-006  | 100        | Ribosomal_L41                            | 2E-008  | protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)<br>- structural constituent of ribosome                                                                        | 0.000   | ribosomal protein      | protein synthesis |
| DT366905                 | F633        | 5                   | 60S acidic ribosomal protein                   | 2E-044  | 84         | 60s_ribosomal                            | 2E-026  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)                                                          | 0.0     | ribosomal protein      | protein synthesis |
| DT366914                 | F348        | 4                   | ENSANGP00000021108<br>Anopheles                | 6E-066  | 95         | Ribosomal_S8                             | 2E-037  | embryonic development (sensu Animalia) - positive regulation of growth rate<br>- larval development (sensu Nematoda)<br>- growth - physiological process - reproduction<br>- gametogenesis | 0.0     | ribosomal protein      | protein synthesis |
| DT366894                 | F730        | 4                   | ENSANGP00000018909<br>Anopheles                | 8E-053  | 93         | Ribosomal_L7Ac                           | 7E-021  | peripheral nervous system development                                                                                                                                                      | 0.0     | ribosomal protein      | protein synthesis |
| DT366889                 | F696        | 3                   | ENSANGP00000011144<br>Anopheles                | 1E-057  | 94         | Ribosomal_L36e                           | 3E-039  | structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)                                                                              | 0.0     | ribosomal protein      | protein synthesis |
| DT366923                 | F207        | 3                   | ENSANGP00000014054<br>Anopheles                | 2E-074  | 81         | Ribosomal_L21e                           | 4E-042  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)                                                          | 0.0     | ribosomal protein      | protein synthesis |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------|-------------------|
| DT367187                 | F908        | 3                   | ribosomal protein S29 <i>Culex pipiens</i>     | 3E-029  | 100        | Ribosomal_S14                            | 1E-008  | intracellular - ribosome<br>- cytosolic small ribosomal subunit (sensu Eukarya)<br>- structural constituent of ribosome - zinc ion binding - RNA binding<br>- regulation of cell shape<br>- protein biosynthesis | 0.0     | ribosomal protein | protein synthesis |
| DT366940                 | F1329-C03   | 3                   | 40S ribosomal protein S28                      | 1E-025  | 92         | Ribosomal_S28e                           | 2E-024  | positive regulation of growth rate - physiological process - reproduction                                                                                                                                        | 0.0     | ribosomal protein | protein synthesis |
| DT366918                 | F237        | 3                   | ENSANGP00000018631 <i>Anopheles</i>            | 3E-037  | 93         | Ribosomal_S21e                           | 3E-029  | embryonic development (sensu Animalia) - larval development (sensu Nematoda) - growth - physiological process - reproduction                                                                                     | 0.0     | ribosomal protein | protein synthesis |
| DT366872                 | F406        | 3                   | ENSANGP00000021870 <i>Anopheles</i>            | 1E-104  | 96         | Ribosomal_S4                             | 7E-024  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)                                                                                      | 0.0     | ribosomal protein | protein synthesis |
| DT366910                 | F1386-G12   | 2                   | ENSANGP00000010955 <i>Anopheles</i>            | 7E-092  | 82         | Ribosomal_L18e                           | 6E-072  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)                                                                                      | 0.0     | ribosomal protein | protein synthesis |
| DT367192                 | F424        | 2                   | ENSANGP00000021862 <i>Anopheles</i>            | 7E-063  | 80         | Ribosomal_L22e                           | 1E-049  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)                                                                                      | 0.0     | ribosomal protein | protein synthesis |
| DT366884                 | F1002       | 2                   | 60S ribosomal protein L31                      | 7E-065  | 100        | Ribosomal_L31e                           | 4E-036  | structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis                                                                                                    | 0.0     | ribosomal protein | protein synthesis |
| DT366932                 | F1021       | 2                   | ENSANGP00000011018 <i>Anopheles</i>            | 1E-085  | 85         | Ribosomal_L6                             | 3E-011  | RNA binding - structural constituent of ribosome - protein biosynthesis - ribosome                                                                                                                               | 0.0     | ribosomal protein | protein synthesis |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------|-------------------|
| DT367196                 | F1098       | 2                   | ENSANGP00000014265<br>Anopheles                | 3E-051  | 68         | Ribosomal_L28e                           | 5E-034  | structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis                                                                                                   | 0.0     | ribosomal protein | protein synthesis |
| DT366937                 | F1316-B02   | 2                   | ENSANGP00000017702<br>Anopheles                | 3E-065  | 91         | Ribosomal_L32e                           | 1E-047  | protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya) - structural constituent of ribosome                                                                                                   | 0.0     | ribosomal protein | protein synthesis |
| DT366887                 | F850        | 2                   | ENSANGP00000016601<br>Anopheles                | 2E-059  | 96         | Ribosomal_S26e                           | 5E-056  | positive regulation of body size - embryonic development (sensu Animalia) - embryonic morphogenesis - larval development (sensu Nematoda) - growth - physiological process - reproduction - locomotory behavior | 0.0     | ribosomal protein | protein synthesis |
| DT366870                 | F374        | 2                   | ENSANGP00000019214<br>Anopheles                | 7E-086  | 94         | Ribosomal_L11                            | 9E-016  | RNA binding - structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya)                                                                                                            | 0.0     | ribosomal protein | protein synthesis |
| DT367060                 | F784        | 2                   | Ribosomal protein L39                          | 9E-022  | 94         | Ribosomal_L39                            | 5E-019  | embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - gametogenesis                                                                      | 0.0     | ribosomal protein | protein synthesis |
| DT366929                 | F271        | 2                   | ENSANGP00000012302<br>Anopheles                | 3E-085  | 99         | ubiquitin                                | 2E-028  | embryonic development (sensu Animalia) - reproduction                                                                                                                                                           | 0.0     | ribosomal protein | protein synthesis |
| DT367225                 | F854        | 2                   | ribosomal protein S17<br>Anopheles             | 2E-062  | 93         | Ribosomal_S17e                           | 1E-053  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)                                                                                     | 0.0     | ribosomal protein | protein synthesis |
| DT366921                 | F388        | 2                   | ENSANGP00000017618<br>Anopheles                | 1E-059  | 90         | Ribosomal_S25                            | 8E-042  | positive regulation of growth rate                                                                                                                                                                              | 0.0     | ribosomal protein | protein synthesis |
| DT367097                 | F581        | 2                   | ENSANGP00000022149<br>Anopheles                | 7E-064  | 76         | Ribosomal_L35Ae                          | 7E-031  | structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - RNA binding                                                                                                            | 0.0     | ribosomal protein | protein synthesis |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------|-------------------|
| DT366892                 | F661        | 2                   | putative large subunit ribosomal prote         | 3E-056  | 99         | Ribosomal_L44                            | 1E-029  | embryonic development (sensu Animalia) - larval development (sensu Nematoda) - growth - reproduction                                                                              | 0.0     | ribosomal protein | protein synthesis |
| DT367088                 | F395        | 1                   | ENSANGP00000021108 Anopheles                   | 1E-053  | 94         | Ribosomal_S8                             | 4E-032  | embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - physiological process - reproduction - gametogenesis | 0.0     | ribosomal protein | protein synthesis |
| DT366899                 | F1287-F03   | 1                   | ENSANGP00000010955 Anopheles                   | 7E-092  | 82         | Ribosomal_L18e                           | 6E-072  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)                                                       | 0.0     | ribosomal protein | protein synthesis |
| DT366939                 | F236        | 1                   | ENSANGP00000021862 Anopheles                   | 7E-063  | 80         | Ribosomal_L22e                           | 1E-049  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)                                                       | 0.0     | ribosomal protein | protein synthesis |
| DT367228                 | F1055       | 1                   | ENSANGP00000013363 Anopheles                   | 1E-044  | 98         | Ribosomal_L37ae                          | 2E-038  | embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth                                                        | 0.0     | ribosomal protein | protein synthesis |
| DT366898                 | F1097       | 1                   | ENSANGP00000017558 Anopheles                   | 3E-058  | 94         | Ribosomal_L29                            | 8E-011  | mRNA binding - structural constituent of ribosome - protein biosynthesis - nucleolus - cytosolic large ribosomal subunit (sensu Eukarya)                                          | 0.0     | ribosomal protein | protein synthesis |
| DT366912                 | F1245-H03   | 1                   | ENSANGP00000021358 Anopheles                   | 1E-110  | 92         | Ribosomal_L15e                           | 5E-089  | positive regulation of growth rate - physiological process - reproduction - gametogenesis                                                                                         | 0.0     | ribosomal protein | protein synthesis |
| DT366926                 | F1376-G02   | 1                   | LP04958p Drosophila melanogaster               | 2E-065  | 78         | S10_pectin                               | 6E-049  | RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)                                                       | 0.0     | ribosomal protein | protein synthesis |
| DT366900                 | F1411-B01   | 1                   | ENSANGP00000023750 Anopheles                   | 1E-122  | 95         | Ribosomal_L10e                           | 8E-091  | cytosolic large ribosomal subunit (sensu Eukarya)                                                                                                                                 | 0.0     | ribosomal protein | protein synthesis |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------|-------------------|
| DT367086                 | F1446-D12   | 1                   | ENSANGP00000019074<br>Anopheles                | 9E-077  | 97         | Ribosomal_S11                            | 3E-035  | intracellular - ribosome<br>- cytosolic small ribosomal subunit (sensu Eukarya)<br>- RNA binding - protein biosynthesis                                         | 0.0     | ribosomal protein | protein synthesis |
| DT367227                 | F1458-E12   | 1                   | ribosomal protein S6 <i>Aedes aegypti</i>      | 3E-061  | 84         | TT_ORF1                                  | 4E-011  | cellular_component<br>unknown                                                                                                                                   | 0.000   | ribosomal protein | protein synthesis |
| DT366917                 | F243        | 1                   | ENSANGP00000015019<br>Anopheles                | 1E-114  | 92         | Ribosomal_L1                             | 1E-043  | embryonic development (sensu Animalia) - positive regulation of growth rate<br>- larval development (sensu Nematoda) - growth<br>- reproduction                 | 0.0     | ribosomal protein | protein synthesis |
| DT366902                 | F276        | 1                   | S7 ribosomal protein <i>Aedes aegypti</i>      | 1E-103  | 99         | Ribosomal_S7e                            | 6E-095  | intracellular - ribosome<br>- cytosolic small ribosomal subunit (sensu Eukarya)<br>- structural constituent of ribosome - RNA binding<br>- protein biosynthesis | 0.0     | ribosomal protein | protein synthesis |
| DT366927                 | F471        | 1                   | ENSANGP00000023750<br>Anopheles                | 1E-122  | 95         | Ribosomal_L10e                           | 8E-091  | cytosolic large ribosomal subunit (sensu Eukarya)                                                                                                               | 0.0     | ribosomal protein | protein synthesis |
| DT366931                 | F555        | 1                   | ENSANGP00000014147<br>Anopheles                | 7E-032  | 53         | Ribosomal_L36                            | 5E-005  | protein biosynthesis<br>- mitochondrial large ribosomal subunit                                                                                                 | 0.0     | ribosomal protein | protein synthesis |
| DT366930                 | F597        | 1                   | ENSANGP00000022284<br>Anopheles                | 9E-065  | 93         | Ribosomal_L7Ae                           | 5E-025  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic small ribosomal subunit (sensu Eukarya)                               | 0.0     | ribosomal protein | protein synthesis |
| DT367064                 | F667        | 1                   | ribosomal protein L31 - forest day mosq        | 4E-067  | 97         | Ribosomal_L34e                           | 6E-042  | RNA binding - structural constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal subunit (sensu Eukarya)                               | 0.0     | ribosomal protein | protein synthesis |
| DT366934                 | F701        | 1                   | ENSANGP00000012582<br>Anopheles                | 3E-031  | 95         | Ribosomal_L38e                           | 9E-028  | positive regulation of growth rate - gametogenesis                                                                                                              | 0.0     | ribosomal protein | protein synthesis |
| DT366901                 | F746        | 1                   | ENSANGP00000021358<br>Anopheles                | 1E-110  | 92         | Ribosomal_L15e                           | 5E-089  | positive regulation of growth rate - physiological process - reproduction<br>- gametogenesis                                                                    | 0.0     | ribosomal protein | protein synthesis |
| DT367202                 | F762        | 1                   | ENSANGP00000010500<br>Anopheles                | 5E-062  | 96         | Ribosomal_L7Ae                           | 3E-023  | neurogenesis                                                                                                                                                    | 0.0     | ribosomal protein | protein synthesis |

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|---------------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|------------------------------------------------|-------------------------|
| DT367203                        | F862        | 1                   | ENSANGP00000016601<br>Anopheles                | 2E-059  | 96         | Ribosomal_S26e                           | 5E-056  | positive regulation of body size - embryonic development (sensu Animalia) - embryonic morphogenesis - larval development (sensu Nematoda) - growth - physiological process - reproduction - locomotory behavior | 0.0     | ribosomal protein                              | protein synthesis       |
| DT366903                        | F972        | 1                   | ENSANGP00000013957<br>Anopheles                | 1E-069  | 83         | Ribosomal_S19                            | 1E-030  | embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - physiological process - reproduction                                               | 0.0     | ribosomal protein                              | protein synthesis       |
| DT366909                        | F976        | 1                   | ENSANGP00000019453<br>Anopheles                | 1E-044  | 97         | Ribosomal_S27e                           | 3E-022  | RNA binding - structural constituent of ribosome - zinc ion binding - protein biosynthesis - signal transduction - cell proliferation - cytosolic small ribosomal subunit (sensu Eukarya)                       | 0.0     | ribosomal protein                              | protein synthesis       |
| DT367084                        | F1181-B11   | 2                   | elongation factor 1 beta <i>Aedes aegypti</i>  | 1E-125  | 100        | EF1BD                                    | 4E-035  | cytosol                                                                                                                                                                                                         | 0       | Probable elongation factor 1-beta - cytosol    | protein synthesis       |
|                                 |             | 142                 |                                                |         |            |                                          |         |                                                                                                                                                                                                                 |         |                                                |                         |
|                                 |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                                                                 |         |                                                |                         |
| <b>transporter/<br/>storage</b> |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                                                                 |         |                                                |                         |
| DT367262                        | F308        | 1                   | ENSANGP00000011786<br>Anopheles                | 7E-024  | 30         | zf-C3HC4                                 | 5E-007  | biological_process unknown - cellular_component unknown                                                                                                                                                         | 0.000   | possible potassium channel modulatory factor   | transporter/<br>storage |
| DT367257                        | F855        | 3                   | transferrin precursor <i>Aedes aegypti</i>     | 0.0     | 99         | transferrin                              | 3E-012  | integral to plasma membrane - iron ion binding                                                                                                                                                                  | 0.0     | transferrin precursor [ <i>Aedes aegypti</i> ] | transporter/<br>storage |
| DT366848                        | F588        | 1                   | ENSANGP00000018348<br>Anopheles                | 1E-135  | 54         | vwd                                      | 0.005   | microtubule binding - heme binding                                                                                                                                                                              | 0.0     | lipophorin - fruit fly                         | transporter/<br>storage |
| DT367261                        | F553        | 1                   | LD22509p <i>Drosophila melanogaster</i>        | 4E-081  | 74         | sugar_tr                                 | 1E-010  | inorganic phosphate transporter activity - phosphate transport - integral to membrane                                                                                                                           | 0.0     | Putative inorganic phosphate cotransporter     | transporter/<br>storage |

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|--------------------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|---------------------------------------------------------------------------------------|---------|-------------------------------------------------------|--------------------------------|
|                                      |             | 6                   |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| <b>odorant biding protein family</b> |             |                     |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| DT366796                             | F192        | 1                   | odorant-binding protein OBP                    | 6E-010  | 34         | PBP_GOBP                                 | 3E-007  | salivary gland cell death - autophagic cell death                                     | 0.000   | odorant-binding protein AgamOBP9                      | odorant binding protein family |
| DT367249                             | F437        | 1                   | putative salivary odorant binding pro          | 8E-018  | 34         | PBP_GOBP                                 | 2E-007  | salivary gland cell death - autophagic cell death                                     | 0.003   | putative salivary odorant binding pro                 | odorant binding protein family |
| DT366797                             | F1353-E03   | 2                   | odorant-binding protein AgamOBP9               | 5E-054  | 69         | PBP_GOBP                                 | 3E-018  | salivary gland cell death - autophagic cell death                                     | 0.0     | odorant-binding protein AgamOBP9                      | odorant binding protein family |
| DT366794                             | F1018       | 2                   | odorant-binding protein AgamOBP9               | 5E-054  | 69         | PBP_GOBP                                 | 3E-018  | salivary gland cell death - autophagic cell death                                     | 0.0     | odorant-binding protein AgamOBP9                      | odorant binding protein family |
| DT366799                             | F410        | 1                   | ENSANGP00000013781 Anopheles                   | 1E-086  | 56         | PBP_GOBP                                 | 0.005   | extracellular space                                                                   | 0.086   | 5'UTR of D7 family member                             | odorant binding protein family |
|                                      |             | 7                   |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| <b>extracellular matrix</b>          |             |                     |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| DT366750                             | F1119       | 1                   | ENSANGP00000021083 Anopheles                   | 1E-088  | 43         | Podocalyxin                              | 3E-008  | extracellular space                                                                   | 0.0     | mucin-like protein                                    | extracellular matrix           |
|                                      |             | 1                   |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| <b>protein modification</b>          |             |                     |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| DT366862                             | F771        | 1                   | ENSANGP00000019887 Anopheles                   | 1E-131  | 95         | HSP70                                    | 4E-075  | heat shock protein activity - neurotransmitter secretion - vesicle-mediated transport | 0       | Molecular chaperones HSP70/HSC70                      | protein modification           |
| DT366861                             | F1138       | 1                   | ENSANGP00000024752 Anopheles                   | 0.0     | 78         | Peptidase_M2                             | 0.0     | peptidyl-di-peptidase A activity - metamorphosis (sensu Insecta)                      | 0       | peptidyl-di-peptidase A activity (based on TIGR data) | protein modification           |
| DT366860                             | F1340-D02   | 1                   | ENSANGP00000024752 Anopheles                   | 0.0     | 78         | Peptidase_M2                             | 0.0     | peptidyl-di-peptidase A activity - metamorphosis (sensu Insecta)                      | 0       | peptidyl-di-peptidase A activity (based on TIGR data) | protein modification           |
| DT366859                             | F558        | 1                   | ENSANGP00000011976 Anopheles                   | 1E-066  | 83         | OST3_OST6                                | 2E-045  | mitochondrion                                                                         | 0.0     | Oligosaccharyltransferase, gamma subunit              | protein modification           |
|                                      |             | 4                   |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |
| <b>signal transduction</b>           |             |                     |                                                |         |            |                                          |         |                                                                                       |         |                                                       |                                |

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|---------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------------------------------------------------------------------------|---------------------|
| DT367250                  | F977        | 1                   | ENSANGP00000014018<br>Anopheles                | 1E-107  | 92         | pkinase                                  | 2E-015  | MAPKKK cascade<br>- protein serine/threonine kinase activity - nucleus - wing morphogenesis - SAP kinase activity - regulation of BMP signaling pathway<br>- regulation of innate immune response | 0.0     | Mitogen-activated protein kinase 14B                                     | signal transduction |
| DT367252                  | F447        | 1                   | ENSANGP00000010163<br>Anopheles                | 2E-090  | 54         |                                          |         | neurogenesis                                                                                                                                                                                      | 0.047   | Fibrillins and related proteins containing Ca2+-binding EGF-like domains | signal transduction |
| DT367251                  | F574        | 1                   |                                                |         |            |                                          |         |                                                                                                                                                                                                   |         | Sex-regulated protein janus-A                                            | signal transduction |
| DT367255                  | fF344       | 1                   | cAMP generating peptide precursor              | 3E-009  | 63         |                                          |         | mitochondrion                                                                                                                                                                                     | 0.000   | cAMP generating peptide precursor                                        | signal transduction |
| DT367254                  | F1416-B11   | 1                   | ENSANGP00000014526<br>Anopheles                | 3E-078  | 70         | Fe_acq                                   | 0.006   | RNA polymerase II transcription factor activity<br>- transcription corepressor activity - regulation of transcription from Pol II promoter - development<br>- cell proliferation                  | 0.0     | similar to cellular repressor of E1                                      | signal transduction |
| DT367253                  | F448        | 1                   | ENSANGP00000018569<br>Anopheles                | 4E-070  | 59         | pkinase                                  | 3E-036  | plasma membrane<br>- protein amino acid phosphorylation - terminal region determination - eggshell pattern formation (sensu Insecta)                                                              | 0.0     | Tyrosine-protein kinase receptor torso precursor                         | signal transduction |
|                           |             | 6                   |                                                |         |            |                                          |         |                                                                                                                                                                                                   |         |                                                                          |                     |
| <b>lipid metabolism</b>   |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                                                   |         |                                                                          |                     |
| DT366785                  | F427        | 1                   | ENSANGP00000010825<br>Anopheles                | 1E-103  | 55         | lipase                                   | 2E-048  | extracellular space                                                                                                                                                                               | 0.0     | lipase                                                                   | lipid metabolism    |
| DT366786                  | F736        | 1                   | ENSANGP00000012410<br>Anopheles                | 0.0     | 74         | FA_desaturase                            | 1E-016  | mitochondrion                                                                                                                                                                                     | 0.0     | Delta-5 fatty acid desaturase                                            | lipid metabolism    |
|                           |             | 2                   |                                                |         |            |                                          |         |                                                                                                                                                                                                   |         |                                                                          |                     |
| <b>nuclear regulation</b> |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                                                   |         |                                                                          |                     |
| DT366789                  | F586        | 1                   | ENSANGP00000012210<br>Anopheles                | 1E-164  | 78         | Peptidase_C1                             | 2E-059  | nuclear export signal receptor activity - protein-nucleus export - nucleus<br>- nuclear pore - cytoplasm                                                                                          | 0       | Exportin                                                                 | nuclear regulation  |

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|---------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------|-------------------------|
| DT366792                  | F291        | 1                   | ENSANGP00000012210<br>Anopheles                | 1E-164  | 78         | Peptidase_C1                             | 2E-059  | nuclear export signal<br>receptor activity - protein-<br>nucleus export - nucleus<br>- nuclear pore - cytoplasm                                                       | 0       | Exportin                                                 | nuclear regulation      |
| DT366791                  | F267        | 2                   | Tamozhennic protein                            | 7E-059  | 48         |                                          |         | molecular_function<br>unknown<br>- spermatogenesis -<br>cytoplasm                                                                                                     | 0.000   | homolog of Dm<br>Tamozhennic protein<br>- nuclear import | nuclear regulation      |
|                           |             | 4                   |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |
| <b>oxidant metabolism</b> |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |
| DT366855                  | F416        | 1                   | ENSANGP00000001916<br>Anopheles                | 1E-113  | 84         | p450                                     | 6E-055  | embryonic development<br>(sensu Animalia)                                                                                                                             | 0.0     | Cytochrome P450 CYP4/<br>CYP19/CYP26 subfamilies         | oxidant metabolism      |
| DT366854                  | F828        | 1                   | ENSANGP000000022709<br>Anopheles               | 4E-030  | 66         |                                          |         | binding - vitamin A<br>metabolism - visual<br>perception - soluble<br>fraction                                                                                        | 0.000   | possible cytochrome P450<br>28a5                         | oxidant metabolism      |
| DT367260                  | F613        | 1                   | ENSANGP000000022709<br>Anopheles               | 4E-030  | 66         |                                          |         | binding - vitamin A<br>metabolism - visual<br>perception - soluble<br>fraction                                                                                        | 0.000   | possible CRALBP                                          | transporter/<br>storage |
|                           |             | 3                   |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |
| <b>immunity</b>           |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |
| DT366753                  | F599        | 1                   |                                                |         |            |                                          |         |                                                                                                                                                                       |         | cecropin A precursor [ <i>Aedes aegypti</i> ]            | immunity                |
| DT366754                  | F572        | 3                   | RE15268p <i>Drosophila melanogaster</i>        | 2E-014  | 35         | lectin_c                                 | 1E-010  | galactose binding                                                                                                                                                     | 0.000   | C-type lectin                                            | immunity                |
| DT366875                  | F837        | 2                   | ENSANGP00000012229<br>Anopheles                | 2E-081  | 96         | Ribosomal_S12                            | 1E-036  | intracellular - ribosome<br>- cytosolic small ribosomal<br>subunit (sensu Eukarya)<br>- structural constituent of<br>ribosome - RNA binding<br>- protein biosynthesis | 0.0     | ribosomal protein                                        | protein synthesis       |
| DT366751                  | F861        | 2                   | defensin A4 <i>Aedes aegypti</i>               | 2E-052  | 100        | Arthro_defensin                          | 6E-011  | antibacterial humoral<br>response (sensu<br>Protostomia) - defense<br>response to bacteria -<br>defense response to Gram-<br>positive bacteria                        | 0.0     | defensin                                                 | immunity                |
|                           |             | 8                   |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |
| <b>lysosomal</b>          |             |                     |                                                |         |            |                                          |         |                                                                                                                                                                       |         |                                                          |                         |

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|------------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------|---------|---------------------------------------------------------------|-----------------------|
| DT366757                     | F538        | 1                   | ENSANGP00000020002<br>Anopheles                | 1E-127  | 90         | Peptidase_C1                             | 1E-091  | salivary gland cell death<br>- autophagic cell death                                                                                    | 0       | Cathepsin L precursor<br>- cathepsin L activity<br>- lysosome | lysosomal             |
|                              |             | 1                   |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| <b>nucleotide metabolism</b> |             |                     |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| DT366788                     | F1092       | 1                   | ENSANGP00000010003<br>Anopheles                | 0.0     | 84         | 5_nucleotidase                           | 1E-117  | 5'-nucleotidase activity<br>- cytosol                                                                                                   | 0       | 5'-nucleotidase, cytosolic                                    | nucleotide metabolism |
| DT366787                     | F1537-D07   | 1                   | ENSANGP00000024036<br>Anopheles                | 0.0     | 86         | FA_desaturase                            | 1E-044  | integral to membrane<br>- fatty acid biosynthesis                                                                                       | 0.0     | fatty acid desaturase                                         | nucleotide metabolism |
|                              |             | 2                   |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| <b>proteasome</b>            |             |                     |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| DT366866                     | F409        | 2                   | ENSANGP00000010608<br>Anopheles                | 1E-123  | 78         | Nin1_C                                   | 4E-057  | endopeptidase activity<br>- proteasome regulatory<br>particle (sensu Eukarya)<br>- proteolysis and<br>peptidolysis                      | 0.0     | 26S proteasome regulatory<br>complex,                         | proteasome            |
| DT366868                     | F823        | 1                   | similar to<br>ENSANGP00000007022 Apis          | 1E-121  | 85         | proteasome                               | 2E-048  | proteasome complex<br>(sensu Eukarya)                                                                                                   | 0       | Proteasome subunit alpha<br>type 7 - 26S proteasome           | proteasome            |
| DT367248                     | F288        | 1                   | similar to Drosophila<br>melanogaster Rp       | 3E-069  | 100        | ubiquitin                                | 2E-028  | embryonic development<br>(sensu Animalia) -<br>reproduction                                                                             | 0.0     | ubiquitin                                                     | ps proteasome         |
|                              |             | 4                   |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| <b>Unknown</b>               |             |                     |                                                |         |            |                                          |         |                                                                                                                                         |         |                                                               |                       |
| DT367265                     | F1410-A12   | 1                   | ENSANGP00000004798<br>Anopheles                | 3E-076  | 75         | DUF852                                   | 2E-054  | mitochondrion                                                                                                                           | 0.0     | unknown conserved                                             | unknown               |
| DT367263                     | F362        | 1                   | ENSANGP00000011689<br>Anopheles                | 1E-036  | 68         |                                          |         |                                                                                                                                         |         | unknown conserved                                             | unknown               |
| DT367264                     | F567        | 1                   | ENSANGP00000009630<br>Anopheles                | 4E-018  | 57         |                                          |         | structural molecule activity<br>- epidermis development<br>- intermediate filament                                                      | 0.000   | unknown conserved                                             | unknown               |
| DT367291                     | F824        | 4                   | ENSANGP00000025118<br>Anopheles                | 4E-047  | 83         | 60s_ribosomal                            | 4E-019  | RNA binding - structural<br>constituent of ribosome<br>- protein biosynthesis<br>- cytosolic large ribosomal<br>subunit (sensu Eukarya) | 0.0     | unknown                                                       | unknown               |
| DT367313                     | F185        | 4                   |                                                |         |            |                                          |         |                                                                                                                                         |         | unknown                                                       | unknown               |
| DT367268                     | F1347-D09   | 3                   | CpCOWP2; cryptosporidium                       | 0.023   | 32         |                                          |         | larval development (sensu<br>Nematoda) - growth -<br>locomotory behavior                                                                | 0.058   | unknown                                                       | unknown               |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------|---------|----------|---------|
| DT367270                 | F1223-F05   | 2                   | Ab2-057 [Rattus norvegicus]                    | 2E-020  | 58         |                                          |         | positive regulation of cell proliferation - hemopoiesis                                 | 0.001   | unknown  | unknown |
| DT367278                 | F1001       | 1                   | ENSANGP00000024365 Anopheles                   | 0.83    | 29         | DUF216                                   | 0.002   |                                                                                         |         | unknown  | unknown |
| DT367290                 | F1016       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367316                 | F1026       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367317                 | F1038       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367306                 | F1051       | 1                   | similar to Y55B1BR.3.p Caenorhabditi           | 6E-006  | 28         | DUF164                                   | 0.031   | biological_process<br>unknown - cellular_<br>component unknown                          | 0.000   | unknown  | unknown |
| DT367288                 | F1056       | 1                   | hypothetical protein                           | 0.40    | 23         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367318                 | F1058       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367319                 | F1115       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367320                 | F1143       | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367281                 | F1190-C08   | 1                   | hypothetical protein                           | 0.022   | 58         | Keratin_B2                               | 0.001   | structural constituent of epidermis - biological_<br>process unknown<br>- extracellular | 0.021   | unknown  | unknown |
| DT367284                 | F1225-F07   | 1                   | ENSANGP00000014861 Anopheles                   | 0.002   | 34         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367272                 | F1298-H02   | 1                   | hypothetical protein Plasmodium fa             | 5.8     | 37         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367286                 | F1320-B06   | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367273                 | F1360-E10   | 1                   | coatamer alpha subunit                         | 7.2     | 24         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367321                 | F1361-E11   | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367322                 | F1362-E12   | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367279                 | F1483-H01   | 1                   | similar to Dictyostelium discoideum            | 4E-028  | 39         | SKN1                                     | 5E-004  | regulation of protein activity, epigenetic                                              | 0.0     | unknown  | unknown |
| DT367305                 | F1497-A03   | 1                   | ENSANGP00000016160 Anopheles                   | 4E-020  | 65         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367277                 | F205        | 1                   | bA99E24.1.1 protocadherin 19                   | 3.6     | 36         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367302                 | F219        | 1                   | ENSANGP00000016495 Anopheles                   | 1E-026  | 60         | DUF227                                   | 6E-014  | protein binding                                                                         | 0.000   | unknown  | unknown |
| DT367285                 | F225        | 1                   | NTPase/helicase maize rayado fino              | 8.2     | 41         |                                          |         |                                                                                         |         | unknown  | unknown |
| DT367323                 | F261        | 1                   |                                                |         |            |                                          |         |                                                                                         |         | unknown  | unknown |

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|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-------------------------------------------------------------------------------------|---------|----------|---------|
| DT367282                 | F306        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367324                 | F350        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367311                 | F380        | 1                   | hypothetical protein Plasmodium fa             | 8.8     | 36         |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367297                 | F402        | 1                   | ENSANGP00000014951 Anopheles                   | 1E-107  | 91         | Ribosomal_S8e                            | 6E-054  | larval development (sensu Nematoda) - growth - physiological process - reproduction | 0.0     | unknown  | unknown |
| DT367298                 | F403        | 1                   | ENSANGP00000014951 Anopheles                   | 1E-107  | 91         | Ribosomal_S8e                            | 6E-054  | larval development (sensu Nematoda) - growth - physiological process - reproduction | 0.0     | unknown  | unknown |
| DT367325                 | F417        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367326                 | F420        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367300                 | F445        | 1                   | ENSANGP00000012302 Anopheles                   | 3E-085  | 99         | ubiquitin                                | 2E-028  | embryonic development (sensu Animalia) - reproduction                               | 0.0     | unknown  | unknown |
| DT367327                 | F450        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367304                 | F533        | 1                   | GM14559p [Drosophila melanogaster]             | 7E-021  | 94         | GTP_EFTU_D3                              | 7E-006  | cytoplasm                                                                           | 0.0     | unknown  | unknown |
| DT367276                 | F547        | 1                   | seven transmembrane helix receptor             | 9E-007  | 29         | Sre                                      | 0.021   |                                                                                     |         | unknown  | unknown |
| DT367308                 | F591        | 1                   | maturase [Narcissus tazetta]                   | 0.82    | 26         |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367328                 | F604        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367287                 | F609        | 1                   | ENSANGP00000018748 Anopheles                   | 3E-047  | 54         | TP2                                      | 0.028   |                                                                                     |         | unknown  | unknown |
| DT367299                 | F638        | 1                   | ENSANGP00000011784 Anopheles                   | 3E-098  | 93         | Ribosomal_L22                            | 4E-026  | RNA binding - structural constituent of ribosome - ribosome                         | 0.0     | unknown  | unknown |
| DT367329                 | F662        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367266                 | F697        | 1                   | ENSANGP00000023084 Anopheles                   | 6E-006  | 38         |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367330                 | F738        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367303                 | F754        | 1                   | ENSANGP00000014492 Anopheles                   | 2E-023  | 59         |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367331                 | F760        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367275                 | F763        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |
| DT367332                 | F768        | 1                   |                                                |         |            |                                          |         |                                                                                     |         | unknown  | unknown |

**Table 1.** *Aedes aegypti* vitellogenic fat body cDNA cluster encoding proteins

| GenBank Accession number | ID sequence | Number of sequences | Best match of AEGY-TIGR to NR protein database | E value | % identity | Best match of AEGY-TIGR to PFAM database | E value | Best match of AEGY-TIGR to GO database                                                                                                              | E value | Comments                                        | class             |
|--------------------------|-------------|---------------------|------------------------------------------------|---------|------------|------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------------------------------------|-------------------|
| DT367293                 | F1045       | 1                   | ENSANGP00000014492<br>Anopheles                | 2E-023  | 59         |                                          |         |                                                                                                                                                     |         | unknown                                         | unknown           |
| DT367310                 | F863        | 1                   | hypothetical protein cgd8_140                  | 4.8     | 48         |                                          |         |                                                                                                                                                     |         | unknown                                         | unknown           |
| DT367279                 | F1483-H01   | 1                   |                                                |         |            |                                          |         |                                                                                                                                                     |         | unknown                                         | unknown           |
| DT367309                 | F970        | 1                   | 100 kDa heme:hemoexin-binding prot             | 2.3     | 20         |                                          |         |                                                                                                                                                     |         | unknown                                         | unknown           |
| DT367295                 | F210        | 1                   | ENSANGP00000002975<br>Anopheles                | 1E-137  | 64         | TMS_TDE                                  | 1E-122  | plasma membrane - integral to membrane                                                                                                              | 0.0     | membrane protein TMS1d                          | unknown           |
| DT367301                 | F1368-F06   | 1                   | ENSANGP000000020785<br>Anopheles               | 1E-074  | 64         | Somatomedin_B                            | 0.001   | extracellular matrix structural constituent - endosome transport - extracellular                                                                    | 0.0     | similar to tubulointerstitial nephritis antigen | unknown           |
| DT366869                 | F575        | 1                   | ribosomal protein P0 [Aedes albopictus]        | 1E-171  | 98         | Ribosomal_L10                            | 1E-030  | structural constituent of ribosome - DNA-(apurinic or apyrimidinic site) lyase activity - cytosolic ribosome (sensu Eukarya) - protein biosynthesis | 0       | ribosomal protein                               | protein synthesis |
| DT367274                 | F338        | 1                   | ribosomal protein P0 [Aedes albopictus]        | 1E-171  | 98         | Ribosomal_L10                            | 1E-030  | structural constituent of ribosome - DNA-(apurinic or apyrimidinic site) lyase activity - cytosolic ribosome (sensu Eukarya) - protein biosynthesis | 0       | unknown                                         | unknown           |
|                          |             | 68                  |                                                |         |            |                                          |         |                                                                                                                                                     |         |                                                 |                   |

The function were based on similarity of the deduced *Ae. aegypti* protein sequences deposited in the NR protein database at NCBI. Translated sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases.

**Table 2.** Division of the transcriptis according to their functions

| Category                       | Number of transcripts | Percentege % |
|--------------------------------|-----------------------|--------------|
| carbohydrate metabolism        | 2                     | 0.57         |
| cytoskeletal                   | 2                     | 0.57         |
| energy metabolism              | 28                    | 7.98         |
| extracellular matrix           | 1                     | 0.28         |
| immunity                       | 8                     | 2.28         |
| lipid metabolism               | 2                     | 0.57         |
| lysosomal                      | 1                     | 0.28         |
| nuclear regulation             | 4                     | 1.14         |
| nucleotide metabolism          | 2                     | 0.57         |
| odorant binding protein family | 7                     | 1.99         |
| oxidant metabolism             | 3                     | 0.85         |
| protease                       | 3                     | 0.85         |
| proteasome                     | 4                     | 1.14         |
| protein export                 | 3                     | 0.85         |
| protein modification           | 4                     | 1.14         |
| protein synthesis              | 142                   | 40.46        |
| signal transduction            | 6                     | 1.71         |
| transcription machinery        | 1                     | 0.28         |
| transporter/storage            | 6                     | 1.71         |
| oogenesis                      | 54                    | 15.38        |
| unknown                        | 68                    | 19.37        |
| total                          | 351                   | 100.00       |

The function were based on similarity of the deduced *Ae. aegypti* protein sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases.

proteins (Hekmat-Safe et al. 2000) are expressed mainly in the insect antennae and they are proposed to bind small hydrophobic odorant molecules, carry and present them to the olfactory receptors. Proteins structurally related to odorant binding proteins have been identified in non-sensory organs and in the hemolymph of insects, where they might carry hydrophobic ligands related to a variety of functions (Biessmann et al. 2002; Calvo et al. 2004). A hemolymph protein with sequence and structural characteristics similar to odorant binding proteins, designated THP12, was described in *Tenebrio molitor* as a carrier protein, associated with the transport of small hydrophobic ligands through the hemolymph (Graham et al. 2001; Rothemund et al. 1999). These *Ae. aegypti* cDNAs may correspond to novel mosquito proteins yet to be described.

Three immunity-related transcripts were found, corresponding to molecules with high amino acid similarity (93%) to the C-type lectin found in *Drosophila melanogaster* (Haq et al. 1996), defensin and cecropin of *Ae. aegypti*. One sequence was identified as a cecropin precursor, also from *Ae. aegypti*. The cecropins have antibacterial and antifungal activity (Zheng and Zheng, 2002) while defensins are antimicrobial peptides that are

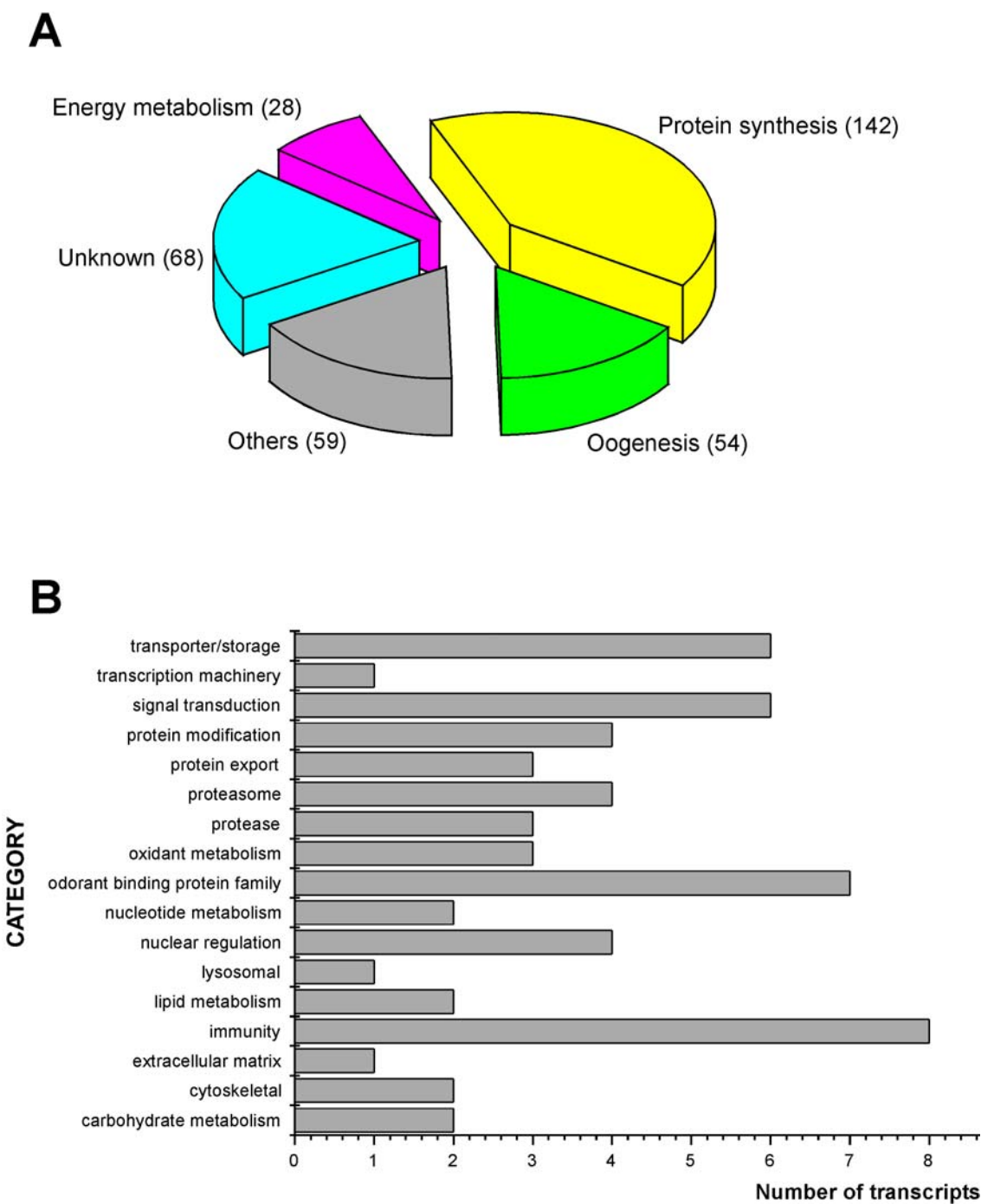
activated in the presence of Gram-positive or negative bacteria. These peptides also are activated by infection with filarial worms (Bartholomay et al. 2004).

Transcripts related to vitellogenesis (Table 1 and Table 2), represent 15.38% of the fat body transcriptome (54 sequences, 22 clusters) at 24 h PBM, and encode vitellogenins, vitellogenic cathepsin-B and vitellogenic carboxypeptidase. These data are in agreement with the previously-described increase of vitellogenic proteins expression in mosquitoes following a blood meal (Kokoza et al., 2001). Transcripts representing three distinct vitellogenins, three cathepsin Bs and two vitellogenic carboxypeptidases were observed in the analyzed set of cDNAs. Multiple vitellogenin genes in the genome of *Ae. aegypti* have been described (Hamblin et al. 1987) while the multiple transcripts with similarity to cathepsin B and vitellogenic carboxypeptidase indicate that these proteins may be encoded by more than one gene as well. It has been already established that vitellogenin, vitellogenic carboxypeptidase and cathepsin B are synthesized by the fat bodies in response to a blood meal, secreted into the hemolymph and accumulate in ovaries, where they are deposited in the oocytes during development (Raikhel, 1987; Raikhel, et al., 2002; Raikhel and Lea, 1983; Ribeiro, 2003).

Final remarks

The sequencing of 800 *Ae. aegypti* fat body-derived cDNAs, and their annotation and organization provides a general picture of the metabolic state of the tissue at 24 h PBM. The insect fat body is a complex tissue where many indispensable processes of intermediate metabolism, storage and immunity occur, however its major function is synthesis and export of proteins (ex. hexamerins, transport proteins and vitellogenic proteins, etc).

This survey revealed strongly-expressed genes in the fat bodies of *Ae. aegypti* and is useful for the purpose of identifying genes whose promoters can be used for driving the expression of anti-pathogen molecules in transgenic mosquitoes, (James et al. 1999). In addition, the use of transgenic mosquito strains in which a female-specific promoter controls the expression of a lethal gene has been proposed as a powerful technique to generate a male-only population useful for a variation of the Sterile Insect Release technique (Thomas et al.



**Figure 1.** Number of sequences of the *Aedes aegypti* fat body cDNA library. The transcript categories are more represented (A) and less represented (B - Others). The sequences were classified in accordance with function based on similarity of the deduced *Ae. aegypti* protein sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSblast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al 2000) databases. See also Table 2.

2000). This requires that a strain of the target organism carry a dominant, sex-specific lethal gene whose expression can be repressed in the laboratory or insectary under controlled conditions. Some of these genes may be suitable targets for new insecticides or strategies to block parasite development within the vector. Other advances could result from the comparative analysis of regulatory *cis*-acting elements found in the promoters of selected genes. For example, searches for conserved motifs may identify common regulatory motifs in the promoters of genes strongly-expressed in the fat bodies of mosquitoes. It is anticipated that genes expressed exclusively in the fat body, mostly those regulated coordinately, may share transcription factor-binding sites in their promoters and other regulatory sequences in the respective UTRs.

## Acknowledgements

We would also like to thank Neusa S. Fernandes and Alexandre Moura for technical assistance. We are grateful to Dr. Hernando A. del Portillo and Dr. Sérgio Verjovski de Almeida for critical reading of the manuscript. We would also like to thank Dr. José Marcos C. Ribeiro for his assistance in the analysis of the transcripts. This work is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) (00/12138-7). Partial support was provided by a grant from the NIH (AI 29746) to A.A. James. F.M.F and E.F.M. are fellows from FAPESP. M.L.C. and A.G. de B. are research fellows from CNPq.

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