

# **A transcriptome analysis reveals iron-regulated genes in *Trichomonas vaginalis*.**

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## **Abstract**

Iron is an essential nutrient for the parasitic protist *Trichomonas vaginalis* and plays a pivotal role in the establishment of *Trichomonas* infections, proliferation, and virulence. To gain insight into the molecular mechanisms underlying these iron-dependent phenomena, we screened iron-regulated genes using the first oligonucleotide microarray for *T. vaginalis* and by comparative EST (expressed sequence tag) sequencing of cDNA libraries derived from trichomonads cultivated under iron-rich (+Fe) and iron-restricted (-Fe) conditions. A comparative analysis of over 19,000 ESTs from both libraries revealed 336 iron-regulated genes, of which 165 were upregulated under +Fe conditions and 171 under -Fe conditions. According to the microarray analysis, 308 of 4,950 unique genes were differentially expressed. Of these, 182 genes were upregulated under +Fe conditions, and 126 were upregulated under -Fe conditions. The results of both methods were congruent concerning the regulatory trends and the representation of gene categories. Under +Fe conditions, the expression of proteins involved in carbohydrate metabolism, particularly in the energetic metabolism of hydrogenosomes, and methionine catabolism was increased. The iron-sulphur cluster assembly machinery and certain cysteine proteases are of particular importance among the proteins upregulated under -Fe conditions. A unique feature of the *T. vaginalis* genome is the presence of multiple paralogous copies for a majority of the genes. Although the origins and reasons for this gene expansion remain unclear, the retention of multiple gene copies could provide an advantage by allowing differential expression during growth in variable environmental conditions. Indeed, for a majority of the genes, iron affected the expression of only some of the paralogous copies, whereas the expression of the other paralogues was iron-independent. This finding indicates a very stringent regulation of the differentially expressed paralogous genes in response to changes in the availability of exogenous nutrients.

## 1. Introduction

The parasitic flagellate *Trichomonas vaginalis* is the causative agent of trichomoniasis, which is the most common sexually transmitted infection of non-viral origin in humans. Although trichomoniasis is usually self-limiting in males, it causes serious health problems for female patients, including an increased risk of cervical cancer, pelvic inflammatory disease, infertility and transmission of the human immunodeficiency virus (Laga et al., 1993; Viikki et al., 2000; Moodley et al., 2002; Zhang and Begg, 1994). Trichomoniasis during pregnancy is associated with low birth weight, the premature rupture of membranes and preterm birth (Cotch et al., 1997). The establishment of a *T. vaginalis* infection as well as infections by other pathogenic microorganisms is dependent on the efficient acquisition of essential nutrients such as iron, from the host environment. Trichomonads can utilise various host iron-containing proteins such as lactoferrin, transferrin, ferritin, haemoglobin and low-molecular-weight-iron complexes (Sutak et al., 2008). Iron is required for critical housekeeping functions such as proteosynthesis, genome duplication and energy fixation. A significant portion of the trichomonad energy metabolism takes place in hydrogenosomes, whose function is particularly dependent on iron (Gorrell, 1985; Vanacova et al., 2001). These specific mitochondria-related organelles contain a number of FeS proteins that catalyse key steps in ferredoxin-linked electron transport, hydrogen production and ATP synthesis at the level of substrate phosphorylation (Hrdy et al., 2004). The formation of FeS clusters in the catalytic centres of apoproteins is mediated by the hydrogenosomal iron-sulphur cluster (ISC) assembly machinery, which consists of approximately 10 proteins, of which cysteine desulfurase (IscS) and the molecular scaffold protein IscU are the main components (Tachezy et al., 2001; Sutak et al., 2004a). Iron is also required for the functions of cytosolic and nuclear FeS proteins such as Rli1p, which is a protein essential for ribosome biogenesis and function (Kispal et al., 2005; Yarunin et al., 2005). This protein

is highly conserved in eukaryotes, including *T. vaginalis* (Smid et al., 2008).

In addition to housekeeping functions, iron affects specific host-pathogen interactions associated with the virulence of the parasite. Experiments performed in vitro have shown that iron regulates the cytoadherence of *T. vaginalis* to target cells (Mundodi et al., 2006) as well as the expression of cysteine proteinases (Sommer et al., 2005; Solano-Gonzalez et al., 2007; Kummer et al., 2008), ecto-ATPases and ecto-phosphatases (De Jesus et al., 2006), and it increases trichomonad resistance to complement-mediated lysis (Alderete et al., 1995). Iron-dependent enhancement of experimental infections in mice was demonstrated with the related bovine parasite *Tritrichomonas foetus* (Kulda et al., 1999). However, little is known about the mechanisms underlying the iron-dependent regulation of these processes. Positive iron regulation at the transcriptional level was observed for the expression of some hydrogenosomal proteins, including malic enzyme (ME) and pyruvate:ferredoxin oxidoreductase (PFOR) (Vanacova et al., 2001). A detailed study of the iron-dependent regulation of hydrogenosomal ME, which may alternatively be present on the cell surface as the adhesin (AP65-1) (Hirt et al., 2007), led to the identification of Myb recognition elements and novel Myb proteins that appear to be components of a multifarious regulatory machinery in *T. vaginalis* (Ong et al., 2006; Ong et al., 2007). Two cysteine proteases, TVCP4 and TVCP12, were recently reported to be regulated at the post-transcriptional level by an IRE/IRP-like system (Solano-Gonzalez et al., 2007; Torres-Romero and Arroyo, 2009).

We combined cDNA microarray analysis with an expressed-sequence-tag (EST) approach to map iron-regulated genes and to reconstruct iron-regulated-pathways in *Trichomonas*

*vaginalis*. Our data revealed numerous iron-responsive genes that are involved in several essential pathways, particularly in cytosolic glycolysis and extended glycolysis in hydrogenosomes, as well genes that encode components of the FeS cluster assembly machinery. Moreover, iron affected the expression of many genes with unknown functions.

## **2. Materials and methods**

### **2.1. Cell cultures**

Axenic cultures of *Trichomonas vaginalis* strain T1 were grown in trypticase-yeast extract-maltose (TYM) medium supplemented with 10% heat-inactivated horse serum, pH 6.2 (Diamond, 1957). Iron-rich medium (+Fe) was supplemented with Fe-nitrilotriacetate to the final Fe concentration of 100  $\mu$ M; iron-restricted medium (-Fe) supplemented with 50  $\mu$ M 2-2-dipyridyl (Sigma) instead of Fe-nitrilotriacetate and cells were subcultured in this medium for ten passages prior to experiment.

### **2.2. cDNA library construction and DNA sequencing**

RNA isolation kit (Pharmacia) was used to extract the total RNA from cells grown under +Fe and -Fe conditions, and contaminating genomic DNA was digested with DNase I. PolyA<sup>+</sup> RNA was isolated using a PolyA<sup>+</sup> tract mRNA isolation kit (Promega). Complementary DNA was synthesised using a ZAP-cDNA synthesis kit after priming with oligo-dT. The cDNA was then directionally cloned into the EcoRI and XhoI sites of the Uni-ZAP XR vector (Stratagene). Single and well-separated plaques were cored out from agar plates and transferred to 96-well microtiter plates containing SM buffer. The phage stocks were used as templates for cDNA insert amplification with T3 and T7 primers (1 nM for each primer). The amplified products were separated in 1.5% agarose gels, and clones that yielded single PCR-amplified bands were collected

for sequencing. Single-pass sequencing from the 5'-end of the cDNA insert was initiated with a T3 primer using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The sequencing products were resolved and analysed on an ABI PRISM 377 (Applied Biosystems) or a MEGABACE DNA Sequencer (GE). The nucleotide sequences obtained were processed with the Phred/Phrap/Consed package (<http://www.phrap.org/phredphrapconsed.html>).

### **2.3. Functional annotations and sequence analysis**

BLAST tools were used to compare the assembled sequence contigs to known *Trichomonas* mRNAs, putative open-reading-frames from the *T. vaginalis* G3 genome (Carlton et al., 2007) and NCBI's non-redundant (nr) nucleotide (E-value  $10^{-15}$ ) and protein database. Genes were functionally annotated based on the Interpro and Gene Ontology databases. Contigs with identity greater than 60% of their length were annotated and assigned to KEGG pathways.

Putative coding regions from the EST data were collected from BLASTX alignments, and the codon usage bias was calculated. A Perl script was also written to locate poly-A tails and to search 10-35 bp upstream for putative mRNA poly-adenylation signals; the script allowed one mismatch and two mismatches from the sequence AAUAAA. Putative signal sequences and transmembrane domains in the coding regions were identified by SignalP 3.0 and TMHMM 2.0 (<http://www.cbs.dtu.dk/services/>), respectively. Putative *Trichomonas* protein kinases and peptidases were identified by sequence comparison with datasets downloaded from the KinBase (<http://www.kinase.com>) and the MEROPS (<http://merops.sanger.ac.uk>) databases, respectively.

### **2.4 Microarray sample preparation**

RNA from trichomonads grown in +Fe and -Fe conditions was isolated using a QuickPrep Total RNA Extraction Kit (Amersham Biosciences) according to the manufacturer's instructions. The

total RNA was then further purified using an RNeasy CleanUp Kit (Quiagen). The RNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The integrity of the RNA was checked by agarose gel electrophoresis. The same RNA samples were used in parallel experiments for cDNA microarray analysis and qRT-PCR. cDNA probes were synthesised using 2 µg of total RNA and primers labelled with Cy3 and Cy5, respectively, according to the manufacturer's instructions (3DNA Array 900 Expression Array Detection Kit, Genisphere). Four independent RNA samples (biological replicates) from *T. vaginalis* strain T1 grown under +Fe and -Fe conditions were compared. A dye-swap design was used to prevent dye-associated effects on cDNA synthesis.

## **2.5 Microarray analysis**

A *T. vaginalis* customised cDNA microarray (TvArray V2.0) was implemented by the Molecular Regulation and Bioinformatics Laboratory, Chang Gung University, Taiwan. The TvArray chip contained PCR products amplified from 7,688 EST clones with an average GC content of 38.36 % and an average length of 384 bps. The cDNA inserts were fabricated on GAPST<sup>™</sup> II Coated Slides (Corning, USA). The microarrays were prehybridised in Coplin chambers using a solution containing 1% BSA, 1% SDS and 3x SSC (1x SSC consists of 0.15 M NaCl with 0.015 M sodium acetate). The slides were incubated at 50°C for 30 min, washed with water and isopropanol and dried by centrifugation at 90 x g for 3 min. Hybridisation with the cDNA hybridisation mix and washes were performed following the protocol for the 3DNA Array 900 Expression Array Detection Kit (Genisphere). cDNA hybridisations were performed in a VersArray Hybridization Chamber (Bio-Rad) at 60°C overnight. Hybridisations with fluorescent 3DNA reagents were performed in the same chamber at 60°C for 4 h. After the final washing step, the slides were dried by

centrifugation at 90 x g for 3 min and scanned using the GenePix 4200A scanner (Axon). GenePix Pro 5.1 software was used to determine the average signal intensity and the local background for each spot. The Cy3/Cy5 fluorescence ratios were log<sub>2</sub> transformed and normalised by the LOWESS normalisation method using the TIGR microarray data analysis system (MIDAS), version 2.19 (Saeed et al., 2003). In total, ten independent hybridisations using samples from four independent cultures and RNA extractions were performed, and data from the independent experiments were combined. A one-class Student's t-test was performed with the TIGR MultiExperiment Viewer 4.0 (Saeed et al., 2006). Genes with expression ratios that changed by a factor of at least 1.3 and with  $P < 0.01$  were considered to be significantly regulated by iron. The results of all experiments are available in the ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress/>) under the array design name TvArray V1.0.

## **2.6 q-RT PCR analysis**

Oligonucleotide primers (Table S1) were designed using Primer Designer software version 1.01 (Scientific and Education software). The primers were tested prior to qRT-PCR analysis using DNA as a template, and single amplicons of the proper size were sequenced. The RT reaction contained 1 µg of total RNA, 500 ng of Oligo(dT) 15 Primer (Invitrogen), 5 mM DTT (Invitrogen), 2 U of RNasin (Invitrogen), 10 U of SuperScriptII reverse transcriptase (Invitrogen) and 500 µM each of dATP, dCTP, dGTP and dTTP. The reactions were incubated at 42°C for 50 min followed by 15 min at 70°C. For qRT-PCR, 1 µl of cDNA was amplified in a 25 µl reaction mixture containing each gene-specific primer at 50 nM and 12.5 µl of iQ<sup>TM</sup> SYBR green Supermix (Bio-Rad). All reactions were performed in triplicate using a RotorGene 2000 Real-Time PCR cycler (Corbett Life Science). The expression levels of each gene were normalised to those of the housekeeping gene  $\beta$ -tubulin, expression of which is not affected by



the availability of iron. Relative quantitative values were obtained by the comparative threshold cycle ( $2^{-\Delta\Delta C_T}$ ) method as described by (Livak and Schmittgen, 2001)

## **2.7 Analysis of 5' untranslated regions**

An application based on the NetBeans Platform (<http://platform.netbeans.org>) was developed to search for MRE-like motifs in 300 bp of the upstream regions of all iron-regulated genes. We searched for the MRE eukaryotic consensus (C/T)AACG(G/T) and the specific MREs of the malic enzyme gene as described by Hsu et al. (2009): MRE1/MRE2r (A(A/T/C/G)AACGAT, CGATA, AACGATA and TAACGA) and MRE2f (TATCGT and TATCGTC).

## **3. Results and Discussion**

### **3.1. Microarray analysis**

To investigate changes in gene expression caused by iron availability, trichomonads were grown in media supplemented with 100 mM Fe-nitrilotriacetate (iron-rich conditions, +Fe) or with the iron chelator 2,2-dipyridyl (50 mM; iron-restricted conditions, -Fe). The total RNA was then isolated from both treatment groups and hybridised onto DNA microarray slides containing PCR products amplified from 7,688 expressed sequenced tag (EST) clones representing 4,950 distinct *T. vaginalis* genes. A direct pairwise comparison strategy was used to identify differentially expressed genes. To establish the cut-off limit and to validate the microarray data, we selected 15 genes for quantitative real-time PCR analyses (qRT-PCR). The selection included genes that were not affected by iron (ferredoxin 6, IscS-2), genes with moderate changes in expression (-1.42- to 1.67-fold change) and genes with a greater than 2-fold change in expression (hydrogenase, alcohol dehydrogenase, PFOR) according to the microarray data (Table 1). In each instance, the qRT-PCR

confirmed regulation trends observed in the microarray analysis. The ratios between paired samples (fold change) determined by qRT-PCR were, however, greater than the fold change obtained for the same gene by microarray analysis. These results are consistent with those of Yuen et al. (2002) and Dallas et al. (2005), who showed that microarray analysis underestimates the changes in gene expression compared with the more quantitative real-time PCR assay, although correlations between microarray and qRT-PCR data are generally strong. Based on these results, we set the cut-off limit to 1.3, and genes with changes in expression that exceeded this limit were classified as significantly regulated. Altogether, 308 genes met this criterion with a fold change  $\geq 1.3$  ( $P < 0.01$ ); 182 and 126 genes were upregulated in cells cultivated under +Fe and -Fe conditions, respectively (Table S2). Genes which change of expression did not reach the cutoff limit (fold change between -1.29 and 1.29) are listed in Table S3, the rest of the genes with no change of expression can be found in the ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress/>) under the array design name TvArray V1.0

### **3.2. Comparative EST analysis.**

As a second technique to identify differentially expressed genes, we employed a comparative EST approach (Lee et al., 1995). 10,042 and 9,032 ESTs were sequenced from two distinct cDNA libraries that were derived from trichomonads grown under +Fe and -Fe conditions, respectively. The relative frequency (RF) of an EST was calculated as the number of ESTs per 10,000 clones. The upregulation index was calculated as the difference between the RF under +Fe conditions and the RF under -Fe conditions. A gene was considered to be significantly upregulated if the difference was greater than or equal to five. Among a total number of 6,381 genes that were generated by the assembly of the ESTs the expression of 336 genes was changed in response to iron availability. Of those genes, 165 were upregulated under +Fe conditions, and 171 were

upregulated under -Fe conditions (Table S4). A representation of the gene categories that were affected by iron availability is shown in Figure 1.

### **3.3. Major iron-regulated pathways**

#### **3.3.1. Glycolysis**

The energy metabolism of *T. vaginalis* relies on fermentative carbohydrate catabolism in the cytosol that is extended to malate or pyruvate degradation in the hydrogenosome (Muller, 2003). At least one (but usually several) of multiple gene copies encoding glycolytic proteins showed significant iron-dependent regulation (Figure 2, Table S5), with similar results obtained from both methods (microarray and EST analysis). The enzymes that supply the glycolytic pathway with substrates (glucokinase, glycogen phosphorylase and phosphoglucomutase) and the successive glycolytic enzymes were significantly upregulated under +Fe conditions. A striking exception was the upregulation of one of the four detected glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes under -Fe conditions according to the EST analysis, as the other three GAPDH genes were upregulated under +Fe conditions (Figure 2, Table S4). The different regulation might suggest that the GAPDH gene copy TVAG\_366380 encodes protein with the distinct function. Multifunctional character of GAPDH was reported in macrophages where GAPDH serves as a surface receptor that is upregulated upon iron depletion (Rawat et al., 2012). Interestingly, *Trichomonas* possesses two types of phosphofructokinase (PFK) that differ in their phosphoryl donor specificity and subcellular localisation (Rada et al., 2011). Two of the three genes that encode the PPi-dependent PFK that is active in the cytosol and one of two genes detected for the hydrogenosomal ATP-dependent PFK were upregulated under +Fe conditions (Table S5). The most significant changes in iron-dependent gene expression were associated with the pathways that follow the conversion of phosphoenolpyruvate (PEP), the branch-point of carbohydrate metabolism. The enzymes that catalyse the formation of malate from PEP via

oxaloacetate [PEP carboxykinase (PEPCK) and malate dehydrogenase] were considerably upregulated under +Fe conditions. A notable increase in expression was detected for PEPCK, where, in contrast to the other glycolytic enzymes, all six genes that encode PEPCK were determined to be highly upregulated by at least one of the two employed methods (Figure 2). In contrast, the pathway that converts malate to lactate was upregulated under -Fe conditions. This pathway involves cytosolic NADP-dependent ME and NADH-dependent lactate dehydrogenase (Figure 2). These findings indicate that under +Fe conditions, malate preferentially enters the hydrogenosome and serves as a substrate for hydrogenosomal energetic metabolism. However under -Fe, when hydrogenosomal metabolism is ceased, malate is metabolised in the cytosol via pyruvate to lactate. Indeed, increased lactate production by *T. vaginalis* grown on -Fe medium was detected (Tachezy, unpublished data). Thus, the ability to switch between hydrogenosomal and cytosolic malate metabolism seems to be important for the ability of trichomonads to quickly adapt to changes in iron availability in their environment. Similar changes in carbohydrate metabolism were reported in *T. vaginalis* that had impaired hydrogenosomal metabolism because of the induction of metronidazole resistance (Kulda et al., 1993).

Besides lactate, *T. vaginalis* also produces low amount of ethanol (Cerkasovová et al., 1986). In our dataset, we identified two types of alcohol dehydrogenases (ADHs) that differ in the metal ion present in the active site of the enzyme. Notably, the iron-containing ADH was upregulated under +Fe conditions, and the zinc-containing ADH was upregulated under -Fe conditions (Figure 2). In the related organism *Tritrichomonas foetus*, acetaldehyde that is reduced by ADH to ethanol is formed from pyruvate by the enzyme pyruvate decarboxylase (Sutak et al., 2004b). However, pyruvate decarboxylase activity was not detected in *T. vaginalis*, and the gene encoding this enzyme was not identified in the genome (Carlton et al., 2007). Thus, the pathway responsible for the formation of acetaldehyde remains unclear.

### **3.3.2. Hydrogenosomal energetic metabolism**

Iron increased the transcription of all critical enzymes in hydrogenosomal carbohydrate catabolism; at least one copy of each gene was significantly upregulated (Figure 3, Table S5). PFOR and ME, which are the enzymes that catalyse the oxidative decarboxylation of the hydrogenosomal substrates pyruvate and malate, respectively, were the most highly upregulated enzymes of the pathway. Three genes encoding PFOR (PFOR-A, BI and BII) displayed the highest upregulation observed in the microarray analysis (4.01, 3.71 and 3.64, respectively), and also had high upregulation scores determined by the EST analysis (32, 18 and 9, respectively) (Table S2, S4). The gene coding for ME-H was the most highly upregulated according to the EST analysis, with an upregulation index value of 63 (Table S4). The reoxidation of NADH resulting from ME activity is mediated by heterodimeric NADH dehydrogenase (remnant of respiratory complex I). The 51-kDa subunit of this enzyme was significantly upregulated under +Fe conditions according to EST analysis, but the upregulation of the 24-kDa subunit did not reach the cut-off limit. Electrons generated by PFOR are transferred via ferredoxin to the hydrogenase responsible for the synthesis of molecular hydrogen. The genes that code for ferredoxin-1 and the 64-kDa [Fe]-hydrogenase were upregulated under +Fe conditions. Finally, the acetyl-CoA that is generated by PFOR activity is used in two steps of ATP synthesis: the reactions catalysed by acetate:succinate-CoA transferase (ASCT) and heterodimeric succinyl-CoA synthase (SCS). Two of the four ASCT genes and all of the genes that encode SCS subunits showed significant upregulation under +Fe conditions.

### **3.3.3. FeS cluster assembly**

The biosynthesis of the ISCs that are necessary for the maturation of FeS proteins is an indispensable process that occurs in the hydrogenosomes of *T. vaginalis*. In contrast to the

components of energetic metabolism, the ISC assembly machinery appeared to be upregulated under -Fe conditions (Figure 3, Table S5). Our screen detected genes that encode two paralogues of the scaffold protein IscA, three Nfu (v tabulce mame NifU –nekonzistentni!) paralogues, and, consistently with the results of Sutak et al. (2004), the cysteine desulfurase IscS-2. Frataxin was previously shown to be upregulated under -Fe conditions using a nuclear run-on assay (Dolezal et al., 2007). This trend was also observed in our analysis, but the cut off limit was not reached (EST upregulation index -2). Interestingly, -Fe conditions also caused a significant increase in the expression of the hydrogenase maturase (Hyd-G) that is required for the assembly of the hydrogenase-specific H cluster (Putz et al., 2006). Altogether, these results suggest a common regulatory mechanism for the genes that encode the multiple components of the ISC assembly machinery that are upregulated under -Fe conditions. This regulation might be related to the sensing of an increased cellular demand for FeS cluster synthesis during -Fe conditions.

#### **3.3.4. Hydrogenosomal membrane proteins**

Hydrogenosomes are surrounded by two membranes that possess a specific set of proteins that facilitate the exchange of metabolites between the cytosol and organelles. We detected three hydrogenosomal membrane proteins [Hmp35 and two ADP/ATP carriers (AACs)] that were differentially expressed in response to iron availability (Table S5). Hmp35, which is a unique form of a  $\beta$ -barrel protein that is localised to the outer hydrogenosomal membrane, was significantly upregulated under +Fe conditions. Hmp35 is a cysteine-rich protein with the cysteine residues clustered near the C terminus, where they form a metal-binding motif (Dyall et al., 2003; Rada et al., 2011). These structural properties, together with the observed upregulation of the protein under +Fe conditions, allow us to speculate that Hmp35 may be involved in iron transport. Five members of the mitochondrial carrier protein family (MCF) that serve as AACs in the inner hydrogenosomal membrane have been identified (Dyall et al., 2000; Rada et al., 2011).

In the present study, two of these proteins showed significant iron-dependent regulation: AAC-1 was upregulated under -Fe conditions, whereas AAC-2 was upregulated under +Fe conditions (Table S5, Figure 3). As described above, active hydrogenosomal energetic metabolism is dependent on iron. Under these conditions, a portion of the ATP synthesised in the hydrogenosomes is directly used by processes such as ISC assembly and HSP-70 dependent protein transport and maturation. Some ATP could also be exported by AACs to the cytosol in exchange for ADP as in mitochondria. However, ATP is also required for hydrogenosomal functions under iron-limiting conditions when the expression of enzymes necessary for ATP synthesis is ceased. Thus, ATP might be imported from the cytosol by AACs. The expression of AAC-1 is increased under -Fe conditions, which renders it the most likely candidate for the transportation of ATP into hydrogenosomes.

### **3.3.5. Amino acid metabolism**

The arginine dihydrolase pathway contributes to energy metabolism in *T. vaginalis* (Yarlett et al., 1996). Two components of the pathway, ornithine carbamoyltransferase and carbamate kinase, were significantly upregulated under +Fe conditions (Table S5), whereas arginine deiminase, which was recently shown to be localised in hydrogenosomes, was not regulated by iron availability (Morada et al., 2011).

Methionine can be directly degraded to  $\alpha$ -ketobutyrate, ammonia and thiols or converted to homocysteine. These two metabolic pathways appear to be regulated by iron in opposing manners. Methionine degradation is catalysed by the unique enzyme methionine  $\gamma$ -lyase (McKie et al., 1998). We found that one of the two genes that encode this enzyme was upregulated under +Fe conditions. In contrast, genes that encode three components of the homocysteine-forming pathway (methionine adenosyltransferase, S-adenosyl methionine-dependent methyltransferase,

and S-adenosyl homocysteine hydrolase) appear to be upregulated under -Fe conditions (Table S5, Figure 4).

### **3.3.6. Proteases**

Multiple proteinases have been found in *T. vaginalis* (Carlton et al., 2007), and many of them have been implicated in the virulence of the parasites (Dailey et al., 1990; Ramon-Luing et al., 2010). The expression of 20 genes coding for cysteine proteases and metallopeptidases was found to be regulated by iron, and 13 of these genes were upregulated under -Fe conditions (Table S5). In two cases (the cysteine protease CP3 and the legumain-like cysteine proteinase-1 LEGU1), we obtained considerably different results between the microarray approach and the EST approach. De Jesus et al. (2007) used comparative proteomics to show that CP3 and LEGU1 are downregulated under -Fe conditions, and their results are consistent with upregulation of these proteins that we observed with EST under +Fe conditions. Contradicting data has been published for another cysteine protease, CP4. Solano-Gonzales et al. (2007) reported that iron had an insignificant effect on CP4 gene transcription but positively regulated the translation of CP4. Kummer et al. (2008) isolated an extracellular protein fraction from *T. vaginalis* that they called CP30 and that contained CP2, CP3, CP4 and CPT, and they demonstrated that trichomonads grown under -Fe conditions had increased CP30 fraction protease activity. These results are consistent with the increased transcription of CP4 and CP2 observed in our experiments (Table S5).

### **3.3.7. Regulation of transcription**

Proteins that are involved in the regulation of transcription and translation, including ribosomal proteins and proteins that affect DNA metabolism, constitute approximately 10% of the iron-regulated genes (Figure 1, Table S5). Of particular interest are the Myb-like transcription factors,



which are exceptionally abundant in the *T. vaginalis* genome and have been suggested to be major regulators of gene transcription (Hsu et al., 2009). In our dataset, we detected the expression of 97 Myb-like DNA-binding proteins (Table S6), six of which were significantly regulated by iron. One gene was upregulated under +Fe conditions, and four genes were upregulated under -Fe conditions (Table S5). To date, four *T. vaginalis* Myb-like proteins have been characterised (Ong et al., 2006; Ong et al., 2007; Hsu et al., 2009; Smith et al., 2011). It was demonstrated that the temporal and iron-inducible transcription of the malic enzyme is regulated by the synergistic or antagonistic actions of three proteins that can selectively bind to two discrete Myb protein recognition sites in the 5' untranslated regions (5'UTR) of the malic enzyme gene. Myb1 and Myb 2, that play the roles of a repressor and an activator, respectively, are able to bind to both sites with affinities that differ over time and according to iron availability (Ong et al., 2006; Ong et al., 2007). Myb3 activates basal and prolonged iron-inducible transcription by binding solely to the MRE1 site (Hsu et al., 2009). In the *T. vaginalis* genome, there are three genes that encode Myb-3-like proteins, and all three products contain conserved base-contacting amino acids. Hsu et al. (2009) showed that the gene copy TVAG\_475500 is upregulated under +Fe conditions. In our screen, we detected a second copy (TVAG\_252420) that was upregulated under -Fe conditions. We also identified a Myb2 gene; however, under our experimental conditions, Myb2 was not regulated by iron, which contradicts the findings of Ong et al. (2007) (Table S6).

Because MRE sites were demonstrated to be essential for the iron-dependent regulation of ME, we searched 300 bp of the 5'UTR of all iron-regulated genes detected in our screen for the presence of MRE motifs. We found numerous genes that had upstream sequences containing the MRE eukaryotic consensus sequence (C/T)AACG(G/T) or specific MRE-like sites similar to those found in the ME gene (Table S7). The genes that contain both MRE1/MRE2r and MRE2f in

their upstream region, and that have the motifs in the same order as in ME upstream region, are listed in Table 2. Interestingly, the 5'UTR of the genes that encode ADH, hypothetical protein TVAG\_383310 and thioredoxin reductase contain both elements at positions similar to those in the 5'UTR sequence of the ME gene (Table 2), which suggests a similar multifarious Myb-mediated regulation of transcription.

### **3.4. Conclusions**

The 160-Mb *T. vaginalis* genome is the largest protozoan genome that has been sequenced thus far (Carlton et al., 2007). A unique feature of the *Trichomonas* genome is the massive expansion of many gene families and preservation of multiple gene copies. The possible origins and functions of these multiple gene copies are not clear. Gene duplication can offset the mutation of one gene by its paralogues and thus contribute to the genetic robustness of the organism (Li et al., 2010). Because the *T. vaginalis* genome appears to be haploid, genetic redundancy might be particularly important to buffer gene mutations. Moreover, the differential expression of paralogous copies could be necessary for optimal growth in response to various environmental conditions (Giaever et al., 2002). We demonstrated that iron affects the composition of the *T. vaginalis* transcriptome, which is consistent with the observation that iron modulates trichomonad growth, metabolic fluxes, and virulence phenotypes such as cytoadherence (Mundodi et al., 2006). However, we found that in most cases, iron regulated the expression of a single gene or several gene copies, but the expression of other paralogous copies of the gene was iron-independent. The highest iron-dependent upregulation was observed for malic enzyme and PFOR, which are enzymes that play critical roles in hydrogenosomal energetic metabolism. Significant iron-dependent changes in the protein expression and enzymatic activity of ME and PFOR have been previously observed (Vanacova et al., 2001; Leitsch et al., 2009). However, there were four and three gene copies that encode malic enzyme and PFOR, respectively, that were not iron-dependent. The only exception

was the enzyme SCS, which mediates the final step of ATP synthesis. The expression of all copies of both SCS subunits appeared to be iron-dependent. Similarly, in the cytosol, PEPCK appeared to be the only enzyme that was completely regulated by iron. Thus, it would be of great interest to compare the expression profiles of trichomonads grown under various conditions such as the absence of external glucose, temperature or oxygen stress, or the induction of the amoeboid form to better insight how the extended gene families are regulated.

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### **Figure legends**

#### **Figure 1:**

Classification of iron-regulated genes by functional category. Gene expression was upregulated under (A) iron-rich and (B) iron-restricted conditions as determined by microarray and EST analyses. The gene numbers in each category are indicated.

#### **Figure 2:**

Iron-dependent regulation of genes encoding enzymes in glycolytic pathway. Each double square represents a single gene copy. Triangles represent the results of the microarray analysis, and circles represent the results of the EST analysis. Upregulation under +Fe and -Fe conditions is indicated by the colours red and green, respectively. The empty squares represent detected, but unregulated gene copies.

#### **Figure 3:**

Iron-dependent regulation of hydrogenosomal metabolism. Each double square represents a single gene copy. Triangles represent the results of the microarray analysis, and circles represent the results of the EST analysis. Upregulation under +Fe and -Fe conditions is indicated by the colours red and green, respectively. The empty squares represent detected, but unregulated gene copies.

#### **Figure 4:**

Iron-regulated genes involved in methionine metabolism. Each double square represents a single gene copy. Triangles represent the results of the microarray analysis, and circles represent the results of the EST analysis. Upregulation under +Fe and -Fe conditions is indicated by the colours

red and green, respectively. The empty squares represent detected, but unregulated gene copies.

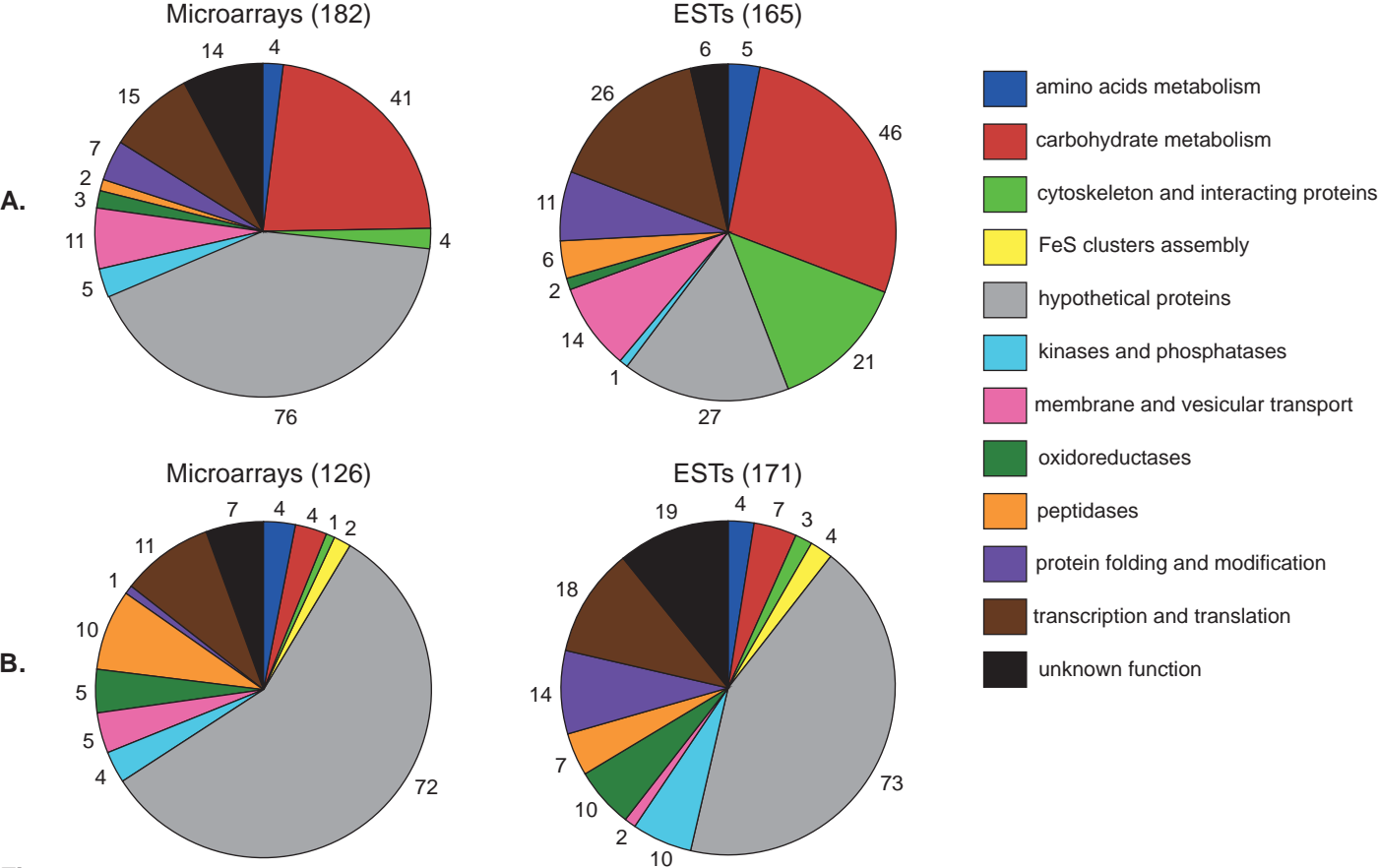
<b>TrichDB 1.2 accession No.</b>	<b>Annotation</b>	<b>Microarrays upregulation rate *</b>	<b>Fold change detected by qRT-PCR**</b>	<b>EST upregulation index</b>
TVAG_239660	IscS-2	NSC	NSC	-5
TVAG_251200	ferredoxin 6	NSC	NSC	0
TVAG_129940	IBP39	1.11	1.88	0
TVAG_348330	glycogen phosphorylase	1.24	1.95	17
TVAG_281070	phosphofructokinase	1.31	4.62	1
TVAG_292710	ferredoxin 4	1.33	2.89	0
TVAG_104250	Hmp-35-2	1.56	2.28	7
TVAG_238830	malic enzyme B	1.67	2.51	33
TVAG_198110	PFO A	4.01	24.96	32
TVAG_037570	iron hydrogenase 64 kDa	2.48	109.64	14
TVAG_422780	alcohol dehydrogenase	2.57	31.44	24
TVAG_165030	malate dehydrogenase	-1.33	-1.62	-1
TVAG_381311	lactate dehydrogenase	-1.81	-5.02	-1
TVAG_361540	IscA-2	-1.36	-2.26	-1
TVAG_008840	NfU-2	-1.42	-1.47	-4

**Table 1.** Validation of regulation trends by qRT-PCR.

\*  $P \leq 0.01$ ; \*\*  $P \leq 0.05$ ; NSC - no significant change of gene expression

<b>TrichDB 1.2 accession No.</b>	<b>Annotation</b>	<b>MRE1/MRE2r</b>	<b>MRE2f</b>
<b>+Fe</b>		<b>ANAACGATA</b>	<b>TATCGT</b>
TVAG_422780	alcohol dehydrogenase (Fe cont.)	<b>-111/-103</b>	<b>-59/-54</b>
		-136/-129	-37/-32
		<b>CGATA</b>	<b>TATCGT</b>
		<b>-107/-103</b>	<b>-59/-54</b>
TVAG_035180	arp2/3 complex subunit	-300/-296	-45/-40
TVAG_218790	conserved hypothetical protein	-230/-226	-110/-105
TVAG_405900	phosphoglucomutase	-286/-282	-199/-194
TVAG_072120	conserved hypothetical protein	<b>TAACGA</b>	<b>TATCGTC</b>
		<b>-110/-105</b>	<b>-59/-53</b>
		-173/-168	-19/-13
<b>-Fe</b>		<b>CGATA</b>	<b>TATCGT</b>
		<b>-107/-103</b>	<b>-59/-54</b>
TVAG_383370	conserved hypothetical protein	-114/-110	-81/-76
TVAG_474980	thioredoxin reductase	-151/-147	-107/-102

**Table 2.** Iron-regulated proteins that contain both MRE1/MRE2r and MRE2f in their upstream regions. The numbers indicate position of the sequence motifs of translation start site. Numbers in bold indicate position of the motifs in the 5'UTR of ME (Tai et al., 2006)



**Figure 1**



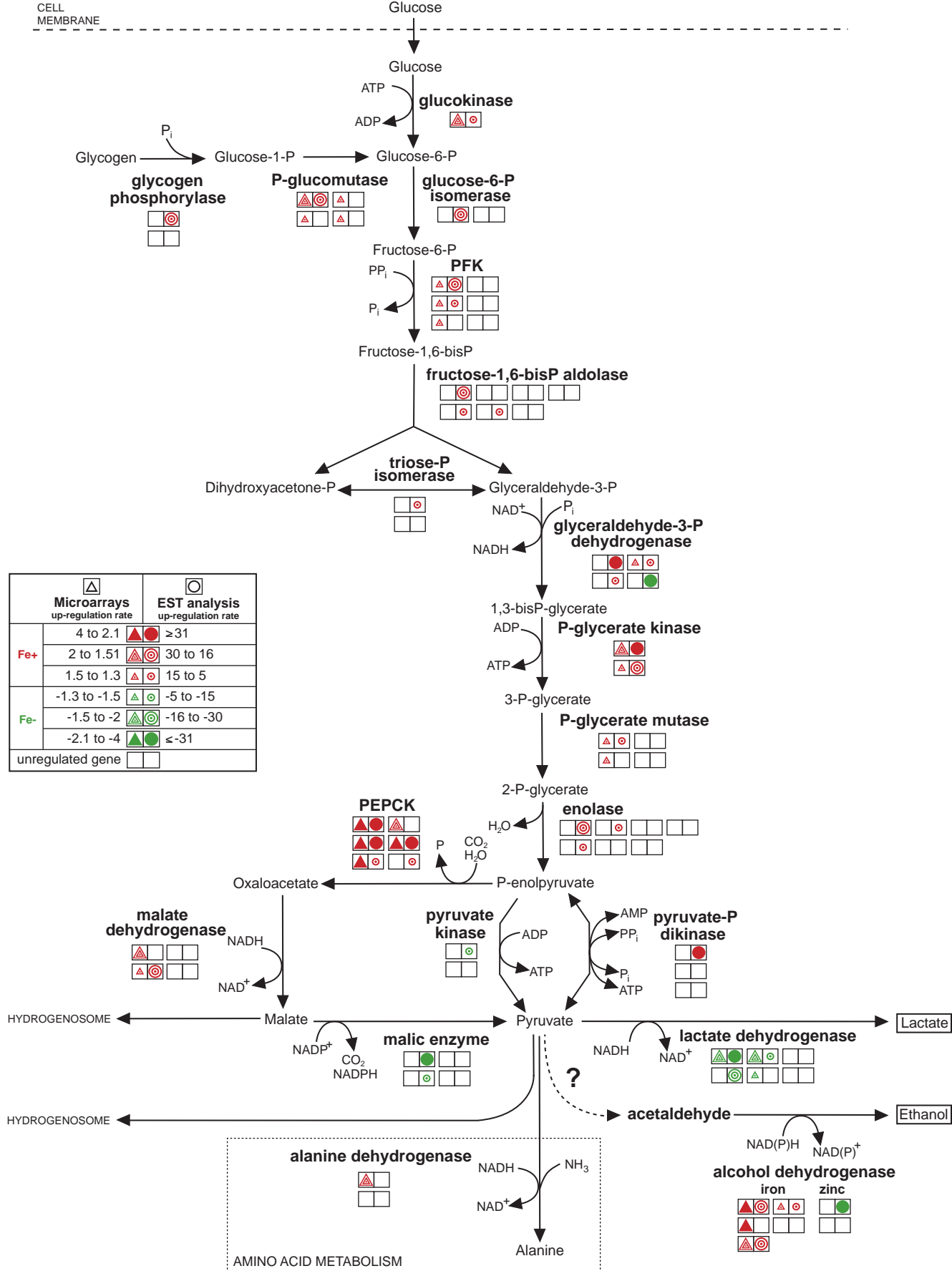
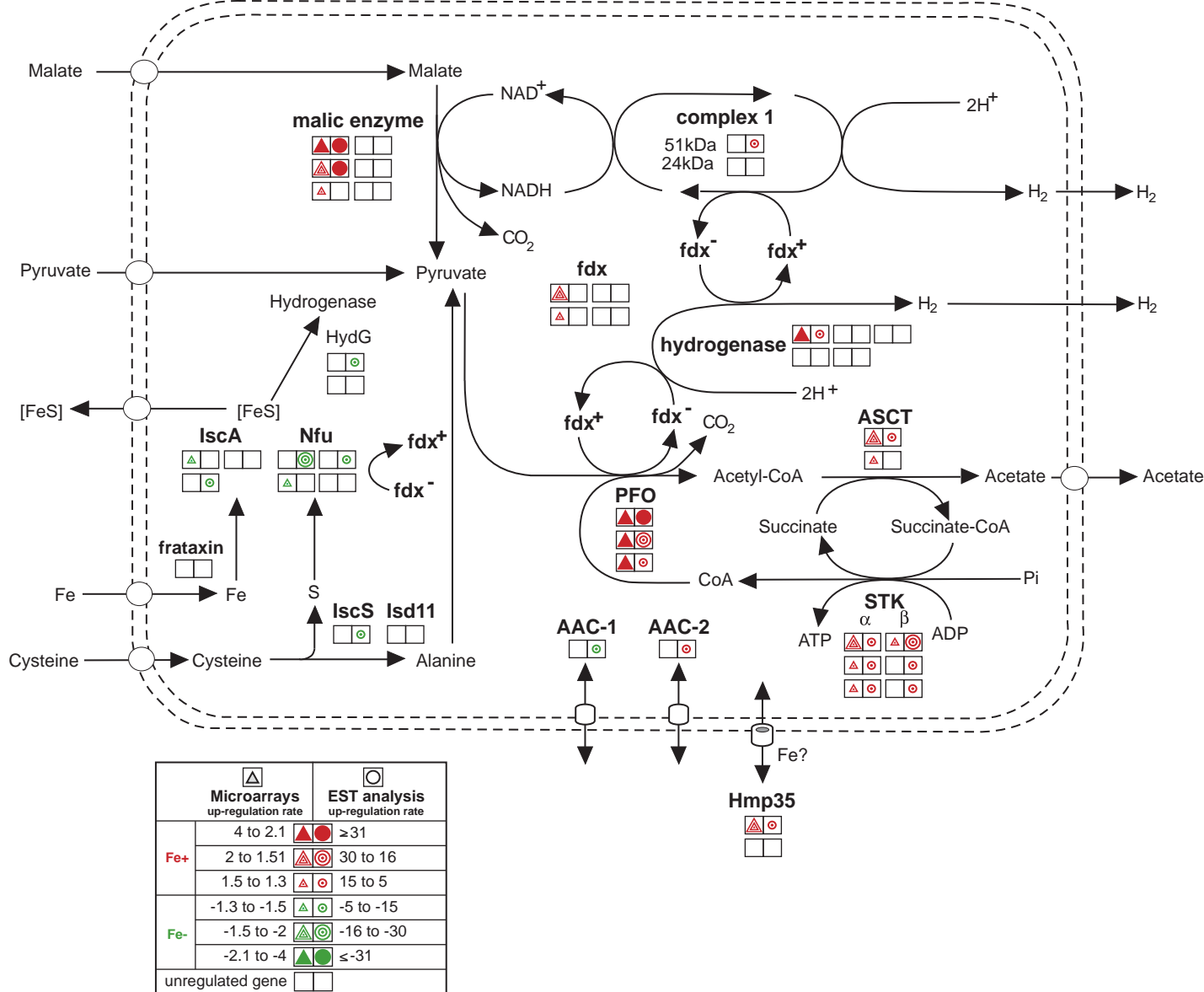
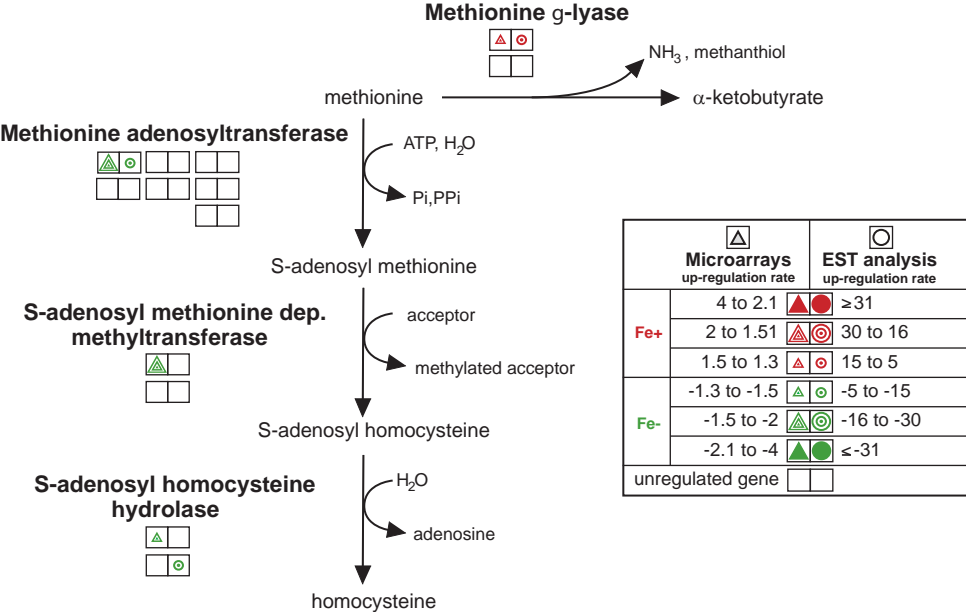


Figure 2



**Figure 3**



**Figure 4**

**Supplementary Table S1.** Oligonucleotide primers used for qRT-PCR analysis

gene	primer sequence
IscS-2 -forward	5'-TTG AAT GTT AGG ATC AGT CT-3'
IscS-2 -reverse	5'-TAA GGA ATC ATA GTA TCG AA-3'
ferredoxin 6 -forward	5'-AAT ACT CAA GAC TTG CTT GT-3'
ferredoxin 6 -reverse	5'-ATC TTA TTG ACG TAC ATG AA-3'
IBP39 -forward	5'-AAG TGA CCT TGC AGA TAT TA-3'
IBP39 -reverse	5'-AGG ACT GTG AAT CAT CAA TA-3'
glycogen phosphorylase -forward	5'-TCA GAG CTC ATG AAG ATC TA-3'
glycogen phosphorylase -reverse	5'-TGT CTT GTT GAT GAA CTT CT-3'
phosphofructokinase -forward	5'-CAG GTC TTA ATG GTG TTA TT-3'
phosphofructokinase -reverse	5'-GCC TTA ACT TCT TCT AAT GA-3'
ferredoxin 4 -forward	5'-CAC AGA ACC TCT ACG TTG TT-3'
ferredoxin 4 -reverse	5'-GAT TGT AGC ACC ATC GAA CT-3'
Hmp-35-2 -forward	5'-GAA CTT CAC ATT CAG TGT CA-3'
Hmp-35-2 -reverse	5'-GTG TTG TTA ACA CCT GTG AT-3'
malic enzyme B -forward	5'-GCT TAC ATC TTC AGT CAA CT-3'
malic enzyme B -reverse	5'-GAT CAC CAT CTT GAA GAA GT-3'
PFO A -forward	5'-ATC AAC ACA TAC GAA GAA CT-3'
PFO A -reverse	5'-TAC TGG TAT GGC TTG TAG TA-3'
iron hydrogenase 64 kDa -forward	5'-CTA CAA GGA AGG TAC AGT CA-3'
iron hydrogenase 64 kDa -reverse	5'-CAG CTT CTT CAC AGA TTG TA-3'
alcohol dehydrogenase -forward	5'-CTC GAG AGA TAT GCT TAC AT-3'
alcohol dehydrogenase -reverse	5'-GCT TGT CTG TGA GTT CAG TA-3'
malate dehydrogenase -forward	5'-CTC TAT TCA AGA ACT CAA GA-3'
malate dehydrogenase -reverse	5'-TTG TAT GAG CTT GTA AGG AA-3'
lactate dehydrogenase -forward	5'-CTC AGA TCT TAT CAG CTC AA-3'
lactate dehydrogenase -reverse	5'-GTA ATA GGC TCT GTT GTG AT-3'
IscA-2 -forward	5'-TTC TAC TCT TGA CTT CAC AA-3'
IscA-2 -reverse	5'-ATG AAT GTA AGT TGG ATT CT-3'
Nfu-2 -forward	5'-CAA GAG AGT TAA CAA GAT CA-3'
Nfu-2 -reverse	5'-CCA TTG TAG AGT GTA TTG TT-3'

**Supplementary Table S2.** Genes that were significantly regulated according to the results of microarray analysis.

TrichDB 1.2 accession No.	Annotation according to TrichDB 1.2 (manually edited)	Upregulation rate
<b>iron rich conditons</b>		
TVAG_392070	conserved hypothetical protein	4,19
TVAG_198110	pyruvate:ferredoxin oxidoreductase A	4,01
TVAG_242960	pyruvate:ferredoxin oxidoreductase BII	3,71
TVAG_230580	pyruvate:ferredoxin oxidoreductase BI	3,64
TVAG_144720	retinal rod rhodopsin-sensitive cGMP 3,5-cyclic phosphodiesterase	3,33
TVAG_479540	phosphoenolpyruvate carboxykinase	3,05
TVAG_469260	conserved hypothetical protein	2,78
TVAG_310250	phosphoenolpyruvate carboxykinase	2,65
TVAG_314830	phosphoenolpyruvate carboxykinase	2,64
TVAG_420390	phosphoenolpyruvate carboxykinase	2,64
TVAG_222330	conserved hypothetical protein	2,61
TVAG_422780	alcohol dehydrogenase (Fe containing)	2,57
TVAG_331060	conserved hypothetical protein	2,53
TVAG_229580	Clan CA, family C19, ubiquitin hydrolase-like cysteine peptidase	2,51
TVAG_475690	beige/BEACH domain containing protein	2,51
TVAG_037570	iron hydrogenase 64 kDa	2,48
TVAG_255570	conserved hypothetical protein	2,45
TVAG_136660	nucleoside diphosphate kinase family protein	2,45
TVAG_439220	N(2),N(2)-dimethylguanosine tRNA methyltransferase	2,45
TVAG_049830	disulfide oxidoreductase family protein	2,37
TVAG_154680	conserved hypothetical protein	2,35
TVAG_306240	conserved hypothetical protein	2,32
TVAG_076610	conserved hypothetical protein	2,24
TVAG_397210	conserved hypothetical protein	2,20
TVAG_340290	malic enzyme H (AP65-1)	2,15
TVAG_026110	conserved hypothetical protein	2,14
TVAG_425970	conserved hypothetical protein	2,14
TVAG_420220	conserved hypothetical protein	2,14
TVAG_013020	alcohol dehydrogenase (Fe containing)	2,13
TVAG_407950	conserved hypothetical protein	2,11
TVAG_270500	conserved hypothetical protein	2,10
TVAG_126240	CAMK family protein kinase	2,09
TVAG_157940	conserved hypothetical protein	2,08
TVAG_091080	ubiquitin-conjugating enzyme family protein	2,05
TVAG_020410	conserved hypothetical protein	2,04
TVAG_227120	conserved hypothetical protein	2,03
TVAG_327470	alcohol dehydrogenase (Fe containing)	2,01
TVAG_427080	conserved hypothetical protein	2,01
TVAG_213710	phosphoenolpyruvate carboxykinase	2,01
TVAG_222040	conserved hypothetical protein	1,96
TVAG_003900	Ferredoxin 1	1,91
TVAG_226870	conserved hypothetical protein	1,90
TVAG_208470	threonyl-tRNA synthetase family protein	1,89
TVAG_254810	conserved hypothetical protein	1,89
TVAG_047080	regulator of nonsense transcripts 1	1,87
TVAG_092750	glucokinase	1,86
TVAG_346820	conserved hypothetical protein	1,85
TVAG_268020	aspartate aminotransferase	1,84
TVAG_239840	sucrose transport protein	1,84
TVAG_470320	conserved hypothetical protein	1,81

TVAG_319000	conserved hypothetical protein	1,80
TVAG_183800	conserved hypothetical protein	1,80
TVAG_023840	conserved hypothetical protein	1,80
TVAG_491860	conserved hypothetical protein	1,79
TVAG_188330	NAD <sup>+</sup> synthetase family protein	1,77
TVAG_263800	disulfide oxidoreductase family protein	1,76
TVAG_395550	acetate:succinate CoA transferase	1,74
TVAG_318870	spermatogenesis associated factor	1,74
TVAG_238830	malic enzyme B	1,67
TVAG_222850	kelch repeat protein	1,67
TVAG_393390	conserved hypothetical protein	1,66
TVAG_214710	calmodulin-like protein	1,65
TVAG_235800	alanine dehydrogenase	1,64
TVAG_268050	phosphoglycerate kinase	1,64
TVAG_047890	succinyl-CoA synthase $\alpha$ -subunit	1,60
TVAG_368740	ornithine carbamoyltransferase	1,58
TVAG_205910	phosphoglucomutase	1,58
TVAG_204360	malate dehydrogenase	1,57
TVAG_085990	agglutinin	1,56
TVAG_474360	centrosomal protein of 135 kDa	1,56
TVAG_083020	conserved hypothetical protein	1,56
TVAG_104250	Hmp-35-2	1,56
TVAG_466000	conserved hypothetical protein	1,56
TVAG_441420	conserved hypothetical protein	1,55
TVAG_165540	CK1 family protein kinase	1,54
TVAG_071040	conserved hypothetical protein	1,54
TVAG_068370	conserved hypothetical protein	1,52
TVAG_487670	conserved hypothetical protein	1,52
TVAG_192290	conserved hypothetical protein	1,52
TVAG_126010	conserved hypothetical protein	1,51
TVAG_455090	conserved hypothetical protein	1,51
TVAG_434470	conserved hypothetical protein	1,50
TVAG_146910	glyceraldehyde 3-phosphate dehydrogenase	1,50
TVAG_141750	16S ribosomal RNA	1,49
TVAG_440840	conserved hypothetical protein	1,49
TVAG_390750	RAB family protein	1,49
TVAG_383940	phosphoglycerate kinase	1,49
TVAG_537380	Myb-like DNA-binding domain containing protein	1,49
TVAG_328940	alcohol dehydrogenase (Fe containing)	1,49
TVAG_209020	phosphoglycerate mutase	1,47
TVAG_430830	phosphofructokinase	1,47
TVAG_589680	conserved hypothetical protein	1,47
TVAG_182130	conserved hypothetical protein	1,46
TVAG_346860	conserved hypothetical protein	1,46
TVAG_362570	glycosyl hydrolase	1,46
TVAG_259190	succinyl-CoA synthase $\beta$ subunit	1,45
TVAG_391450	conserved hypothetical protein	1,45
TVAG_165340	succinyl-CoA synthase $\alpha$ -subunit	1,45
TVAG_339280	CAMK family protein kinase	1,44
TVAG_350920	conserved hypothetical protein	1,44
TVAG_280820	conserved hypothetical protein	1,44
TVAG_219770	macrophage migration inhibitory factor-like protein	1,44
TVAG_139450	hypothetical protein	1,43
TVAG_481370	AMP dependent ligase/synthetase	1,43
TVAG_182700	conserved hypothetical protein	1,43
TVAG_232840	tryparedoxin like protein	1,43

TVAG_212800	Clan MG, family M24, aminopeptidase P-like metallopeptidase	1,42
TVAG_217320	conserved hypothetical protein	1,42
TVAG_135190	cytidine deaminase family protein	1,42
TVAG_147790	methionine gamma-lyase-2	1,41
TVAG_340700	conserved hypothetical protein	1,41
TVAG_293510	ankyrin repeat protein	1,41
TVAG_010490	viral A-type inclusion protein	1,40
TVAG_211310	coiled-coil domain-containing protein	1,39
TVAG_125450	beige/BEACH domain containing protein	1,39
TVAG_051730	conserved hypothetical protein	1,39
TVAG_064460	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	1,39
TVAG_090440	conserved hypothetical protein	1,39
TVAG_384480	ribokinase	1,38
TVAG_262010	ankyrin repeat protein	1,37
TVAG_387680	ankyrin repeat protein	1,37
TVAG_007280	conserved hypothetical protein	1,37
TVAG_054830	phosphoglucomutase	1,37
TVAG_412220	malic enzyme D	1,37
TVAG_194770	conserved hypothetical protein	1,37
TVAG_364620	phosphofructokinase	1,37
TVAG_090740	conserved hypothetical protein	1,36
TVAG_345280	sugar transporter	1,36
TVAG_259990	calmoduline	1,36
TVAG_405900	phosphoglucomutase, putative	1,36
TVAG_253650	malate dehydrogenase	1,36
TVAG_024780	ammonium transporter family protein	1,36
TVAG_122360	conserved hypothetical protein	1,36
TVAG_424960	conserved hypothetical protein	1,35
TVAG_156680	actinin	1,35
TVAG_102390	utp-glucose-1-phosphate uridylyltransferase	1,35
TVAG_264290	conserved hypothetical protein	1,35
TVAG_264700	ubiquitin	1,35
TVAG_370340	CAMK family protein kinase	1,35
TVAG_178680	conserved hypothetical protein	1,34
TVAG_212500	conserved hypothetical protein	1,34
TVAG_420260	ATP synthase beta subunit	1,34
TVAG_300510	phosphoglucomutase	1,34
TVAG_039200	sucrose transport protein	1,34
TVAG_016710	ankyrin repeat protein	1,34
TVAG_318670	succinyl-CoA synthase $\alpha$ -subunit	1,34
TVAG_485570	synaptotagmin	1,33
TVAG_166950	conserved hypothetical protein	1,33
TVAG_178580	alpha-amylase	1,33
TVAG_292710	Ferredoxin 4	1,33
TVAG_567260	conserved hypothetical protein	1,33
TVAG_486110	conserved hypothetical protein	1,33
TVAG_244120	actin-binding protein	1,33
TVAG_366060	hypothetical protein	1,32
TVAG_246940	hypothetical protein	1,32
TVAG_247060	40S ribosomal protein S17	1,32
TVAG_005660	conserved hypothetical protein	1,32
TVAG_183510	conserved hypothetical protein	1,32
TVAG_080000	glykosyl hydrolase	1,32
TVAG_467850	conserved hypothetical protein	1,32
TVAG_018450	conserved hypothetical protein	1,32
TVAG_212480	conserved hypothetical protein	1,32

TVAG_164890	acetate:succinate CoA transferase	1,31
TVAG_347470	conserved hypothetical protein	1,31
TVAG_281070	phosphofructokinase (PPi dependent)	1,31
TVAG_121630	conserved hypothetical protein	1,31
TVAG_021420	coronin	1,31
TVAG_276400	cysteinyl-tRNA synthetase	1,31
TVAG_193090	conserved hypothetical protein	1,31
TVAG_437940	Myb-like DNA-binding domain containing protein	1,31
TVAG_003280	conserved hypothetical protein	1,31
TVAG_106780	conserved hypothetical protein	1,30
TVAG_310210	conserved hypothetical protein	1,30
TVAG_453580	clathrin coat assembly protein	1,30
TVAG_037610	vacuolar ATP synthase subunit C	1,30
TVAG_297440	conserved hypothetical protein	1,30
TVAG_097660	ubiquitin family protein	1,30
TVAG_297650	GrpE protein homolog	1,30
TVAG_212740	phosphoglycerate mutase	1,30
TVAG_291460	40S ribosomal protein S17-B	1,30
TVAG_044510	cytoplasmic heat shock protein 70	1,30
TVAG_347810	conserved hypothetical protein	1,30
iron restricted conditions		
TVAG_436530	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	-2,58
TVAG_338230	dihydroorotate dehydrogenase	-2,46
TVAG_184270	ankyrin repeat protein	-2,44
TVAG_167830	conserved hypothetical protein	-2,35
TVAG_359510	conserved hypothetical protein	-2,26
TVAG_110660	glycosyl hydrolase	-2,20
TVAG_415420	zinc-iron transporter	-2,16
TVAG_032750	conserved hypothetical protein	-1,98
TVAG_403830	methionine adenosyltransferase	-1,94
TVAG_311620	conserved hypothetical protein	-1,92
TVAG_191070	conserved hypothetical protein	-1,90
TVAG_266310	conserved hypothetical protein	-1,90
TVAG_091450	conserved hypothetical protein	-1,83
TVAG_360110	conserved hypothetical protein	-1,81
TVAG_287330	conserved hypothetical protein	-1,80
TVAG_406980	conserved hypothetical protein	-1,80
TVAG_304360	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	-1,78
TVAG_464270	conserved hypothetical protein	-1,76
TVAG_381310	lactate dehydrogenase	-1,76
TVAG_410430	conserved hypothetical protein	-1,73
TVAG_139510	conserved hypothetical protein	-1,70
TVAG_057000	TVCP2	-1,70
TVAG_245250	conserved hypothetical protein	-1,68
TVAG_354300	conserved hypothetical protein	-1,67
TVAG_343240	conserved hypothetical protein	-1,66
TVAG_108920	conserved hypothetical protein	-1,64
TVAG_383130	conserved hypothetical protein	-1,58
TVAG_464150	conserved hypothetical protein	-1,58
TVAG_125070	conserved hypothetical protein	-1,58
TVAG_365110	ribokinase	-1,58
TVAG_256150	conserved hypothetical protein	-1,58
TVAG_575220	conserved hypothetical protein	-1,58
TVAG_171100	lactate dehydrogenase	-1,56
TVAG_242850	Clan CA, family C1, cathepsin L, S or H-like cysteine peptidase	-1,56
TVAG_321740	conserved hypothetical protein	-1,55



TVAG_116180	DNA excision repair protein	-1,55
TVAG_006140	6-phosphogluconate dehydrogenase	-1,54
TVAG_265530	conserved hypothetical protein	-1,54
TVAG_479680	2-nitropropane dioxygenase family protein	-1,53
TVAG_354010	conserved hypothetical protein	-1,52
TVAG_535110	conserved hypothetical protein	-1,51
TVAG_415010	S-adenosyl methionine dep. methyltransferase	-1,51
TVAG_191250	conserved hypothetical protein	-1,49
TVAG_097580	conserved hypothetical protein	-1,48
TVAG_411870	aldo-keto reductase family protein	-1,48
TVAG_496270	phenylalanyl-tRNA synthetase beta chain	-1,47
TVAG_181910	conserved hypothetical protein	-1,47
TVAG_157460	conserved hypothetical protein	-1,45
TVAG_227750	res subunit family protein	-1,45
TVAG_031330	protein phosphatase-1	-1,43
TVAG_038090	thioredoxin peroxidase	-1,43
TVAG_542460	ABC transporter family protein	-1,43
TVAG_035540	conserved hypothetical protein	-1,43
TVAG_008840	Nfu-2	-1,42
TVAG_110950	polyadenylate-binding protein	-1,42
TVAG_363130	conserved hypothetical protein	-1,41
TVAG_180100	conserved hypothetical protein	-1,41
TVAG_013870	60S ribosomal protein L35	-1,41
TVAG_465230	oxidoreductase, aldo/keto reductase family protein	-1,41
TVAG_190580	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,41
TVAG_252420	Myb-like DNA-binding domain containing protein	-1,41
TVAG_469010	conserved hypothetical protein	-1,40
TVAG_413130	conserved hypothetical protein	-1,40
TVAG_398870	conserved hypothetical protein	-1,40
TVAG_493150	conserved hypothetical protein	-1,40
TVAG_203290	conserved hypothetical protein	-1,40
TVAG_147860	conserved hypothetical protein	-1,40
TVAG_351790	conserved hypothetical protein	-1,40
TVAG_012750	conserved hypothetical protein	-1,39
TVAG_429790	conserved hypothetical protein	-1,39
TVAG_385340	TVLEGU2	-1,39
TVAG_506330	conserved hypothetical protein	-1,39
TVAG_320080	conserved hypothetical protein	-1,38
TVAG_098820	aminotransferase, classes I and II family protein	-1,37
TVAG_114360	conserved hypothetical protein	-1,37
TVAG_463220	dynein heavy chain	-1,37
TVAG_482210	conserved hypothetical protein	-1,37
TVAG_090100	TVCP3	-1,37
TVAG_483750	conserved hypothetical protein	-1,37
TVAG_284870	conserved hypothetical protein	-1,37
TVAG_405240	S-adenosyl homocysteine hydrolase	-1,37
TVAG_426660	TVLEGU1	-1,37
TVAG_452580	serine/threonine protein phosphatase	-1,36
TVAG_110370	ankyrin repeat protein	-1,36
TVAG_361540	IscA	-1,36
TVAG_397410	conserved hypothetical protein	-1,35
TVAG_437250	conserved hypothetical protein	-1,35
TVAG_487470	40S ribosomal protein S6	-1,35
TVAG_321430	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,35
TVAG_306880	conserved hypothetical protein	-1,35
TVAG_393400	conserved hypothetical protein	-1,35

TVAG_265070	conserved hypothetical protein	-1,34
TVAG_144520	protein phosphatase-1	-1,34
TVAG_417660	cortactin-binding protein	-1,34
TVAG_055940	40S ribosomal protein S15	-1,34
TVAG_133500	conserved hypothetical protein	-1,34
TVAG_497440	conserved hypothetical protein	-1,34
TVAG_165030	lactate dehydrogenase	-1,33
TVAG_402660	conserved hypothetical protein	-1,33
TVAG_427280	conserved hypothetical protein	-1,33
TVAG_369130	conserved hypothetical protein	-1,33
TVAG_491150	conserved hypothetical protein	-1,33
TVAG_391010	conserved hypothetical protein	-1,32
TVAG_361780	translation initiation factor 5A	-1,32
TVAG_153990	conserved hypothetical protein	-1,32
TVAG_182700	conserved hypothetical protein	-1,32
TVAG_541500	conserved hypothetical protein	-1,32
TVAG_184870	conserved hypothetical protein	-1,32
TVAG_144700	flavodoxin family protein	-1,32
TVAG_192480	60S ribosomal protein L35a	-1,32
TVAG_176480	structural maintenance of chromosomes 5,6	-1,32
TVAG_224190	conserved hypothetical protein	-1,32
TVAG_437930	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,32
TVAG_201190	conserved hypothetical protein	-1,32
TVAG_533610	conserved hypothetical protein	-1,32
TVAG_551850	conserved hypothetical protein	-1,31
TVAG_070850	ankyrin repeat protein	-1,31
TVAG_462940	14-3-3 protein	-1,31
TVAG_255940	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,31
TVAG_475640	ankyrin repeat protein	-1,31
TVAG_481960	conserved hypothetical protein	-1,30
TVAG_402410	sugar transporter	-1,30
TVAG_467970	TVCP4	-1,30
TVAG_448940	conserved hypothetical protein	-1,30
TVAG_442350	conserved hypothetical protein	-1,30
TVAG_177320	conserved hypothetical protein	-1,30

**Supplementary Table S3.** Genes with the upregulation rate under the cut of limit 1,3 according to the results of microarray analysis.

TrichDB accession No.	Annotation according to TrichDB	Upregulation rate
TVAG_077610	conserved hypothetical proteinTIGR hypothetical protein	1,29
TVAG_139240	Branched chain amino acid transferase	1,29
TVAG_191490	Loricrin (keratinocyte cell envelope protein)	1,29
TVAG_212500	ATP-dependent Zn protease	1,29
TVAG_222340	Thioredoxin type domain	1,29
TVAG_414880	Thioredoxin domain 2	1,29
TVAG_440230	C2 domain	1,29
TVAG_451990	Ankyrin repeat domain	1,29
TVAG_487600	Enolase	1,29
TVAG_056850	RNA-binding region RNP-1	1,28
TVAG_205910	Phosphoglucomutase	1,28
TVAG_230400	Chaperone Hsp33 protein	1,28
TVAG_293170	conserved hypothetical proteinNO HIT: TvC075H06	1,28
TVAG_449290	Trichohyalin	1,28
TVAG_484000	conserved hypothetical proteinTIGR hypothetical protein	1,28
TVAG_024690	Isoleucyl tRNA synthetase partial cds [T.vaginalis]	1,27
TVAG_060470	Chromodomain-helicase-DNA-binding protein 8 (CHD-8)	1,27
TVAG_108330	Zn-finger protein C2H2 type	1,27
TVAG_210250	Iron-containing alcohol dehydrogenase	1,27
TVAG_271570	Nucleoside family protein equilibrative nucleoside	1,27
TVAG_309120	conserved hypothetical proteinTIGR hypothetical protein	1,27
TVAG_360020	Outer membrane protein repeat	1,27
TVAG_464170	Enolase (Eno) mRNA [T.vaginalis]	1,27
TVAG_537380	Myb-like DNA-binding domain	1,27
TVAG_025610	Peptidase C19 Ubiquitin-carboxyl-terminal hydrolase	1,26
TVAG_043500	Enolase mRNA [T.vaginalis]	1,26
TVAG_073860	Pyruvate phosphate dikinase (PPDK) Tv_PPDK1	1,26
TVAG_096490	3-oxo-5-alpha-steroid 4-dehydrogenase	1,26
TVAG_146790	conserved hypothetical proteinNO HIT: TvE038E09	1,26
TVAG_163000	Chaperone Hsp70 (TVCChaperone Hsp70) cytosolic [T.vaginalis]	1,26
TVAG_168500	Protein kinase: Casein kinase I epsilon isoform (CK1d/C)	1,26
TVAG_204360	Malate dehydrogenase cytosolic [T.vaginalis]	1,26
TVAG_248630	Calcium-binding EF-hand G-protein beta WD-40 repeat	1,26
TVAG_346790	conserved hypothetical proteinNO HIT: TvE041B01	1,26
TVAG_381690	NAD-dependent epimerase/dehydratase family protein	1,26
TVAG_393480	Major Facilitator Superfamily protein (membrane sugar)	1,26
TVAG_454490	Purine nucleoside phosphorylase	1,26
TVAG_055200	Cytochrome b/b6 N-terminal	1,25
TVAG_080820	conserved hypothetical proteinNO HIT: TvG065D03	1,25
TVAG_113710	Phosphoglycerate mutase (EC 5.4.2.1) (Tv_PGAM1B)	1,25
TVAG_144730	Adhesin protein AP51-2 (Succinyl-CoA ligase) [T.vaginalis]	1,25
TVAG_198390	conserved hypothetical proteinNO HIT: TvG101A10	1,25
TVAG_258220	Glycosyl transferase group 1 family protein	1,25
TVAG_270160	conserved hypothetical proteinTIGR hypothetical protein	1,25
TVAG_409990	Oxysterol binding protein-related	1,25
TVAG_512770	conserved hypothetical proteinTIGR hypothetical protein	1,25
TVAG_183500	Adhesin protein AP51-3 (Succinyl-CoA synthetase) [T.vaginalis]	1,24
TVAG_185650	conserved hypothetical proteinTIGR hypothetical protein	1,24
TVAG_209020	Phosphoglycerate mutase (EC 5.4.2.1) (Tv_PGAM1A)	1,24
TVAG_248540	TONB Box N terminus (lactoferrin/transferrin/vitamin B12)	1,24
TVAG_348330	Glycogen phosphorylase [T.vaginalis]	1,24

TVAG_378240	Protein kinase: (SGK2/AKT1) RAC-family serine/threonine	1,24
TVAG_457230	Ankyrin-1 (Erythrocyte ankyrin) (Ankyrin R)	1,24
TVAG_034730	Chaperone Hsp90 cytosolic-type[T.vaginalis]	1,23
TVAG_049140	Iron superoxide dismutase Tv_SOD8	1,23
TVAG_064640	60S Ribosomal protein L5 [T.vaginalis]	1,23
TVAG_106650	Peptidase S8 and S54 subtilisin kexin sedolisin	1,23
TVAG_151790	BspA-like leucine rich repeat	1,23
TVAG_162630	conserved hypothetical proteinTIGR hypothetical protein	1,23
TVAG_253650	Malate dehydrogenase cytosolic [T.vaginalis]	1,23
TVAG_336890	conserved hypothetical proteinTIGR hypothetical protein	1,23
TVAG_396970	BspA-like leucine rich repeat	1,23
TVAG_056920	Helix-turn-helix AraC type protein	1,22
TVAG_083490	Thymidine kinase	1,22
TVAG_118820	conserved hypothetical proteinNO HIT: TvC036F12	1,22
TVAG_170290	conserved hypothetical proteinNO HIT: TvG125A10	1,22
TVAG_187170	Ubiquitin-like protein	1,22
TVAG_227630	conserved hypothetical proteinTIGR hypothetical protein	1,22
TVAG_252620	Protein kinase: Casein kinase I delta isoform like (CK1a	1,22
TVAG_276310	1 4-alpha-glucan branching enzyme IIB chloroplast prec	1,22
TVAG_330340	conserved hypothetical proteinNO HIT: TvC008B12	1,22
TVAG_347500	Zn-finger protein C2H2 type	1,22
TVAG_383940	Phosphoglycerate kinase (EC 2.7.2.3) (Tv_PGK2)	1,22
TVAG_410620	conserved hypothetical proteinScaffold ORF : AAHC010	1,22
TVAG_085980	Myb-like DNA-binding domain	1,21
TVAG_086470	Thioredoxin domain 2	1,21
TVAG_104540	conserved hypothetical proteinTIGR hypothetical protein	1,21
TVAG_155010	Chaperone Hsp90 cytosolic-type[T.vaginalis]	1,21
TVAG_163840	Phosphatidylinositol 3-kinase 1 (EC 2.7.1.137) (PI3-kinase	1,21
TVAG_164890	Acetyl-CoA hydrolase (EC 3.1.2.1) (Acetyl-CoA deacylas	1,21
TVAG_263800	Pyridine nucleotide-disulphide oxidoreductase	1,21
TVAG_286760	conserved hypothetical proteinTIGR hypothetical protein	1,21
TVAG_300510	Phosphoglucomutase (EC 5.4.2.2) Tv_PGM1B	1,21
TVAG_330860	conserved hypothetical proteinNO HIT: TvC081F01	1,21
TVAG_392920	conserved hypothetical proteinTIGR hypothetical protein	1,21
TVAG_399980	Ankyrin repeat domain	1,21
TVAG_486850	conserved hypothetical proteinScaffold HIT : AAHC0100	1,21
TVAG_RG_DS114240_2	conserved hypothetical proteinTIGR hypothetical protein	1,21
TVAG_013590	conserved hypothetical proteinTIGR hypothetical protein	1,2
TVAG_292920	conserved hypothetical proteinTIGR hypothetical protein	1,2
TVAG_400750	Ankyrin repeat domain	1,2
TVAG_434490	Short-chain dehydrogenase/reductase SDR	1,2
TVAG_491670	Malic enzyme gene [T.vaginalis]	1,2
TVAG_004570	RNA-binding region RNP-1	1,19
TVAG_104840	Protein kinase: (MARK1/CG6114) Serine/threonine-prote	1,19
TVAG_146910	Glyceraldehyde 3-phosphate dehydrogenase	1,19
TVAG_206270	Chaperone Hsp70 (TVCCChaperone Hsp70) cytosolic [	1,19
TVAG_237930	G-protein beta WD-40 repeat	1,19
TVAG_247170	Actin [T.vaginalis]	1,19
TVAG_276400	Cysteinyl-tRNA synthetase family protein	1,19
TVAG_350520	Tetratrico peptide repeat (TPR): 92429.m00107	1,19
TVAG_450680	Phosphoglucomutase (EC 5.4.2.2) Tv_PGM2B	1,19
TVAG_080240	BspA-like leucine rich repeat	1,18
TVAG_170230	conserved hypothetical proteinTIGR hypothetical protein	1,18
TVAG_239540	H+-transporting two-sector ATPase alpha/beta subunit	1,18
TVAG_288230	conserved hypothetical proteinScaffold HIT : AAHC0100	1,18
TVAG_360700	Fructose-1 6-bisphosphate aldolase (EC 4.1.2.13)	1,18

TVAG_498820	DNA clamp loader	1,18
TVAG_RG_DS113210_36	Protein phosphatase (serine/threonine) Metallo-phosphatase	1,18
TVAG_045310	Inositol-3-phosphate synthase (EC 5.5.1.4) (Myo-inositol 3-phosphatase)	1,17
TVAG_139320	Chaperone dnaK heat shock-related protein	1,17
TVAG_148590	Myb-like DNA-binding domain	1,17
TVAG_178210	Nucleotidyl transferase family protein	1,17
TVAG_293540	Sugar transporter superfamily	1,17
TVAG_309990	Protein kinase: (BRSK2/sad-1) BR serine/threonine-protein kinase	1,17
TVAG_337850	Long-chain-fatty-acid--CoA ligase 1 (EC 6.2.1.3) (Long-chain-fatty-acid-CoA ligase)	1,17
TVAG_388450	conserved hypothetical proteinTIGR hypothetical protein	1,17
TVAG_412780	Glyceraldehyde 3-phosphate dehydrogenase	1,17
TVAG_087120	Protein phosphatase (serine/threonine) PP1-gamma catalytic subunit	1,16
TVAG_225470	conserved hypothetical proteinTIGR hypothetical protein	1,16
TVAG_226100	conserved hypothetical proteinTIGR hypothetical protein	1,16
TVAG_234120	RNA-binding region RNP-1 Endoplasmic reticulum target	1,16
TVAG_371050	conserved hypothetical proteinTIGR hypothetical protein	1,16
TVAG_382020	Ubiquitin-conjugating enzyme E2 1 (EC 6.3.2.19) (Ubiquitin-conjugating enzyme E2)	1,16
TVAG_476290	conserved hypothetical proteinNO HIT: TvG015E07	1,16
TVAG_006110	Ubiquitin-activating enzyme E1	1,15
TVAG_100720	Nuclear transport factor 2 (NTF2)	1,15
TVAG_101870	Seryl-tRNA synthetase family protein	1,15
TVAG_340390	Chaperone Hsp70 mitochondrial type	1,15
TVAG_347440	Rho Rhodopsin C-terminal tail XYPPX repeat family protein	1,15
TVAG_472760	conserved hypothetical proteinTIGR hypothetical protein	1,15
TVAG_054030	Actin type 6 [T.vaginalis]	1,14
TVAG_441630	Conserved hypothetical protein 698	1,14
TVAG_154760	conserved hypothetical proteinNO HIT: TvG114G10	1,13
TVAG_243900	conserved hypothetical proteinTIGR hypothetical protein	1,13
TVAG_444560	conserved hypothetical proteinTIGR hypothetical protein	1,13
TVAG_476020	ATPase AAA family protein (ATPases Associated with Cytoskeleton)	1,13
TVAG_RG_DS114631_1	Ferredoxin	1,13
TVAG_094060	F-actin capping protein beta subunit containing protein	1,12
TVAG_446720	Cytochrome c heme-binding site (Transmembrane)	1,12
TVAG_491170	conserved hypothetical proteinNO HIT: TvG009B06	1,12
TVAG_172200	conserved hypothetical proteinTIGR hypothetical protein	1,11
TVAG_345360	Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13)	1,11
TVAG_353000	Glycerol kinase	1,11
TVAG_098350	ABC transporter	1,1
TVAG_110580	Human Rev interacting-like protein (hRIP) ARF GTPase	1,08
TVAG_248610	Ankyrin repeat domain	1,07
TVAG_320050	conserved hypothetical proteinTIGR hypothetical protein	1,07
TVAG_314870	Thioredoxin type domain	-1,06
TVAG_106650	Peptidase S8 and S54 subtilisin kexin sedolisin	-1,07
TVAG_238120	Protein of unknown function DUF852	-1,07
TVAG_251200	Ferredoxin6	-1,07
TVAG_476350	conserved hypothetical proteinTIGR hypothetical protein	-1,07
TVAG_005570	conserved hypothetical proteinNO HIT: TvG019D07	-1,08
TVAG_204380	Root hair defective 3 GTP-binding required for regulated growth	-1,08
TVAG_239370	conserved hypothetical proteinTIGR hypothetical protein	-1,08
TVAG_252710	conserved hypothetical proteinTIGR hypothetical protein	-1,08
TVAG_419930	Protein phosphatase Dual specificity protein phosphatase	-1,08
TVAG_103380	conserved hypothetical proteinTIGR hypothetical protein	-1,09
TVAG_161110	conserved hypothetical proteinNO HIT: TvG065B04	-1,09
TVAG_299380	40S Ribosomal protein S14	-1,09
TVAG_299410	conserved hypothetical proteinTIGR hypothetical protein	-1,09
TVAG_308170	conserved hypothetical proteinTIGR hypothetical protein	-1,09

TVAG_040820	Basic-leucine zipper (bZIP) transcription factor	-1,1
TVAG_078700	Basic helix-loop-helix dimerization domain bHLH	-1,1
TVAG_090410	conserved hypothetical proteinTIGR hypothetical protein	-1,1
TVAG_093600	60S Ribosomal protein L23	-1,1
TVAG_175450	conserved hypothetical proteinNO HIT: TvG039B02	-1,1
TVAG_177740	Peptidase C19 Ubiquitin-carboxyl-terminal hydrolase	-1,1
TVAG_254840	60S Ribosomal protein L12	-1,1
TVAG_260820	N-6 Adenine-specific DNA methylase	-1,1
TVAG_394720	Nucleotidyl transferase family protein	-1,1
TVAG_413140	conserved hypothetical proteinNO HIT: TvG082A06	-1,1
TVAG_499010	Histone H4-3 [T.vaginalis]	-1,1
TVAG_054140	conserved hypothetical proteinTIGR hypothetical protein	-1,11
TVAG_062600	conserved hypothetical proteinTIGR hypothetical protein	-1,11
TVAG_111570	Secretory carrier membrane protein	-1,11
TVAG_160060	Actin type 6 [T.vaginalis]	-1,11
TVAG_201980	Ras GTPase superfamily	-1,11
TVAG_216800	Peptidase M8 leishmanolysin gp63 (EC 3.4.24.36) (Cell	-1,11
TVAG_340280	conserved hypothetical proteinNO HIT: TvG031F04	-1,11
TVAG_410840	Neurofilament protein	-1,11
TVAG_424990	conserved hypothetical proteinNO HIT: TvG077D06	-1,11
TVAG_464040	60S Rbosomal protein	-1,11
TVAG_479120	Kinesin motor domain	-1,11
TVAG_491520	conserved hypothetical proteinNO HIT: TvC046H06	-1,11
TVAG_062280	conserved hypothetical proteinTIGR hypothetical protein	-1,12
TVAG_078350	BTB/POZ domain	-1,12
TVAG_083260	60S Ribosomal protein L22	-1,12
TVAG_093070	Bromodomain	-1,12
TVAG_108410	Meichroacidin	-1,12
TVAG_182560	Protein phosphatase (serine/threonine) PP1 isozyme 8 (E	-1,12
TVAG_212620	60S Ribosomal L37ae protein	-1,12
TVAG_235070	BspA-like leucine rich repeat	-1,12
TVAG_248360	conserved hypothetical proteinTIGR hypothetical protein	-1,12
TVAG_249090	40S Ribosomal protein S23	-1,12
TVAG_271230	60S Ribosomal protein L11	-1,12
TVAG_315940	conserved hypothetical proteinTIGR hypothetical protein	-1,12
TVAG_348090	40S Ribosomal S3Ae family protein	-1,12
TVAG_394230	conserved hypothetical proteinTIGR hypothetical protein	-1,12
TVAG_417430	conserved hypothetical proteinTIGR hypothetical protein	-1,12
TVAG_006950	TONB Box N terminus (lactoferrin/transferrin/vitamin B12	-1,13
TVAG_016810	Cytochrome c heme-binding site	-1,13
TVAG_025730	Kelch repeat	-1,13
TVAG_045690	G-protein beta WD-40 repeat	-1,13
TVAG_074600	Alanine aminotransferase 2 (EC 2.6.1.2) (GPT) (Glutami	-1,13
TVAG_126410	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,13
TVAG_139430	Glycine-rich protein Rv2741-related	-1,13
TVAG_151210	conserved hypothetical proteinTIGR hypothetical protein	-1,13
TVAG_163810	conserved hypothetical proteinNO HIT: TvG006F07	-1,13
TVAG_199250	conserved hypothetical proteinTIGR hypothetical protein	-1,13
TVAG_224650	Histone H3-1 [T.vaginalis]	-1,13
TVAG_267550	Histidine acid phosphatase	-1,13
TVAG_294880	conserved hypothetical proteinTIGR hypothetical protein	-1,13
TVAG_321400	Histone H2A-IV	-1,13
TVAG_344040	Metacaspase 2-related	-1,13
TVAG_351390	conserved hypothetical proteinTIGR hypothetical protein	-1,13
TVAG_416000	conserved hypothetical proteinTIGR hypothetical protein	-1,13
TVAG_453630	Zn-finger protein ZPR1 (Zinc finger protein 259)	-1,13

TVAG_466650	60S Ribosomal protein L5 [T.vaginalis]	-1,13
TVAG_RG_DS114005_6	WW/Rsp5/WWP domain cytoskeletal protein	-1,13
TVAG_007510	Ankyrin repeat domain	-1,14
TVAG_013720	conserved hypothetical proteinNO HIT: TvG051E06	-1,14
TVAG_029100	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_074580	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_099970	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_103010	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_110530	40S Ribosomal protein S10p/S20e	-1,14
TVAG_110970	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_121140	G-protein beta WD-40 repeat	-1,14
TVAG_142440	40S ribosomal protein S2 [T.vaginalis]	-1,14
TVAG_160890	Protein phosphatase (serine/threonine) PP1 (EC 3.1.3.16)	-1,14
TVAG_205030	Protein disulfide-isomerase precursor (EC 5.3.4.1) (PDI)	-1,14
TVAG_237700	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_251660	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_280820	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_282960	40S Ribosomal protein S6e	-1,14
TVAG_341990	BspA-like leucine rich repeat	-1,14
TVAG_351500	Ras GTPase superfamily Tv_RabX5 (AY896277)	-1,14
TVAG_361070	conserved hypothetical proteinNO HIT: TvC003G12	-1,14
TVAG_378080	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_389550	conserved hypothetical proteinNO HIT: TvC057F09	-1,14
TVAG_407280	conserved hypothetical proteinScaffold ORF : AAHC010	-1,14
TVAG_430570	Haloacid dehalogenase-like hydrolase	-1,14
TVAG_444550	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_449850	Ankyrin repeat domain	-1,14
TVAG_488500	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_540000	conserved hypothetical proteinTIGR hypothetical protein	-1,14
TVAG_008850	PX domain containing protein	-1,15
TVAG_021170	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_036220	40S Ribosomal protein S16	-1,15
TVAG_050400	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_067400	Elongation factor 1 alpha (tef1) [T.vaginalis]	-1,15
TVAG_083700	Cytochrome c heme-binding site	-1,15
TVAG_100030	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_102410	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_113950	40S Ribosomal protein S18	-1,15
TVAG_121720	Serine protease K12H4.7 precursor (EC 3.4.-.-)	-1,15
TVAG_126180	60S Ribosomal protein L44	-1,15
TVAG_126400	Gamma-gliadin precursor.-related	-1,15
TVAG_132670	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_144550	60S Ribosomal protein L10a [T.vaginalis]	-1,15
TVAG_151580	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_180170	conserved hypothetical proteinNO HIT: TvG090D05	-1,15
TVAG_182290	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_190450	Alpha-actinin (AACTI) mRNA [T.vaginalis]	-1,15
TVAG_199230	60S Ribosomal protein L27a	-1,15
TVAG_223480	conserved hypothetical proteinNO HIT: TvG021D09	-1,15
TVAG_224480	Surface immunogen P270 [T.vaginalis]	-1,15
TVAG_242760	Isd11 homologue	-1,15
TVAG_247340	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_249940	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_262240	60S Ribosomal protein L31e	-1,15
TVAG_268400	conserved hypothetical proteinNO HIT: TvC019B09	-1,15
TVAG_301220	ADP-ribosylation factor Tv_ARF2	-1,15

TVAG_316870	Ankyrin repeat domain	-1,15
TVAG_330620	Glycerophosphoryl diester phosphodiesterase	-1,15
TVAG_381680	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_385850	Protein kinase: (CDC2/CDK2) Cell division protein kinase	-1,15
TVAG_392560	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,15
TVAG_405300	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_411440	60S Ribosomal protein P2	-1,15
TVAG_428220	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_469150	Ankyrin-3 (ANK-3) (Ankyrin G)	-1,15
TVAG_483980	conserved hypothetical proteinTIGR hypothetical protein	-1,15
TVAG_530030	BspA-like leucine rich repeat	-1,15
TVAG_060380	Exonuclease II (EC 3.1.11.-) (Exo II) (P140)	-1,16
TVAG_061920	40S Ribosomal protein S26	-1,16
TVAG_073990	conserved hypothetical proteinNO HIT: TvC053G07	-1,16
TVAG_075420	conserved hypothetical proteinNO HIT: TvC065F08	-1,16
TVAG_076150	40S ribosomal protein S13	-1,16
TVAG_106750	Peptidase M49 dipeptidyl-peptidase III	-1,16
TVAG_107240	conserved hypothetical proteinScaffold ORF : AAHC010	-1,16
TVAG_120480	conserved hypothetical proteinNO HIT: TvE082B01	-1,16
TVAG_146090	conserved hypothetical proteinNO HIT: TvE055F02	-1,16
TVAG_171620	Zn-finger protein C-x8-C-x5-C-x3-H type	-1,16
TVAG_199370	conserved hypothetical proteinScaffold HIT : AAHC0102	-1,16
TVAG_208960	40S Ribosomal protein S19 Protein phosphatase 2C su	-1,16
TVAG_216890	Vacuolar protein sorting-associated protein 35 containing	-1,16
TVAG_224370	60S Ribosomal protein L39	-1,16
TVAG_243080	Thioredoxin domain 2	-1,16
TVAG_255140	60S Ribosomal protein L6	-1,16
TVAG_261950	Ricin B lectin domain QXW lectin repeat	-1,16
TVAG_287170	conserved hypothetical proteinTIGR hypothetical protein	-1,16
TVAG_327540	conserved hypothetical proteinTIGR hypothetical protein	-1,16
TVAG_345520	conserved hypothetical proteinNO HIT: TvC060E02	-1,16
TVAG_366620	40S Ribosomal protein S29	-1,16
TVAG_376330	Zn-finger protein RING	-1,16
TVAG_399070	Serine protease F56F10.1 precursor (EC 3.4.-.-) (carbox	-1,16
TVAG_459150	Hydrogenosomal malic enzyme subunit B proprotein (ma	-1,16
TVAG_463860	Rho Rhodopsin-like G-protein-coupled receptor (GPCR)	-1,16
TVAG_008040	conserved hypothetical proteinNO HIT: TvC121C10	-1,17
TVAG_009310	Asp/Glu racemase	-1,17
TVAG_020480	40S Ribosomal protein S18e	-1,17
TVAG_034390	Uridine phosphorylase	-1,17
TVAG_041350	40S Ribosomal protein S4	-1,17
TVAG_067100	60S Ribosomal protein L37-B	-1,17
TVAG_072730	conserved hypothetical proteinNO HIT: TvC074D06	-1,17
TVAG_125000	conserved hypothetical proteinTIGR hypothetical protein	-1,17
TVAG_136530	Adapter-related protein complex 2 beta 1 subunit (Beta-a	-1,17
TVAG_167170	conserved hypothetical proteinTIGR hypothetical protein	-1,17
TVAG_211390	60S Ribosomal protein L31e ADP-ribosylation factor	-1,17
TVAG_258150	40S Ribosomal protein S17-B Ubiquitin	-1,17
TVAG_263480	conserved hypothetical proteinNO HIT: TvG087E03	-1,17
TVAG_276010	60S Ribosomal protein L44 Enolase	-1,17
TVAG_301220	ADP-ribosylation factor Tv_ARF2	-1,17
TVAG_303020	conserved hypothetical proteinTIGR hypothetical protein	-1,17
TVAG_339340	Protein kinase: (EG:22E5.8/AMPKa2) EG:22E5.8	-1,17
TVAG_349660	conserved hypothetical proteinNO HIT: TvG054D05	-1,17
TVAG_354940	Malate dehydrogenase	-1,17
TVAG_419040	40S Ribosomal protein S13p/S18e Homeobox	-1,17



TVAG_436100	Cytochrome c heme-binding site	-1,17
TVAG_436370	conserved hypothetical proteinTIGR hypothetical protein	-1,17
TVAG_450470	conserved hypothetical proteinNO HIT: TvC023E04	-1,17
TVAG_454260	60S Ribosomal protein L37-B	-1,17
TVAG_455100	Chaperone dnaK heat shock-related protein	-1,17
TVAG_464120	40S Ribosomal protein S14	-1,17
TVAG_470330	60S Ribosomal protein L32	-1,17
TVAG_566400	conserved hypothetical proteinTIGR hypothetical protein	-1,17
TVAG_RG_DS113183_26	conserved hypothetical proteinNO HIT: TvG125E04	-1,17
TVAG_006250	40S Ribosomal protein S15a [T.vaginalis]	-1,18
TVAG_019240	Polyubiquitin (UbA) mRNA partial cds [T.vaginalis]	-1,18
TVAG_038050	60S Ribosomal protein L26	-1,18
TVAG_061670	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_087660	Ubiquitin-conjugating enzyme family protein	-1,18
TVAG_109110	conserved hypothetical proteinScaffold ORF : AAHC010	-1,18
TVAG_114180	conserved hypothetical proteinNO HIT: TvG045D06	-1,18
TVAG_129450	BspA-like leucine rich repeat	-1,18
TVAG_131210	40S Ribosomal protein S4-C	-1,18
TVAG_155060	60S Ribosomal protein L12	-1,18
TVAG_166730	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,18
TVAG_174510	Ubiquitin-associated domain UBA	-1,18
TVAG_204060	Ankyrin repeat domain	-1,18
TVAG_207280	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_224610	60S Ribosomal protein L7Ae	-1,18
TVAG_240680	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_311610	RasGEF domain	-1,18
TVAG_319800	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_347250	60S Ribosomal protein L18ae	-1,18
TVAG_403850	MATE efflux family protein	-1,18
TVAG_410930	Sec1 domain	-1,18
TVAG_412910	conserved hypothetical proteinNO HIT: TvC123E11	-1,18
TVAG_434090	Ankyrin repeat domain	-1,18
TVAG_444580	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_449770	conserved hypothetical proteinTIGR hypothetical protein	-1,18
TVAG_452780	40S Ribosomal protein S27a Ubiquitin	-1,18
TVAG_459760	Immunoglobulin MHC-like region	-1,18
TVAG_517010	Flavodoxin family protein NAD(P)H dehydrogenase (qui	-1,18
TVAG_573910	Ankyrin repeat domain	-1,18
TVAG_583060	40S Ribosomal protein S4-C	-1,18
TVAG_006490	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_026460	60S Ribosomal protein L14 glyceraldehyde-3-phosphate	-1,19
TVAG_027840	conserved hypothetical proteinNO HIT: TvC023D05	-1,19
TVAG_046860	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_074670	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_122640	BspA-like leucine rich repeat	-1,19
TVAG_127550	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_142060	40S Ribosomal S3Ae family protein	-1,19
TVAG_146890	Protein phosphatase (serine/threonine) PP1-gamma cata	-1,19
TVAG_186020	Ras GTPase superfamily	-1,19
TVAG_190890	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_228780	Iron-containing alcohol dehydrogenase (Adh) gene [T.va	-1,19
TVAG_231250	60S Ribosomal protein L21e	-1,19
TVAG_249100	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_272260	Peptidase C1A papain family cysteine protease inhibito	-1,19
TVAG_347960	40S Ribosomal protein S26	-1,19
TVAG_371270	60S Ribosomal protein L8	-1,19

TVAG_425200	Guanylate cyclase (EC 4.6.1.3) (NPR-A) (Atrial natriuretic peptide receptor)	-1,19
TVAG_433130	Chaperone Hsp70 (TvaghyChaperone Hsp70) [T.vagina]	-1,19
TVAG_438290	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_440760	Acylphosphatase	-1,19
TVAG_463220	Dynein heavy chain family protein	-1,19
TVAG_498870	conserved hypothetical proteinTIGR hypothetical protein	-1,19
TVAG_499570	Protein kinase: (BRSK2/BRSK1) BR serine/threonine-protein kinase	-1,19
TVAG_571370	Ankyrin repeat domain	-1,19
TVAG_RG_DS121890_1	conserved hypothetical proteinNO HIT: TvG077E06	-1,19
TVAG_109530	40S Ribosomal protein S23	-1,2
TVAG_146620	Protein phosphatase (serine/threonine) PP1-2 (EC 3.1.3.1)	-1,2
TVAG_153250	conserved hypothetical proteinNO HIT: TvC046D07	-1,2
TVAG_183440	conserved hypothetical proteinNO HIT: TvG037G08	-1,2
TVAG_210740	conserved hypothetical proteinNO HIT: TvG010A05	-1,2
TVAG_219390	Ankyrin repeat domain	-1,2
TVAG_232820	60S Ribosomal protein L10 Cytochrome c heme-binding protein	-1,2
TVAG_262730	60S Ribosomal protein L14	-1,2
TVAG_284260	conserved hypothetical proteinTIGR hypothetical protein	-1,2
TVAG_302890	Concanavalin A-like lectin/glucanase	-1,2
TVAG_329530	conserved hypothetical proteinTIGR hypothetical protein	-1,2
TVAG_339320	60S Ribosomal protein L37-B	-1,2
TVAG_343970	Alpha-tubulin 1 [T.vaginalis]	-1,2
TVAG_354720	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,2
TVAG_371800	EGF-like domain	-1,2
TVAG_388110	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,2
TVAG_390570	Phosphatidylinositol 3-kinase 1 (EC 2.7.1.137) (PI3-kinase)	-1,2
TVAG_395680	conserved hypothetical proteinTIGR hypothetical protein	-1,2
TVAG_396820	conserved hypothetical proteinNO HIT: TvG075C09	-1,2
TVAG_438370	60S Ribosomal protein L13e	-1,2
TVAG_440820	Nascent polypeptide-associated complex (NAC) protein	-1,2
TVAG_444300	conserved hypothetical proteinTIGR hypothetical protein	-1,2
TVAG_471390	40S Ribosomal protein S5	-1,2
TVAG_494060	60S Ribosomal protein L30	-1,2
TVAG_RG_DS113195_8	conserved hypothetical proteinTIGR hypothetical protein	-1,2
TVAG_RG_DS113790_1	conserved hypothetical proteinNO HIT: TvG011A05	-1,2
TVAG_RG_DS114071_8	conserved hypothetical proteinNO HIT: TvC122A10	-1,2
TVAG_RG_DS114526_1	Peptidase M neutral zinc metalloproteinases zinc-binding	-1,2
TVAG_042520	60S Ribosomal protein L36e	-1,21
TVAG_098270	Transcriptional repressor tup11.-related	-1,21
TVAG_119450	conserved hypothetical proteinTIGR hypothetical protein	-1,21
TVAG_143310	MATE efflux family protein	-1,21
TVAG_150040	Multicopper oxidase type 1	-1,21
TVAG_191230	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,21
TVAG_226860	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,21
TVAG_245580	conserved hypothetical proteinTIGR hypothetical protein	-1,21
TVAG_246600	60S Ribosomal protein L39	-1,21
TVAG_264140	Protein phosphatase (serine/threonine) PP2A catalytic subunit	-1,21
TVAG_277940	40S Ribosomal protein S26	-1,21
TVAG_298310	conserved hypothetical proteinNO HIT: TvC054G07	-1,21
TVAG_361420	Ras GTPase superfamily Tv_RabX3 (AY896275)	-1,21
TVAG_419520	Phosphoadenosine phosphosulfate reductase	-1,21
TVAG_432800	conserved hypothetical proteinNO HIT: TvG031G04	-1,21
TVAG_496920	conserved hypothetical proteinNO HIT: TvC008B02	-1,21
TVAG_005920	60S Ribosomal protein L30	-1,22
TVAG_055320	ISCS (Iron-sulfur cluster assembly accessory protein)	-1,22
TVAG_057380	40S Ribosomal protein S28e	-1,22

TVAG_112100	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_154540	conserved hypothetical proteinNO HIT: TvC120B07	-1,22
TVAG_164860	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_177910	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_185570	conserved hypothetical proteinNO HIT: TvE048F11	-1,22
TVAG_185720	Amino acid transporter (AAP family)	-1,22
TVAG_194850	Alpha-tubulin 1 [T.vaginalis]	-1,22
TVAG_212110	Dynein light chain Tctex-1 family protein	-1,22
TVAG_246200	40S Ribosomal protein S5-B	-1,22
TVAG_269280	Cys-rich domain containing protein	-1,22
TVAG_277140	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_331060	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_360400	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_371240	40S Ribosomal protein S24e	-1,22
TVAG_379480	Clathrin adaptor complex medium chain	-1,22
TVAG_386680	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_472390	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_484120	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_485880	Peptidase C1A papain family cysteine protease	-1,22
TVAG_498580	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_525700	conserved hypothetical proteinTIGR hypothetical protein	-1,22
TVAG_RG_DS113186_5	conserved hypothetical proteinNO HIT: TvC019A06	-1,22
TVAG_006170	60S Ribosomal protein L19-3	-1,23
TVAG_028310	conserved hypothetical proteinNO HIT: TvC041A09	-1,23
TVAG_040760	Protein phosphatase (serine/threonine) PP2A catalytic su	-1,23
TVAG_065320	conserved hypothetical proteinNO HIT: TvG045E08	-1,23
TVAG_069820	conserved hypothetical proteinNO HIT: TvG057F04	-1,23
TVAG_119180	conserved hypothetical proteinTIGR hypothetical protein	-1,23
TVAG_162990	conserved hypothetical proteinNO HIT: TvC074F08	-1,23
TVAG_165290	conserved hypothetical proteinNO HIT: TvC021H06	-1,23
TVAG_166770	HD domain containing protein	-1,23
TVAG_169060	Cdc42 homolog	-1,23
TVAG_180530	conserved hypothetical proteinNO HIT: TvC038D05	-1,23
TVAG_206000	Endoplasmic reticulum targeting sequence C-terminal s	-1,23
TVAG_222640	RNA-binding region RNP-1	-1,23
TVAG_258230	conserved hypothetical proteinTIGR hypothetical protein	-1,23
TVAG_277090	Ubiquitin-conjugating enzyme family protein	-1,23
TVAG_277620	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,23
TVAG_298360	RabGAP/TBC domain	-1,23
TVAG_298420	Protein phosphatase (serine/threonine) PP2A catalytic su	-1,23
TVAG_313260	40S Ribosomal protein S28e	-1,23
TVAG_340090	conserved hypothetical proteinNO HIT: TvC048F02	-1,23
TVAG_356830	conserved hypothetical proteinTIGR hypothetical protein	-1,23
TVAG_371220	Polyadenylate-binding protein cytoplasmic and nuclear (	-1,23
TVAG_418120	Peptidase M49 dipeptidyl-peptidase III	-1,23
TVAG_422670	PT repeat	-1,23
TVAG_442870	conserved hypothetical proteinNO HIT: TvG058G01	-1,23
TVAG_448160	conserved hypothetical proteinTIGR hypothetical protein	-1,23
TVAG_453350	conserved hypothetical proteinTIGR hypothetical protein	-1,23
TVAG_474410	40S Ribosomal protein S2 :Myo-inositol-1-phosphate sy	-1,23
TVAG_028290	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,24
TVAG_087160	conserved hypothetical proteinNO HIT: TvG045G12	-1,24
TVAG_107660	40S Ribosomal protein S29	-1,24
TVAG_140190	Peptidase M3A and M3B thimet/oligopeptidase F	-1,24
TVAG_159500	conserved hypothetical proteinTIGR hypothetical protein	-1,24
TVAG_172900	Zn-finger protein RING Protein prenyltransferase alpha	-1,24

TVAG_178150	Galactose-binding like	-1,24
TVAG_178890	Legume lectin alpha domain	-1,24
TVAG_183300	Phosphoserine aminotransferase (EC 2.6.1.52) (PSAT)	-1,24
TVAG_212650	conserved hypothetical proteinNO HIT: TvG042A05	-1,24
TVAG_215920	Laminin A family protein lysosomal membrane glycoprotein	-1,24
TVAG_265950	60S Ribosomal protein L32	-1,24
TVAG_268390	conserved hypothetical proteinNO HIT: TvE069H11	-1,24
TVAG_361330	conserved hypothetical proteinTIGR hypothetical protein	-1,24
TVAG_371540	conserved hypothetical proteinNO HIT: TvC125C08	-1,24
TVAG_402510	60S Ribosomal protein P1	-1,24
TVAG_413770	Myb-like DNA-binding domain	-1,24
TVAG_416540	conserved hypothetical proteinTIGR hypothetical protein	-1,24
TVAG_429250	Loricrin (keratinocyte cell envelope protein)	-1,24
TVAG_454070	conserved hypothetical proteinTIGR hypothetical protein	-1,24
TVAG_492790	Rho GAP domain kinesin and myosin motor	-1,24
TVAG_493590	conserved hypothetical proteinTIGR hypothetical protein	-1,24
TVAG_RG_DS125540_1	conserved hypothetical proteinNO HIT: TvG058G10	-1,24
TVAG_011510	conserved hypothetical proteinNO HIT: TvC027E03	-1,25
TVAG_058740	conserved hypothetical proteinTIGR hypothetical protein	-1,25
TVAG_066340	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,25
TVAG_146860	Protein kinase: (BRSK1/CG11870) Carbon catabolite de	-1,25
TVAG_163440	40S Ribosomal protein S7	-1,25
TVAG_176480	RecF/RecN/SMC N terminal domain containing protein	-1,25
TVAG_192930	Phosphopantetheine attachment site	-1,25
TVAG_193770	Ras GTPase superfamily	-1,25
TVAG_214700	conserved hypothetical proteinNO HIT: TvG078E03	-1,25
TVAG_266710	conserved hypothetical proteinScaffold HIT : AAHC0100	-1,25
TVAG_277900	conserved hypothetical proteinTIGR hypothetical protein	-1,25
TVAG_299910	BspA-like leucine rich repeat	-1,25
TVAG_349780	conserved hypothetical proteinTIGR hypothetical protein	-1,25
TVAG_388060	Saposin-like protein B region 1 (Tv_SAPLIP1B)	-1,25
TVAG_388870	Protein Phoaphatase Low molecular weight phosphotyros	-1,25
TVAG_401590	60S Ribosomal protein L18	-1,25
TVAG_446490	Thioredoxin type domain	-1,25
TVAG_460730	Dynein 1-beta heavy chain flagellar inner arm I1 comple	-1,25
TVAG_464780	Protein kinase: (RSK2/NEK5) CBL-interacting serine/thre	-1,25
TVAG_522520	conserved hypothetical proteinTIGR hypothetical protein	-1,25
TVAG_020030	Eukaryotic release factor 1 mRNA partial cds [T.vaginali	-1,26
TVAG_020530	60S Ribosomal protein L36e	-1,26
TVAG_101030	Regulator of chromosome condensation (RCC)	-1,26
TVAG_125100	Protein kinase: (mnbl/HIPK2) Protein kinase YAK1 (EC 2	-1,26
TVAG_154500	conserved hypothetical proteinTIGR hypothetical protein	-1,26
TVAG_174130	Cys-rich domain containing protein	-1,26
TVAG_206880	Glycine-rich protein TomR2-related	-1,26
TVAG_213390	conserved hypothetical proteinTIGR hypothetical protein	-1,26
TVAG_222970	conserved hypothetical proteinTIGR hypothetical protein	-1,26
TVAG_392990	60S Rbosomal protein	-1,26
TVAG_468310	HIT domain containing protein	-1,26
TVAG_474660	Protein phosphatase (serine/threonine) PP-Z1 (EC 3.1.3.	-1,26
TVAG_480190	Myb-like DNA-binding domain	-1,26
TVAG_484350	Peptidase C1A papain family cysteine protease 2.6.1.1	-1,26
TVAG_047960	Peptidase C1A papain family cysteine protease	-1,27
TVAG_093850	BspA-like leucine rich repeat	-1,27
TVAG_097930	conserved hypothetical proteinTIGR hypothetical protein	-1,27
TVAG_098440	Class I nuclease-related	-1,27
TVAG_114310	Thioredoxin peroxidase (trxp gene) strain G3 [T.vaginali	-1,27

TVAG_280860	Tetraspanin	-1,27
TVAG_319320	Silent information regulator protein Sir2	-1,27
TVAG_341050	conserved hypothetical proteinNO HIT: TvE034E01	-1,27
TVAG_343240	Thymidylate synthase (EC 2.1.1.45) (TS) (TSase)	-1,27
TVAG_390510	Nucleotide binding protein	-1,27
TVAG_405890	conserved hypothetical proteinNO HIT: TvG054B06	-1,27
TVAG_435990	conserved hypothetical proteinNO HIT: TvG011D08	-1,27
TVAG_444760	conserved hypothetical proteinTIGR hypothetical protein	-1,27
TVAG_RG_DS113319_23	conserved hypothetical proteinTIGR hypothetical protein	-1,27
TVAG_014960	conserved hypothetical proteinNO HIT: TvC033D07	-1,28
TVAG_051330	Ubiquitin-carboxyl-terminal hydrolase	-1,28
TVAG_067980	60S ribosomal protein L7-2	-1,28
TVAG_091430	conserved hypothetical proteinTIGR hypothetical protein	-1,28
TVAG_145540	Glycoside hydrolase family 2 beta-galactosidase	-1,28
TVAG_159580	Peptidase M67 Mov34/MPN/PAD-1 family	-1,28
TVAG_184530	40S Ribosomal protein S18e Glutamine amidotransferase	-1,28
TVAG_344880	Iron-containing alcohol dehydrogenase 2 mRNA partial	-1,28
TVAG_376760	conserved hypothetical proteinNO HIT: TvC070A12	-1,28
TVAG_015780	60S Ribosomal protein L12 Growth factor cystine knot	-1,29
TVAG_034660	60S Ribosomal protein L44	-1,29
TVAG_071160	Protein of unknown function DM15	-1,29
TVAG_076370	60S Ribosomal protein L39	-1,29
TVAG_246270	Ubiquitin-associated domain	-1,29
TVAG_298080	Peptidase C1A papain family cysteine protease CP4 m	-1,29
TVAG_317020	Peptidase M24A methionine aminopeptidase subfamily	-1,29
TVAG_434010	conserved hypothetical proteinNO HIT: TvE081D10	-1,29
TVAG_440110	conserved hypothetical proteinTIGR hypothetical protein	-1,29

**Supplementary Table S4.** Genes that were significantly regulated according to the results of comparative EST analysis.

TrichDB 1.2 accession No.	Annotation according to TrichDB 1.2 (manually edited)	Upregulation index
<b>upregulated in iron rich conditions</b>		
TVAG_239310	alpha-actinin	68
TVAG_340290	malic enzyme H (AP65-1)	63
TVAG_479540	phosphoenolpyruvate carboxykinase	51
TVAG_314830	phosphoenolpyruvate carboxykinase	48
TVAG_310250	phosphoenolpyruvate carboxykinase	48
TVAG_268050	phosphoglycerate kinase	47
TVAG_276410	elongation factor 2	46
TVAG_204360	malate dehydrogenase	44
TVAG_049830	disulfide oxidoreductase	43
TVAG_463940	elongation factor 1-alpha	41
TVAG_067400	elongation factor 1-alpha	41
TVAG_073860	pyruvate, phosphate dikinase	38
TVAG_054030	actin	36
TVAG_080000	glykosyl hydrolase	36
TVAG_238830	malic enzyme B	33
TVAG_172680	actin	32
TVAG_198110	pyruvate:ferredoxin oxidoreductase A	32
TVAG_337240	actin	31
TVAG_412780	glyceraldehyde 3-phosphate dehydrogenase	31
TVAG_464170	enolase	29
TVAG_383940	phosphoglycerate kinase	28
TVAG_253650	malate dehydrogenase	28
TVAG_534990	actin	26
TVAG_310030	actin	26
TVAG_092490	heat shock protein 70kD	26
TVAG_205910	phosphoglucomutase	25
TVAG_208470	threonyl-tRNA synthetase	25
TVAG_512800	actin	24
TVAG_422780	alcohol dehydrogenase (Fe containing)	24
TVAG_126970	RAB GDP-dissociation inhibitor	21
TVAG_218800	conserved hypothetical protein	20
TVAG_300000	fructose-bisphosphate aldolase	18
TVAG_259190	succinyl-CoA syntahse $\beta$ subunit	18
TVAG_242960	pyruvate:ferredoxin oxidoreductase BII	18
TVAG_061930	glucose-6-phosphate isomerase	18
TVAG_348330	glycogen phosphorylase	17
TVAG_183500	succinyl-CoA syntahse $\beta$ subunit	17
TVAG_420260	ATP synthase beta subunit	17
TVAG_271570	equilibrative nucleoside transporter	17
TVAG_430830	phosphofructokinase	16
TVAG_327470	alcohol dehydrogenase (Fe containing)	16
TVAG_324980	ATP synthase	16
TVAG_051160	ribosomal protein L10	16
TVAG_245580	conserved hypothetical protein	16
TVAG_312330	alpha-tubulin 1	15
TVAG_262750	vacuolar ATP synthase subunit H	15
TVAG_395550	acetate:succinate CoA transferase	14
TVAG_037570	iron hydrogenase 64 kDa	14
TVAG_420390	phosphoenolpyruvate carboxykinase	13
TVAG_139300	phosphoenolpyruvate carboxykinase	13
TVAG_092750	glucokinase	13

TVAG_139320	dnaK	13
TVAG_268020	aspartate aminotransferase	12
TVAG_043070	fructose-bisphosphate aldolase	12
TVAG_298080	TVCPT	12
TVAG_261970	carbamate kinase	11
TVAG_156680	actinin	11
TVAG_359120	40S ribosomal protein S2	11
TVAG_044510	cytoplasmic heat shock protein 70	11
TVAG_462940	14-3-3 protein	11
TVAG_573910	conserved hypothetical protein	11
TVAG_333110	conserved hypothetical protein	11
TVAG_090470	actin	10
TVAG_367130	Clan MA, family M8, leishmanolysin-like metallopeptidase	10
TVAG_407250	adenylyl cyclase-associated protein	10
TVAG_145570	guanine nucleotide-binding protein beta subunit	10
TVAG_364620	phosphofructokinase	9
TVAG_263740	enolase	9
TVAG_230580	pyruvate:ferredoxin oxidoreductase BI	9
TVAG_047890	succinyl-CoA synthase $\alpha$ -subunit	9
TVAG_043500	enolase	9
TVAG_516070	clathrin heavy chain	9
TVAG_064640	ribosomal protein L5	9
TVAG_429360	WD domain containing protein	9
TVAG_386690	conserved hypothetical protein	9
TVAG_147790	methionine gamma-lyase-2	8
TVAG_041310	ornithine carbamoyltransferase	8
TVAG_360700	fructose-bisphosphate aldolase	8
TVAG_209020	phosphoglycerate mutase	8
TVAG_144730	succinyl-CoA synthase $\beta$ subunit	8
TVAG_474360	centrosomal protein of 135 kDa, putative	8
TVAG_344700	ribosomal protein L10	8
TVAG_305400	seryl-tRNA synthetase	8
TVAG_125550	ribosomal protein L3p	8
TVAG_155010	heat shock protein 82	8
TVAG_045310	myo-inositol-1 phosphate synthase	8
TVAG_367140	conserved hypothetical protein	8
TVAG_340570	conserved hypothetical protein	8
TVAG_360870	alpha-tubulin 1	7
TVAG_200190	actin	7
TVAG_062880	beta-tubulin	7
TVAG_475220	glyceraldehyde 3-phosphate dehydrogenase	7
TVAG_318670	succinyl-CoA synthase $\alpha$ -subunit	7
TVAG_212020	transketolase	7
TVAG_453110	ATP synthase alpha subunit B	7
TVAG_104250	Hmp-35-2	7
TVAG_051820	ACC-2	7
TVAG_426660	TVLEGU1	7
TVAG_224980	Clan MH, family M20, peptidase T-like metallopeptidase	7
TVAG_454000	ribosomal protein L5	7
TVAG_128790	ribosomal protein	7
TVAG_569810	conserved hypothetical protein	7
TVAG_474550	conserved hypothetical protein	7
TVAG_218820	conserved hypothetical protein	7
TVAG_218790	conserved hypothetical protein	7
TVAG_402260	coactosin	6
TVAG_249200	actin	6

TVAG_035180	arp2/3 complex subunit, putative	6
TVAG_008680	beta-tubulin	6
TVAG_462920	phosphofructokinase (ATP dependent)	6
TVAG_165340	succinyl-CoA synthase $\alpha$ -subunit	6
TVAG_096350	triosephosphate isomerase	6
TVAG_558650	clathrin heavy chain	6
TVAG_485580	synaptotagmin	6
TVAG_032440	Clan MA, family M8, leishmanolysin-like metallopeptidase	6
TVAG_583060	40S ribosomal protein S4	6
TVAG_258380	glutaminyl-tRNA synthetase	6
TVAG_156640	aspartyl/lysyl-tRNA synthetase	6
TVAG_397610	cytoplasmic heat shock protein 70	6
TVAG_464010	calreticulin	6
TVAG_496040	conserved hypothetical protein	6
TVAG_477640	conserved hypothetical protein	6
TVAG_166850	conserved hypothetical protein	6
TVAG_157940	conserved hypothetical protein	6
TVAG_150430	conserved hypothetical protein	6
TVAG_072570	conserved hypothetical protein	6
TVAG_072120	conserved hypothetical protein	6
TVAG_276530	cysteine synthase	5
TVAG_206890	alpha-tubulin 1	5
TVAG_160060	actin	5
TVAG_159160	profilin	5
TVAG_073810	beta-tubulin	5
TVAG_328940	alcohol dehydrogenase (Fe containing)	5
TVAG_146910	glyceraldehyde 3-phosphate dehydrogenase	5
TVAG_133030	complex1, Tvh47	5
TVAG_102390	utp-glucose-1-phosphate uridylyltransferase	5
TVAG_547230	adaptin N terminal region family protein	5
TVAG_344080	adaptin N terminal region family protein	5
TVAG_148040	ADP-ribosylation factor 1	5
TVAG_036010	hydrogenosomal oxygen reductase	5
TVAG_090100	TVCP3	5
TVAG_490240	ribosomal protein L5	5
TVAG_476810	60S ribosomal protein L4	5
TVAG_416510	adenosine deaminase	5
TVAG_414510	40S ribosomal protein S3a	5
TVAG_240050	40S ribosomal protein sa	5
TVAG_200220	60S ribosomal protein L15	5
TVAG_113720	ribosomal protein L5	5
TVAG_098450	40S ribosomal protein S4	5
TVAG_054130	60S ribosomal protein L7	5
TVAG_024110	50S ribosomal protein L14p	5
TVAG_398480	chaperonin-60kD	5
TVAG_340390	HSP70	5
TVAG_206270	cytosolic heat shock protein 70	5
TVAG_153560	heat shock protein	5
TVAG_258220	glycosyltransferase	5
TVAG_396480	conserved hypothetical protein	5
TVAG_393400	conserved hypothetical protein	5
TVAG_369900	conserved hypothetical protein	5
TVAG_366360	conserved hypothetical protein	5
TVAG_212500	conserved hypothetical protein	5
TVAG_180570	conserved hypothetical protein	5
TVAG_088640	conserved hypothetical protein	5



TVAG_038420	conserved hypothetical protein	5
TVAG_000810	conserved hypothetical protein	5
<b>upregulated in iron restricted conditions</b>		
TVAG_321740	conserved hypothetical protein	-79
TVAG_228520	cytosolic malic enzyme	-74
TVAG_171100	lactate dehydrogenase	-60
TVAG_256720	conserved hypothetical protein	-58
TVAG_366380	glyceraldehyde 3-phosphate dehydrogenase	-57
TVAG_228780	alcohol dehydrogenase (Zinc containing)	-50
TVAG_386080	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-48
TVAG_057000	TVCP2	-48
TVAG_146250	conserved hypothetical protein	-45
TVAG_114310	thioredoxin peroxidase	-44
TVAG_215420	conserved hypothetical protein	-29
TVAG_146780	NifU-like protein	-28
TVAG_222490	conserved hypothetical protein	-28
TVAG_264700	polyubiquitin	-25
TVAG_454100	conserved hypothetical protein	-24
TVAG_442590	conserved hypothetical protein	-24
TVAG_335250	conserved hypothetical protein	-22
TVAG_171090	lactate dehydrogenase	-19
TVAG_361410	conserved hypothetical protein	-19
TVAG_441960	polyubiquitin	-18
TVAG_410120	conserved hypothetical protein	-17
TVAG_436700	glykosyl hydrolase	-16
TVAG_343660	conserved hypothetical protein	-16
TVAG_059980	conserved hypothetical protein	-16
TVAG_069570	polyubiquitin	-15
TVAG_077200	conserved hypothetical protein	-15
TVAG_210320	S-adenosyl homocysteine hydrolase	-14
TVAG_352520	40S ribosomal protein S19, putative	-14
TVAG_319530	histone-lysine N-methyltransferase	-14
TVAG_070850	ankyrin repeat protein	-14
TVAG_246920	hypothetical protein	-14
TVAG_491130	conserved hypothetical protein	-14
TVAG_386680	conserved hypothetical protein	-14
TVAG_009450	conserved hypothetical protein	-14
TVAG_437930	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-13
TVAG_184170	polyubiquitin	-13
TVAG_159980	polyubiquitin	-13
TVAG_110540	polyubiquitin	-13
TVAG_083710	ubiquitin-protein ligase, putative	-13
TVAG_365620	ankyrin repeat protein	-13
TVAG_354010	conserved hypothetical protein	-13
TVAG_003130	conserved hypothetical protein	-13
TVAG_381310	lactate dehydrogenase	-12
TVAG_389740	thioredoxin	-12
TVAG_419710	Myb-like DNA-binding domain containing protein	-12
TVAG_474980	thioredoxin reductase	-11
TVAG_165690	thiol peroxidase	-11
TVAG_350540	thioredoxin peroxidase	-11
TVAG_038090	thioredoxin peroxidase	-11
TVAG_209010	Clan CA, family C40, NlpC/P60 superfamily cysteine peptidase	-11
TVAG_184150	polyubiquitin	-11
TVAG_462360	2-hydroxyacid dehydrogenase	-11
TVAG_575220	conserved hypothetical protein	-11

TVAG_525930	conserved hypothetical protein	-11
TVAG_331060	conserved hypothetical protein	-11
TVAG_226530	conserved hypothetical protein	-11
TVAG_110300	conserved hypothetical protein	-11
TVAG_157210	conserved hypothetical protein	-11
TVAG_196270	alpha tubulin	-10
TVAG_437020	50S ribosomal protein L10e	-10
TVAG_028740	elongation protein 3 homolog	-10
TVAG_046120	ankyrin repeat protein	-10
TVAG_360540	conserved hypothetical protein	-10
TVAG_068370	conserved hypothetical protein	-10
TVAG_028320	conserved hypothetical protein	-10
TVAG_476160	Clan MG, family M24, aminopeptidase P-like metalloprotein	-9
TVAG_202090	Clan CA, family C1, cathepsin L-like cysteine peptidase	-9
TVAG_339710	ubiquitin-conjugating enzyme E2	-9
TVAG_469020	HydG, Fe-hydrogenase assembly protein	-9
TVAG_205430	ankyrin repeat-containing protein	-9
TVAG_417070	conserved hypothetical protein	-9
TVAG_204060	conserved hypothetical protein	-9
TVAG_167830	conserved hypothetical protein	-9
TVAG_133500	conserved hypothetical protein	-9
TVAG_107080	conserved hypothetical protein	-9
TVAG_045970	conserved hypothetical protein	-9
TVAG_387920	cysteine synthase	-8
TVAG_403830	methionine adenosyltransferase	-8
TVAG_474660	serine/threonine protein phosphatase	-8
TVAG_373720	pyruvate kinase	-8
TVAG_436530	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	-8
TVAG_193770	Ras family protein	-8
TVAG_479680	2-nitropropane dioxygenase family protein	-8
TVAG_312130	ubiquitin-conjugating enzyme family protein	-8
TVAG_256570	ankyrin repeat protein	-8
TVAG_437250	conserved hypothetical protein	-8
TVAG_145670	conserved hypothetical protein	-8
TVAG_182990	hypothetical protein	-8
TVAG_123670	conserved hypothetical protein	-8
TVAG_032750	hypothetical protein	-8
TVAG_145660	conserved hypothetical protein	-8
TVAG_354020	actin family protein	-7
TVAG_493290	pyridoxal kinase family protein	-7
TVAG_154840	serine/threonine protein phosphatase	-7
TVAG_125500	thioredoxin	-7
TVAG_473150	pelota-like protein	-7
TVAG_278520	polyadenylate-binding protein	-7
TVAG_366320	pyrazinamidase/nicotinamidase	-7
TVAG_407170	myo-inositol-1 phosphate synthase	-7
TVAG_389850	ankyrin repeat protein	-7
TVAG_291510	ankyrin repeat protein	-7
TVAG_498870	conserved hypothetical protein	-7
TVAG_444300	conserved hypothetical protein	-7
TVAG_430700	conserved hypothetical protein	-7
TVAG_345110	conserved hypothetical protein	-7
TVAG_323540	conserved hypothetical protein	-7
TVAG_280090	conserved hypothetical protein	-7
TVAG_266310	conserved hypothetical protein	-7
TVAG_246270	conserved hypothetical protein	-7

TVAG_022220	conserved hypothetical protein	-7
TVAG_451860	NifU-like protein	-6
TVAG_009460	cytosolic malic enzyme	-6
TVAG_298420	serine/threonine protein phosphatase	-6
TVAG_266900	TKL family protein kinase	-6
TVAG_417810	60S ribosomal protein L34e	-6
TVAG_142060	40S ribosomal protein S3Ae	-6
TVAG_110950	polyadenylate-binding protein	-6
TVAG_230590	ankyrin repeat protein	-6
TVAG_151040	COP9 signalosome complex subunit	-6
TVAG_026290	oxysterol-binding protein	-6
TVAG_496030	conserved hypothetical protein	-6
TVAG_452310	conserved hypothetical protein	-6
TVAG_417000	conserved hypothetical protein	-6
TVAG_383370	conserved hypothetical protein	-6
TVAG_311620	conserved hypothetical protein	-6
TVAG_300010	conserved hypothetical protein	-6
TVAG_265530	conserved hypothetical protein	-6
TVAG_226630	conserved hypothetical protein	-6
TVAG_226520	conserved hypothetical protein	-6
TVAG_203290	conserved hypothetical protein	-6
TVAG_182700	conserved hypothetical protein	-6
TVAG_158760	conserved hypothetical protein	-6
TVAG_117200	conserved hypothetical protein	-6
TVAG_064650	conserved hypothetical protein	-6
TVAG_060160	conserved hypothetical protein	-6
TVAG_053720	conserved hypothetical protein	-6
TVAG_303150	XRN 5'-3' exonuclease N-terminus family protein	-5
TVAG_174180	ubiquitin	-5
TVAG_359090	tubulin beta chain, putative	-5
TVAG_319320	transcriptional regulator, Sir2 family protein	-5
TVAG_204390	thioredoxin	-5
TVAG_145440	thioredoxin	-5
TVAG_452580	serine/threonine protein phosphatase	-5
TVAG_301840	serine/threonine protein phosphatase	-5
TVAG_264140	serine/threonine protein phosphatase	-5
TVAG_216210	phosphotyrosyl phosphatase activator	-5
TVAG_030480	Phage shock protein E precursor	-5
TVAG_259200	Myb-like DNA-binding domain containing protein	-5
TVAG_242750	Myb-like DNA-binding domain containing protein	-5
TVAG_225230	longevity-assurance protein	-5
TVAG_239660	IscS-2	-5
TVAG_456770	IscA2-1	-5
TVAG_214040	hypothetical protein	-5
TVAG_336320	Hybrid cluster protein-2	-5
TVAG_196700	glutamate dehydrogenase	-5
TVAG_354110	elongation factor 1 alpha	-5
TVAG_595150	conserved hypothetical protein	-5
TVAG_425470	conserved hypothetical protein	-5
TVAG_259010	conserved hypothetical protein	-5
TVAG_172200	conserved hypothetical protein	-5
TVAG_111510	conserved hypothetical protein	-5
TVAG_090860	conserved hypothetical protein	-5
TVAG_090070	conserved hypothetical protein	-5
TVAG_106750	Clan M-, family M49, dipeptidylpeptidase III-like metallopeptidase	-5
TVAG_169060	Cdc42 homolog	-5

TVAG_223730	ankyrin repeat protein	-5
TVAG_217780	ankyrin repeat protein	-5
TVAG_237680	AAC-1	-5
TVAG_477470	60S ribosomal protein L10a	-5
TVAG_471390	40S ribosomal protein S5	-5
TVAG_074310	40S ribosomal protein S15	-5

**Supplementary Table S5.** All annotated iron-regulated genes sorted by functional categories.

TrichDB 1.2 accession No.	Annotation according to TrichDB 1.2 (manually edited)	Microarrays upregulation rate	EST upregulation index
<b>AMINO ACID METABOLISM</b>			
methionine metabolism			
TVAG_147790	methionine gamma-lyase-2	1,41	8
TVAG_403830	methionine adenosyltransferase	-1,94	-8
TVAG_415010	S-adenosyl methionine dep. methyltransferase	-1,51	-
TVAG_405240	S-adenosyl homocysteine hydrolase	-1,37	-
TVAG_210320	S-adenosyl homocysteine hydrolase	-	-14
arginine dihydrolase pathway			
TVAG_041310	ornithine carbamoyltransferase	-	8
TVAG_368740	ornithine carbamoyltransferase	1,6	2
TVAG_261970	carbamate kinase	-	11
others			
TVAG_235800	alanine dehydrogenase	1,64	-
TVAG_268020	aspartate aminotransferase	1,84	12
TVAG_276530	cysteine synthase	-	5
TVAG_387920	cysteine synthase	-	-8
TVAG_196700	glutamate dehydrogenase	-	-5
TVAG_098820	aminotransferase, classes I and II family protein	-1,37	-
<b>CARBOHYDRATE METABOLISM</b>			
glycogen metabolism			
TVAG_348330	glycogen phosphorylase	1,24	17
TVAG_102390	utp-glucose-1-phosphate uridylyltransferase	1,35	5
TVAG_178580	alpha-amylase	1,33	-
glycolysis			
TVAG_092750	glucokinase	1,86	13
TVAG_205910	phosphoglucomutase	1,58	25
TVAG_054830	phosphoglucomutase	1,37	1
TVAG_405900	phosphoglucomutase	1,36	-1
TVAG_300510	phosphoglucomutase	1,34	2
TVAG_061930	glucose-6-phosphate isomerase	-	18
TVAG_430830	phosphofructokinase (PPi dependent)	1,47	16
TVAG_364620	phosphofructokinase (PPi dependent)	1,37	9
TVAG_281070	phosphofructokinase (PPi dependent)	1,31	1
TVAG_300000	fructose-bisphosphate adolase	-	18
TVAG_360700	fructose-bisphosphate aldolase	1,24	8
TVAG_043070	fructose-bisphosphate aldolase	-	12
TVAG_096350	triosephosphate isomerase	-	6
TVAG_146910	glyceraldehyde 3-phosphate dehydrogenase	1,5	5
TVAG_412780	glyceraldehyde-3-phosphate dehydrogenase	1,17	31
TVAG_475220	glyceraldehyde-3-phosphate dehydrogenase	-	7
TVAG_366380	glyceraldehyde-3-phosphate dehydrogenase	-	-57
TVAG_268050	phosphoglycerate kinase	1,64	47
TVAG_383940	phosphoglycerate kinase	1,49	28
TVAG_209020	phosphoglycerate mutase	1,47	8
TVAG_212740	phosphoglycerate mutase	1,3	4
TVAG_464170	enolase	1,27	29
TVAG_043500	enolase	1,26	9
TVAG_263740	enolase	-	9
TVAG_373720	pyruvate kinase	-	-8

TVAG_073860	pyruvate, phosphate dikinase	1,26	38
TVAG_479540	phosphoenolpyruvate carboxykinase	3,05	51
TVAG_310250	phosphoenolpyruvate carboxykinase	2,65	48
TVAG_420390	phosphoenolpyruvate carboxykinase	2,64	13
TVAG_213710	phosphoenolpyruvate carboxykinase	2,01	-
TVAG_314830	phosphoenolpyruvate carboxykinase	2,64	48
TVAG_139300	phosphoenolpyruvate carboxykinase	-	13
TVAG_204360	malate dehydrogenase	1,57	-
TVAG_253650	malate dehydrogenase	1,36	28
TVAG_228520	malic enzyme	-	-74
TVAG_009460	malic enzyme	-	-6
TVAG_171100	lactate dehydrogenase	-1,56	-60
TVAG_165030	lactate dehydrogenase	-1,33	-1
TVAG_171090	lactate dehydrogenase	-	-19
TVAG_381310	lactate dehydrogenase	-1,76	-12
TVAG_013020	alcohol dehydrogenase (Fe containing)	2,13	-
TVAG_422780	alcohol dehydrogenase (Fe containing)	2,57	24
TVAG_327470	alcohol dehydrogenase (Fe containing)	2,01	16
TVAG_328940	alcohol dehydrogenase (Fe containing)	1,49	5
TVAG_228780	alcohol dehydrogenase (Zinc containing)	-1,19	-50
hydrogenosomal energy metabolism			
TVAG_340290	malic enzyme H (AP65-1)	2,15	63
TVAG_238830	malic enzyme B	1,67	33
TVAG_412220	malic enzyme D	1,37	-1
TVAG_198110	pyruvate:ferredoxin oxidoreductase A	4,01	32
TVAG_242960	pyruvate:ferredoxin oxidoreductase BII	3,71	18
TVAG_230580	pyruvate:ferredoxin oxidoreductase BI	3,64	9
TVAG_037570	iron hydrogenase 64kDa	2,48	14
TVAG_003900	ferredoxin 1	1,91	3
TVAG_292710	ferredoxin 4	1,33	-
TVAG_395550	acetate:succinate CoA transferase	1,74	14
TVAG_164890	acetate:succinate CoA transferase	1,31	3
TVAG_047890	succinyl-CoA synthase $\alpha$ -subunit	1,6	9
TVAG_165340	succinyl-CoA synthase $\alpha$ -subunit	1,45	6
TVAG_318670	succinyl-CoA syntahse $\alpha$ -subunit	1,34	7
TVAG_259190	succinyl-CoA syntahse $\beta$ subunit	1,45	18
TVAG_183500	succinyl-CoA syntahse $\beta$ subunit	1,24	17
TVAG_144730	succinyl-CoA syntahse $\beta$ subunit	1,25	8
TVAG_133030	complex1, Tvh47	-	5
TVAG_462920	phosphofructokinase (ATP dependent)	-	6
others			
TVAG_006140	6-phosphogluconate dehydrogenase	-1,54	-
TVAG_212020	transketolase	-	7

#### CYTOSKELETON AND INTERACTING PROTEINS

TVAG_160060	actin	1,28	5
TVAG_249200	actin	-	6
TVAG_200190	actin	-1,1	7
TVAG_090470	actin	-	10
TVAG_512800	actin	-	24
TVAG_534990	actin	-	26
TVAG_310030	actin	-	26
TVAG_337240	actin	-	31
TVAG_172680	actin	-	32

TVAG_054030	actin	-	36
TVAG_244120	actin-binding protein	1,33	4
TVAG_156680	actinin	1,35	11
TVAG_239310	alpha-actinin	-	68
TVAG_035180	arp2/3 complex subunit	-	6
TVAG_259990	calmoduline	1,36	-
TVAG_402260	coactosin	-	6
TVAG_021420	coronin	1,31	4
TVAG_159160	profilin	-	5
TVAG_312330	alpha-tubulin 1	-	15
TVAG_206890	alpha-tubulin 1	-	5
TVAG_073810	beta-tubulin	-	5
TVAG_008680	beta-tubulin	-	6
TVAG_360870	alpha-tubulin 1	-	7
TVAG_062880	beta-tubulin	-	7
TVAG_354020	centractin, putative	-	-7
TVAG_417660	cortactin-binding protein	-1,34	-
TVAG_196270	tubulin beta chain	-	-10
TVAG_359090	tubulin beta chain	-	-5
TVAG_463220	dynein heavy chain	-1,37	

#### FeS CLUSTERS ASSEMBLY

TVAG_146780	Nfu	-	-28
TVAG_008840	Nfu	-1,42	-4
TVAG_451860	Nfu	-	-6
TVAG_361540	IscA2	-1,36	-1
TVAG_456770	IscA2	-	-5
TVAG_239660	IscS-2, cystein desulfurase	-	-5
TVAG_469020	HydG, Fe-hydrogenase assembly protein	-	-9

#### KINASES AND PHOSPHATASES

kinases			
TVAG_126240	CAMK family protein kinase	2,09	-
TVAG_339280	CAMK family protein kinase	1,44	-
TVAG_370340	CAMK family protein kinase	1,35	-
TVAG_165540	CK1 family protein kinase	1,54	-
TVAG_384480	ribokinase	1,38	-
TVAG_365110	ribokinase	-1,58	-
TVAG_493290	pyridoxal kinase family protein	-	-7
TVAG_266900	TKL family protein kinase	-	-6
phosphatases			
TVAG_474660	serine/threonine protein phosphatase	-	-8
TVAG_154840	serine/threonine protein phosphatase	-	-7
TVAG_298420	serine/threonine protein phosphatase	-	-6
TVAG_264140	serine/threonine protein phosphatase	-	-5
TVAG_452580	serine/threonine protein phosphatase	-1,36	-5
TVAG_301840	serine/threonine protein phosphatase	-	-5
TVAG_216210	phosphotyrosyl phosphatase activator	-	-5
TVAG_144520	protein phosphatase-1	-1,34	-

#### MEMBRANE AND VESICULAR TRANSPORT

transporters			
TVAG_324980	ATP synthase	-	16
TVAG_262750	vacuolar ATP synthase subunit H	-	15
TVAG_453110	ATP synthase alpha subunit B	-	7
TVAG_064460	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	1,39	1

TVAG_345280	sugar transporter	1,36	4
TVAG_420260	ATP synthase beta subunit	1,34	17
TVAG_239840	sucrose transport protein	1,84	-
TVAG_039200	sucrose transport protein	1,34	1
TVAG_037610	vacuolar ATP synthase subunit C	1,30	3
TVAG_271570	equilibrative nucleoside transporter	1,27	17
TVAG_024780	ammonium transporter family protein	1,36	-
TVAG_402410	sugar transporter	-1,30	-
TVAG_542460	ABC transporter family protein	-1,43	4
TVAG_415420	zinc-iron transporter	-2,16	-
TVAG_304360	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	-1,8	-
TVAG_436530	Na <sup>+</sup> driven multidrug efflux pump (MatE family)	-2,58	-8
hydrogenosomal transporters			
TVAG_104250	Hmp35-2	1,56	7
TVAG_051820	ACC-2	-	7
TVAG_237680	ACC-1	-	-5
intracellular membrane trafficking			
TVAG_390750	RAB family protein	1,49	-
TVAG_126970	RAB GDP-dissociation inhibitor	-	21
TVAG_148040	ADP-ribosylation factor 1	-	5
TVAG_344080	adaptin N terminal region family protein	-	5
TVAG_547230	adaptin N terminal region family protein	-	5
TVAG_485580	synaptotagmin	-	6
TVAG_485570	synaptotagmin	1,33	-
TVAG_516070	clathrin heavy chain	-	9
TVAG_558650	clathrin heavy chain	-	6
TVAG_453580	clathrin coat assembly protein	1,30	1
TVAG_193770	Ras family protein	-1,25	-8
TVAG_169060	Cdc42 homolog	-1,23	-5

## OXIDOREDUCTASES

TVAG_049830	disulfide oxidoreductase	2,37	43
TVAG_263800	disulfide oxidoreductase	1,76	3
TVAG_465230	oxidoreductase, aldo/keto reductase family protein	-1,41	1
TVAG_144700	flavodoxin family protein	-1,32	-3
TVAG_479680	2-nitropropane dioxygenase family protein	-1,53	-8
TVAG_411870	aldo-keto reductase family protein	-1,48	-4

## cytosolic thioredoxin linked antioxidant system

TVAG_232840	tryparedoxin like protein	1,43	-
TVAG_145440	thioredoxin	-	-5
TVAG_204390	thioredoxin	-	-5
TVAG_125500	thioredoxin	-	-7
TVAG_350540	thioredoxin peroxidase	-	-11
TVAG_038090	thioredoxin peroxidase	-1,43	-11
TVAG_474980	thioredoxin reductase	-	-11
TVAG_389740	thioredoxin	-	-12
TVAG_114310	thioredoxin peroxidase	-1,27	-44

## hydrogenosomal antioxidant system

TVAG_036010	hydrogensomal oxygen reductase	-	5
TVAG_165690	thiol peroxidase	-	-11

## KINASES AND PHOSPHATASES

kinases			
TVAG_126240	CAMK family protein kinase	2,09	-
TVAG_339280	CAMK family protein kinase	1,44	-



TVAG_370340	CAMK family protein kinase	1,35	-
TVAG_165540	CK1 family protein kinase	1,54	-
TVAG_384480	ribokinase	1,38	-
TVAG_102390	utp-glucose-1-phosphate uridylyltransferase	-	5
TVAG_365110	ribokinase	-1,58	-
TVAG_493290	phosphomethylpyrimidine kinase, putative	-	-7
TVAG_266900	TKL family protein kinase	-	-6
phosphatases			
TVAG_474660	serine/threonine protein phosphatase	-	-8
TVAG_154840	serine/threonine protein phosphatase	-	-7
TVAG_298420	serine/threonine protein phosphatase	-	-6
TVAG_264140	serine/threonine protein phosphatase	-	-5
TVAG_452580	serine/threonine protein phosphatase	-1,36	-5
TVAG_301840	serine/threonine protein phosphatase	-	-5
TVAG_216210	phosphotyrosyl phosphatase activator	-	-5
TVAG_031330	protein phosphatase-1	-1,43	-
TVAG_144520	protein phosphatase-1	-1,34	-

## PEPTIDASES

TVAG_229580	Clan CA, family C19, ubiquitin hydrolase-like cysteine peptidase	2,51	-
TVAG_212800	Clan MG, family M24, aminopeptidase P-like metallopeptidase	1,42	1
TVAG_367130	Clan MA, family M8, leishmanolysin-like metallopeptidase	-	10
TVAG_032440	Clan MA, family M8, leishmanolysin-like metallopeptidase	-	6
TVAG_224980	Clan MH, family M20, peptidase T-like metallopeptidase	-	7
TVAG_090100	TVCP3	-1,37	5
TVAG_298080	TVCPT	-1,29	12
TVAG_426660	TVLEGU1	-1,37	7
TVAG_385340	TVLEGU2	-1,39	2
TVAG_057000	TVCP2	-1,7	-48
TVAG_467970	TVCP4	-1,3	-1
TVAG_202090	clan CA, family C1, cathepsin-L like cysteine peptidase	-	-9
TVAG_209010	Clan CA, family C40, NlpC/P60 superfamily cysteine peptidase	-	-11
TVAG_242850	Clan CA, family C1, cathepsin L, S or H-like cysteine peptidase	-1,56	-
TVAG_106750	Clan M-, family M49, dipeptidylpeptidase III-like metallopeptidase	-1,23	-5
TVAG_386080	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-	-48
TVAG_437930	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,32	-13
TVAG_476160	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-	-9
TVAG_255940	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,31	-2
TVAG_321430	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,35	-1
TVAG_190580	Clan MG, family M24, aminopeptidase P-like metallopeptidase	-1,41	-

## PROTEIN FOLDING AND MODIFICATION

chaperones - cytosolic			
TVAG_092490	heat shock protein 70kD	-	26
TVAG_139320	dnaK	1,28	13
TVAG_044510	cytoplasmic heat shock protein 70	1,3	11
TVAG_155010	heat shock protein 82	1,23	8
TVAG_397610	cytoplasmic heat shock protein 70	-	6
TVAG_464010	calreticulin	-	6
TVAG_398480	chaperonin-60kD	-	5
TVAG_153560	heat shock protein	-	5
TVAG_206270	cytosolic heat shock protein 70	1,19	5
TVAG_091080	ubiquitin-conjugating enzyme family protein	2,05	2
TVAG_297650	GrpE protein homolog	1,3	-1
chaperones - hydrogenosomal			

TVAG_340390	HSP70	-	5
ubiquitins			
TVAG_097660	ubiquitin family protein	1,3	1
TVAG_264700	ubiquitin	1,35	-25
TVAG_441960	polyubiquitin	-	-18
TVAG_069570	polyubiquitin	-	-15
TVAG_110540	polyubiquitin	-	-13
TVAG_159980	polyubiquitin	-	-13
TVAG_083710	ubiquitin-protein ligase	-	-13
TVAG_184170	polyubiquitin	-	-13
TVAG_184150	polyubiquitin	-	-11
TVAG_339710	ubiquitin-conjugating enzyme E2	-	-9
TVAG_312130	ubiquitin-conjugating enzyme family protein	-	-8
TVAG_174180	ubiquitin	-	-5
glycosylation			
TVAG_080000	glycosyl hydrolase	1,32	36
TVAG_362570	glycosyl hydrolase	1,46	-
TVAG_258220	glycosyltransferase	-	5
TVAG_110660	glycosyl hydrolase	-2,20	-
TVAG_436700	glycosyl hydrolase	-	-16

## TRANSCRIPTION AND TRANSLATION

regulation of transcription			
TVAG_537380	Myb-like DNA-binding domain containing protein	1,49	3
TVAG_047080	regulator of nonsense transcripts 1	1,87	2
TVAG_437940	Myb-like DNA-binding domain containing protein	1,31	-1
TVAG_259200	Myb-like DNA-binding domain containing protein	-	-5
TVAG_252420	Myb3	-1,41	-1
TVAG_419710	Myb-like DNA-binding domain containing protein	-	-12
TVAG_242750	Myb-like DNA-binding domain containing protein	-	-5
TVAG_361780	translation initiation factor 5A	-1,32	1
TVAG_028740	elongation protein 3 homolog	-	-10
TVAG_354110	elongation factor 1 alpha	-	-5
TVAG_319320	transcriptional regulator, Sir2 family protein	-1,27	-5
translation			
TVAG_463940	elongation factor 1-alpha	-	41
TVAG_276410	elongation factor 2	-	46
TVAG_067400	elongation factor 1-alpha	-	41
TVAG_496270	phenylalanyl-tRNA synthetase beta chain	-1,47	-
TVAG_208470	threonyl-tRNA synthetase	1,89	25
TVAG_305400	seryl-tRNA synthetase	-	8
TVAG_156640	aspartyl/lysyl-tRNA synthetase	-	6
TVAG_258380	glutamyl-tRNA synthetase	-	6
TVAG_276400	cysteiny-tRNA synthetase	1,31	0
TVAG_439220	N(2),N(2)-dimethylguanosine tRNA methyltransferase	2,45	-
TVAG_473150	translation factor (pelota-like protein)	-	-7
ribosomal proteins			
TVAG_051160	ribosomal protein L10	-	16
TVAG_359120	40S ribosomal protein S2	-	11
TVAG_064640	ribosomal protein L5	-	9
TVAG_344700	ribosomal protein L10	-	8
TVAG_125550	ribosomal protein L3p	-	8
TVAG_454000	ribosomal protein L5	-	7
TVAG_128790	60S ribosomal protein L4	-	7

TVAG_583060	30S/40S ribosomal protein S4	-	6
TVAG_490240	ribosomal protein L5	1,23	5
TVAG_476810	60S ribosomal protein L4	-	5
TVAG_414510	40S ribosomal protein S3a	-	5
TVAG_240050	40S ribosomal protein sa	-	5
TVAG_200220	60S ribosomal protein L15	-	5
TVAG_113720	ribosomal protein L5	-	5
TVAG_098450	40S ribosomal protein S4	-	5
TVAG_054130	60S ribosomal protein L7	-	5
TVAG_024110	50S ribosomal protein L14p	-	5
TVAG_247060	40S ribosomal protein S17	1,32	1
TVAG_141750	16S ribosomal RNA	1,49	-
TVAG_291460	40S ribosomal protein S17	1,30	-
TVAG_352520	40S ribosomal protein S19	-	-14
TVAG_437020	50S ribosomal protein L10e	-	-10
TVAG_142060	40S ribosomal protein S3a	-1,19	-6
TVAG_417810	60S ribosomal protein L34	-	-6
TVAG_074310	40S ribosomal protein S15	-	-5
TVAG_471390	40S ribosomal protein S5	-1,2	-5
TVAG_477470	60S ribosomal protein L10a	-	-5
TVAG_487470	40S ribosomal protein S6	-1,35	-2
TVAG_013870	60S ribosomal protein L35	-1,41	-2
TVAG_055940	40S ribosomal protein S15	-1,34	-1
TVAG_192480	60S ribosomal protein L35a	-1,32	-

#### DNA

TVAG_306240	DNA double-strand break repair Rad50 ATPase, putative	2,32	-
TVAG_474360	centrosomal protein of 135 kDa	1,56	8
TVAG_010490	viral A-type inclusion protein	1,40	-
TVAG_278520	polyadenylate-binding protein	-	-7
TVAG_110950	polyadenylate-binding protein	-1,42	-6
TVAG_227750	res subunit family protein	-1,45	-
TVAG_116180	DNA excision repair protein	-1,55	1
TVAG_319530	histone-lysine N-methyltransferase	-	-14
TVAG_176480	structural maintenance of chromosomes 5,6	-1,32	2

#### nucleotide metabolism

TVAG_136660	nucleoside diphosphate kinase family protein	2,45	-1
TVAG_188330	NAD <sup>+</sup> synthetase family protein	1,77	1
TVAG_135190	cytidine deaminase family protein	1,42	1
TVAG_416510	adenosine deaminase	-	5
TVAG_338230	dihydroorotate dehydrogenase	-2,46	-
TVAG_366320	pyrazinamidase/nicotinamidase	-	-7
TVAG_145570	guanine nucleotide-binding protein beta subunit	-	10

#### UNKNOWN FUNCTION

TVAG_475690	beige/BEACH domain containing protein	2,51	-
TVAG_481370	AMP dependent ligase/synthetase	1,43	-
TVAG_144720	retinal rod rhodopsin-sensitive cGMP 3,5-cyclic phosphodiesterase	3,33	-
TVAG_318870	spermatogenesis associated factor	1,74	-
TVAG_222850	kelch repeat protein	1,67	-
TVAG_214710	calmodulin-like protein	1,65	-
TVAG_085990	agglutinin	1,56	-
TVAG_219770	macrophage migration inhibitory factor-like protein	1,44	-
TVAG_293510	ankyrin repeat protein	1,41	-
TVAG_211310	coiled-coil domain-containing protein	1,39	-

TVAG_125450	beige/BEACH domain containing protein	1,4	-
TVAG_262010	ankyrin repeat protein	1,37	-
TVAG_387680	ankyrin repeat protein	1,37	-
TVAG_016710	ankyrin repeat protein	1,34	-
TVAG_045310	myo-inositol-1 phosphate synthase	1,17	8
TVAG_429360	WD domain containing protein	-	9
TVAG_407250	adenylyl cyclase-associated protein	-	10
TVAG_462940	14-3-3 protein	-1,31	11
TVAG_184270	ankyrin repeat protein	-2,44	-
TVAG_475640	ankyrin repeat protein	-1,31	-
TVAG_070850	ankyrin repeat protein	-1,31	-14
TVAG_110370	ankyrin repeat protein	-1,36	-
TVAG_365620	ankyrin repeat protein	-	-13
TVAG_462360	2-hydroxyacid dehydrogenase	-	-11
TVAG_046120	ankyrin repeat protein	-	-10
TVAG_205430	ankyrin repeat protein	-	-9
TVAG_256570	ankyrin repeat protein	-	-8
TVAG_407170	myo-inositol-1 phosphate synthase	-	-7
TVAG_389850	ankyrin repeat protein	-	-7
TVAG_291510	ankyrin repeat protein	-	-7
TVAG_230590	ankyrin repeat protein	-	-6
TVAG_151040	COP9 signalosome complex subunit	-	-6
TVAG_026290	oxysterol-binding protein	-	-6
TVAG_225230	longevity-assurance protein	-	-5
TVAG_223730	ankyrin repeat protein	-	-5
TVAG_217780	ankyrin repeat protein	-	-5
TVAG_030480	Phage shock protein E precursor, putative	-	-5
hydrogenosomal			
TVAG_336320	Hybrid cluster protein-2	-	-5

**Supplementary Table S6.** List of detected Myb-like proteins.

<b>TrichDB 1.2 accession No.</b>	<b>Annotation</b>	<b>Microarrays upregulation rate</b>	<b>EST upregulation index</b>
TVAG_537380	Myb-like DNA-binding domain containing protein	1,49	3
TVAG_437940	Myb-like DNA-binding domain containing protein	1,31	-1
TVAG_180160	Myb-like DNA-binding domain containing protein	-	2
TVAG_225360	Myb-like DNA-binding domain containing protein	-	2
TVAG_307780	Myb-like DNA-binding domain containing protein	-	2
TVAG_339390	Myb-like DNA-binding domain containing protein	-	2
TVAG_385810	Myb-like DNA-binding domain containing protein	-	2
TVAG_026280	Myb-like DNA-binding domain containing protein	-	1
TVAG_072460	Myb-like DNA-binding domain containing protein	-	1
TVAG_084520	Myb-like DNA-binding domain containing protein	-	1
TVAG_085980	Myb-like DNA-binding domain containing protein	1,21	1
TVAG_092320	Myb-like DNA-binding domain containing protein	-	1
TVAG_093690	Myb-like DNA-binding domain containing protein	-	1
TVAG_111520	Myb-like DNA-binding domain containing protein	-	1
TVAG_120830	Myb-like DNA-binding domain containing protein	-	1
TVAG_165400	Myb-like DNA-binding domain containing protein	-	1
TVAG_167820	Myb-like DNA-binding domain containing protein	-	1
TVAG_198210	Myb-like DNA-binding domain containing protein	-	1
TVAG_210910	Myb-like DNA-binding domain containing protein	-	1
TVAG_219560	Myb-like DNA-binding domain containing protein	-	1
TVAG_240540	Myb-like DNA-binding domain containing protein	-	1
TVAG_308040	Myb-like DNA-binding domain containing protein	-	1
TVAG_320410	Myb-like DNA-binding domain containing protein	-	1
TVAG_371340	Myb-like DNA-binding domain containing protein	-	1
TVAG_373330	Myb-like DNA-binding domain containing protein	-	1
TVAG_443020	Myb-like DNA-binding domain containing protein	-	1
TVAG_463870	Myb-like DNA-binding domain containing protein	-	1
TVAG_483210	Myb-like DNA-binding domain containing protein	-	1
TVAG_485190	Myb-like DNA-binding domain containing protein	-	1
TVAG_491010	Myb-like DNA-binding domain containing protein	-	1
TVAG_497080	Myb-like DNA-binding domain containing protein	-	1
TVAG_476290	MYB	1.16	-
TVAG_148590	Myb-like DNA-binding domain containing protein	1,17	-
TVAG_419710	Myb-like DNA-binding domain containing protein	-	-12
TVAG_242750	Myb-like DNA-binding domain containing protein	-	-5
TVAG_259200	Myb-like DNA-binding domain containing protein	-	-5
TVAG_485240	Myb-like DNA-binding domain containing protein	-	-4
TVAG_035980	Myb-like DNA-binding domain containing protein	-	-3
TVAG_300280	Myb-like DNA-binding domain containing protein	-	-3
TVAG_309650	Myb-like DNA-binding domain containing protein	-	-3
TVAG_429940	Myb-like DNA-binding domain containing protein	-	-3
TVAG_139500	Myb-like DNA-binding domain containing protein	-	-2
TVAG_170470	Myb-like DNA-binding domain containing protein	-	-2
TVAG_228280	Myb-like DNA-binding domain containing protein	-	-2
TVAG_240720	Myb-like DNA-binding domain containing protein	-	-2
TVAG_294950	Myb-like DNA-binding domain containing protein	-	-2
TVAG_346310	Myb-like DNA-binding domain containing protein	-	-2
TVAG_421410	Myb-like DNA-binding domain containing protein	-	-2
TVAG_429530	Myb-like DNA-binding domain containing protein	-	-2
TVAG_459370	Myb-like DNA-binding domain containing protein	-	-2
TVAG_480190	Myb-like DNA-binding domain	-1.26	-

TVAG_413770	Myb-like DNA-binding domain containing protein	-1,24	-
TVAG_473470	Myb1	-	-1
TVAG_211210	Myb2	-	-1
TVAG_252420	Myb3	-1,41	-1
TVAG_031450	Myb-like DNA-binding domain containing protein	-	-1
TVAG_042270	Myb-like DNA-binding domain containing protein	-	-1
TVAG_047940	Myb-like DNA-binding domain containing protein	-	-1
TVAG_048980	Myb-like DNA-binding domain containing protein	-	-1
TVAG_055240	Myb-like DNA-binding domain containing protein	-	-1
TVAG_073430	Myb-like DNA-binding domain containing protein	-	-1
TVAG_090040	Myb-like DNA-binding domain containing protein	-	-1
TVAG_097250	Myb-like DNA-binding domain containing protein	-	-1
TVAG_101370	Myb-like DNA-binding domain containing protein	-	-1
TVAG_118720	Myb-like DNA-binding domain containing protein	-	-1
TVAG_145460	Myb-like DNA-binding domain containing protein	-	-1
TVAG_158960	Myb-like DNA-binding domain containing protein	-	-1
TVAG_169810	Myb-like DNA-binding domain containing protein	-	-1
TVAG_180180	Myb-like DNA-binding domain containing protein	-	-1
TVAG_196880	Myb-like DNA-binding domain containing protein	-	-1
TVAG_198240	Myb-like DNA-binding domain containing protein	-	-1
TVAG_212330	Myb-like DNA-binding domain containing protein	-	-1
TVAG_214320	Myb-like DNA-binding domain containing protein	-	-1
TVAG_220980	Myb-like DNA-binding domain containing protein	-	-1
TVAG_221940	Myb-like DNA-binding domain containing protein	-	-1
TVAG_237090	Myb-like DNA-binding domain containing protein	-	-1
TVAG_245910	Myb-like DNA-binding domain containing protein	-	-1
TVAG_257400	Myb-like DNA-binding domain containing protein	-	-1
TVAG_268910	Myb-like DNA-binding domain containing protein	-	-1
TVAG_274330	Myb-like DNA-binding domain containing protein	-	-1
TVAG_303430	Myb-like DNA-binding domain containing protein	-	-1
TVAG_303880	Myb-like DNA-binding domain containing protein	-	-1
TVAG_317030	Myb-like DNA-binding domain containing protein	-	-1
TVAG_336370	Myb-like DNA-binding domain containing protein	-	-1
TVAG_371630	Myb-like DNA-binding domain containing protein	-	-1
TVAG_380940	Myb-like DNA-binding domain containing protein	-	-1
TVAG_413160	Myb-like DNA-binding domain containing protein	-	-1
TVAG_429510	Myb-like DNA-binding domain containing protein	-	-1
TVAG_449770	Myb-like DNA-binding domain containing protein	-1,18	-1
TVAG_476050	Myb-like DNA-binding domain containing protein	-	-1
TVAG_480710	Myb-like DNA-binding domain containing protein	-	-1
TVAG_482180	Myb-like DNA-binding domain containing protein	-	-1
TVAG_485270	Myb-like DNA-binding domain containing protein	-	-1
TVAG_488440	Myb-like DNA-binding domain containing protein	-	-1
TVAG_495930	Myb-like DNA-binding domain containing protein	-	-1
TVAG_592190	Myb-like DNA-binding domain containing protein	-	-1

**Supplementary table S7.** Iron-regulated proteins that contain both MRE-like sequences in their upstream regions. The numbers indicate position of the motifs upstream of translation start site. Numbers in bold indicate position of the motifs in the 5'UTR of ME (Tai et al., 2006)

<b>MRE eukaryotic consensus: (C/T)AACG(G/T)</b>					
<b>+Fe</b>		<b>1. position</b>		<b>2. position</b>	
TVAG_347810	conserved hypothetical protein	-18	-12		
TVAG_244120	actin-binding protein	-35	-29		
TVAG_018450	conserved hypothetical protein	-47	-41		
TVAG_073810	beta-tubulin	-63	-57		
TVAG_144730	succinyl-CoA synthase $\beta$ subunit	-76	-70		
TVAG_227120	conserved hypothetical protein	-105	-99	-279	-273
TVAG_390750	RAB family protein	-106	-100		
TVAG_026110	conserved hypothetical protein	-113	-107	-231	-225
TVAG_165340	succinyl-CoA synthase $\alpha$ -subunit	-118	-112		
TVAG_387680	ankyrin repeat protein	-141	-135		
TVAG_305400	seryl-tRNA synthetase	-180	-174		
TVAG_441420	conserved hypothetical protein	-198	-192		
TVAG_159160	profilin	-242	-236		
TVAG_088640	conserved hypothetical protein	-257	-251		
<b>-Fe</b>					
TVAG_475640	ankyrin repeat protein	-24	-18		
TVAG_535110	conserved hypothetical protein	-72	-66		
TVAG_338230	dihydroorotate dehydrogenase	-82	-76		
TVAG_215420	conserved hypothetical protein	-95	-89		
TVAG_280090	conserved hypothetical protein	-100	-94		
TVAG_201190	conserved hypothetical protein	-127	-121		
TVAG_028740	elongator complex protein, putative	-137	-131		
TVAG_259010	conserved hypothetical protein	-153	-147		
TVAG_139510	conserved hypothetical protein	-164	-158		
TVAG_352520	40S ribosomal protein S19	-171	-165		
TVAG_242850	Clan CA, family C1, cathepsin L, S or	-204	-198	-218	-212
TVAG_202090	Clan CA, family C1, cathepsin L-like d	-205	-199		
TVAG_230590	ankyrin repeat protein	-213	-207		
TVAG_145670	conserved hypothetical protein	-219	-213		
TVAG_407170	myo-inositol-1 phosphate synthase, p	-281	-275		
TVAG_146780	conserved hypothetical protein	-293	-287		
<b>MRE1/MRE2r A(A/T/C/G)AACGAT - Myb1 binding</b>		<b>-111</b>	<b>-104</b>		
<b>+Fe</b>					
TVAG_369900	conserved hypothetical protein	-18	-11		
TVAG_212020	transketolase	-33	-26		
TVAG_049830	disulfide oxidoreductase	-38	-31		
TVAG_054130	60S ribosomal protein L7	-72	-65		
TVAG_268020	aspartate aminotransferase	-76	-69		
TVAG_402260	coactosin	-77	-70		
TVAG_479540	phosphoenolpyruvate carboxykinase	-78	-71		
TVAG_328940	alcohol dehydrogenase (Fe)	-82	-75		
TVAG_178580	alpha-amylase	-83	-76		
TVAG_156640	aspartyl/lysyl-tRNA synthetase	-88	-81		
TVAG_327470	alcohol dehydrogenase (Fe)	-95	-88		
TVAG_126970	RAB GDP-dissociation inhibitor	-102	-95		
TVAG_139320	dnaK	-112	-105		
TVAG_347470	conserved hypothetical protein	-114	-107		
TVAG_440840	conserved hypothetical protein	-133	-126		

TVAG_422780	alcohol dehydrogenase (Fe)	-136	-129
TVAG_368740	ornithine carbamoyltransferase	-148	-141
TVAG_393390	conserved hypothetical protein	-169	-162
TVAG_318670	succinyl-CoA syntahse $\alpha$ -subunit	-171	-164
TVAG_474550	conserved hypothetical protein	-175	-168
TVAG_088640	conserved hypothetical protein	-178	-171
TVAG_146910	glyceraldehyde 3-phosphate dehydro	-187	-180
TVAG_412780	glyceraldehyde 3-phosphate dehydro	-221	-214
TVAG_139300	phosphoenolpyruvate carboxykinase	-229	-222
TVAG_281070	phosphofructokinase (PPi dependent)	-235	-228
TVAG_135190	cytidine deaminase family protein	-243	-236
TVAG_013020	alcohol dehydrogenase (Fe)	-257	-250
TVAG_133030	complex1, Tvh47	-258	-251
TVAG_383940	phosphoglycerate kinase	-260	-253
TVAG_244120	actin-binding protein	-278	-271
TVAG_064640	ribosomal protein L5	-292	-285

**-Fe**

TVAG_008840	conserved hypothetical protein	-35	-28
TVAG_196700	glutamate dehydrogenase	-40	-33
TVAG_157210	conserved hypothetical protein	-65	-58
TVAG_145670	conserved hypothetical protein	-195	-188
TVAG_222490	conserved hypothetical protein	-232	-225
TVAG_144700	flavodoxin family protein	-254	-247
TVAG_323540	conserved hypothetical protein	-278	-271
TVAG_452310	conserved hypothetical protein	-291	-284

MRE2r		CGATA-Myb2 binding site		-107	-103	AACGATA	
+Fe		1. position		2.position			
TVAG_366360	conserved hypothetical protein	-5	-1	-194	-190		
TVAG_569810	conserved hypothetical protein	-6	-2				
TVAG_369900	conserved hypothetical protein	-14	-10			-16	-10
TVAG_346820	conserved hypothetical protein	-17	-13				
TVAG_339280	CAMK family protein kinase	-19	-15				
TVAG_150430	conserved hypothetical protein	-19	-15				
TVAG_133030	complex1, Tvh47	-20	-16				
TVAG_235800	alanine dehydrogenase	-22	-18				
TVAG_128790	60S ribosomal protein L4	-25	-21				
TVAG_037570	iron hydrogenase 64kDa	-26	-22			-28	-22
TVAG_212020	transketolase	-29	-25			-31	-25
TVAG_188330	NAD <sup>+</sup> synthetase family protein	-29	-25			-31	-25
TVAG_268020	aspartate aminotransferase	-30	-26			-32	-26
TVAG_226870	conserved hypothetical protein	-30	-26				
TVAG_247060	40S ribosomal protein S17	-31	-27	-65	-61		
TVAG_368740	ornithine carbamoyltransferase	-32	-28	-134	-130		
TVAG_291460	40S ribosomal protein S17	-32	-28				
TVAG_208470	threonyl-tRNA synthetase	-32	-28				
TVAG_073810	beta-tubulin	-32	-28				
TVAG_047080	regulator of nonsense transcripts 1	-32	-28				
TVAG_292710	Ferredoxin 4	-38	-34				
TVAG_244120	actin-binding protein	-38	-34				
TVAG_091080	ubiquitin conjugating enzyme family p	-40	-36				
TVAG_000810	conserved hypothetical protein	-43	-39	-57	-53	-59	-53
TVAG_219770	macrophage migration inhibitory facto	-51	-47				
TVAG_485570	synaptotagmin	-56	-52	-143	-139		
TVAG_085990	agglutinin	-58	-54				
TVAG_276410	elongation factor 2	-60	-56				
TVAG_384480	ribokinase	-61	-57				



TVAG_135190	cytidine deaminase family protein	-61	-57	-111	-107	-63	-57
TVAG_092750	glucokinase	-61	-57				
TVAG_224980	Clan MH, family M20, peptidase T-like	-63	-59				
TVAG_214710	calmodulin-like protein	-63	-59				
TVAG_297650	GrpE protein homolog	-67	-63				
TVAG_402260	coactosin	-73	-69			-75	-69
TVAG_453110	ATP synthase alpha subunit B	-74	-70			-76	-70
TVAG_218820	conserved hypothetical protein	-76	-72				
TVAG_178580	alpha-amylase	-79	-75			-81	-75
TVAG_481370	AMP dependent ligase/synthetase	-82	-78				
TVAG_156640	aspartyl/lysyl-tRNA synthetase	-84	-80			-86	-80
TVAG_310030	actin	-86	-82				
TVAG_212480	conserved hypothetical protein	-87	-83				
TVAG_126970	RAB GDP-dissociation inhibitor	-93	-89	-98	-94	-100	-94
TVAG_383940	phosphoglycerate kinase	-97	-93	-216	-212		
TVAG_479540	phosphoenolpyruvate carboxykinase	-101	-97				
TVAG_238830	malic enzyme B	-105	-101				
TVAG_166850	conserved hypothetical protein	-107	-103			-109	-103
TVAG_474550	conserved hypothetical protein	-108	-104	-171	-167	-173	-167
TVAG_098450	40S ribosomal protein S4	-110	-106	-256	-252		
TVAG_155010	heat shock protein 82	-114	-110				
TVAG_324980	ATP synthase	-115	-111				
TVAG_268050	phosphoglycerate kinase	-115	-111	-251	-247		
TVAG_430830	phosphofructokinase (PPi dependent)	-125	-121	-296	-292		
TVAG_422780	alcohol dehydrogenase (Fe containing)	-132	-128			-134	-128
TVAG_061930	glucose-6-phosphate isomerase	-141	-137				
TVAG_032440	Clan MA, family M8, leishmanolysin-li	-141	-137	-185	-181		
TVAG_072120	conserved hypothetical protein	-143	-139				
TVAG_397610	cytoplasmic heat shock protein 70	-146	-142				
TVAG_270500	conserved hypothetical protein	-147	-143	-223	-219		
TVAG_258380	glutaminyt-tRNA synthetase	-147	-143				
TVAG_054030	actin	-149	-145			-151	-145
TVAG_262010	ankyrin repeat protein	-151	-147	-251	-247		
TVAG_209020	phosphoglycerate mutase	-156	-152				
TVAG_104250	conserved hypothetical protein	-163	-159				
TVAG_206270	cytosolic heat shock protein 70	-173	-169				
TVAG_217320	conserved hypothetical protein	-175	-171				
TVAG_051820	ACC-2	-176	-172				
TVAG_463940	elongation factor 1-alpha	-180	-176				
TVAG_146910	glyceraldehyde 3-phosphate dehydro	-183	-179			-185	-179
TVAG_054130	60S ribosomal protein L7	-214	-210				
TVAG_347810	conserved hypothetical protein	-218	-214				
TVAG_154680	conserved hypothetical protein	-224	-220				
TVAG_305400	seryl-tRNA synthetase	-226	-222				
TVAG_398480	chaperonin-60kD	-228	-224				
TVAG_364620	phosphofructokinase (PPi dependent)	-228	-224				
TVAG_344700	ribosomal protein L10	-230	-226				
TVAG_218790	conserved hypothetical protein	-230	-226				
TVAG_281070	phosphofructokinase (PPi dependent)	-231	-227			-233	-227
TVAG_340570	conserved hypothetical protein	-234	-230				
TVAG_312330	alpha-tubulin 1	-239	-235	-272	-268		
TVAG_331060	conserved hypothetical protein	-241	-237				
TVAG_136660	nucleoside diphosphate kinase family	-248	-244				
TVAG_407950	conserved hypothetical protein	-249	-245				
TVAG_043500	enolase	-250	-246				
TVAG_414510	40S ribosomal protein S3a	-251	-247				

TVAG_013020	alcohol dehydrogenase (Fe containing)	-253	-249			-255	-249
TVAG_183510	conserved hypothetical protein	-261	-257				
TVAG_390750	RAB family protein	-272	-268				
TVAG_359120	40S ribosomal protein S2	-274	-270				
TVAG_230580	pyruvate:ferredoxin oxidoreductase B1	-285	-281				
TVAG_405900	phosphoglucomutase	-286	-282				
TVAG_026110	conserved hypothetical protein	-290	-286				
TVAG_071040	conserved hypothetical protein	-294	-290				
TVAG_035180	arp2/3 complex subunit	-300	-296				
<b>-Fe</b>		<b>1. position</b>		<b>2. position</b>			
TVAG_352520	40S ribosomal protein S19	-24	-20				
TVAG_167830	conserved hypothetical protein	-25	-21				
TVAG_246920	hypothetical protein	-25	-21				
TVAG_259200	Myb-like DNA-binding domain contain	-25	-21				
TVAG_125070	conserved hypothetical protein	-27	-23				
TVAG_360540	conserved hypothetical protein	-30	-26				
TVAG_008840	conserved hypothetical protein	-31	-27	-67	-63	-33	-27
TVAG_319530	histone-lysine N-methyltransferase	-38	-34	-239	-235		
TVAG_145670	conserved hypothetical protein	-42	-38	-239	-235		
TVAG_464270	conserved hypothetical protein	-46	-42			-48	-42
TVAG_444300	conserved hypothetical protein	-47	-43				
TVAG_181910	conserved hypothetical protein	-56	-52	-226	-222	-228	-222
TVAG_006140	6-phosphogluconate dehydrogenase	-68	-64				
TVAG_165030	lactate dehydrogenase	-70	-66				
TVAG_266310	conserved hypothetical protein	-70	-66				
TVAG_259010	conserved hypothetical protein	-73	-69				
TVAG_144700	flavodoxin family protein	-76	-72	-96	-92	-98	-92
TVAG_157210	conserved hypothetical protein	-77	-73				
TVAG_405240	S-adenosyl homocysteine hydrolase	-82	-78				
TVAG_226630	conserved hypothetical protein	-84	-80				
TVAG_210320	S-adenosyl homocysteine hydrolase	-93	-89				
TVAG_090070	conserved hypothetical protein	-100	-96				
TVAG_064650	conserved hypothetical protein	-108	-104				
TVAG_383370	conserved hypothetical protein	-114	-110				
TVAG_117200	conserved hypothetical protein	-120	-116				
TVAG_300010	conserved hypothetical protein	-124	-120				
TVAG_038090	thioredoxin peroxidase	-125	-121	-236	-232		
TVAG_417070	conserved hypothetical protein	-133	-129	-254	-250	-256	-250
TVAG_411870	aldo-keto reductase family protein	-134	-130				
TVAG_059980	conserved hypothetical protein	-149	-145				
TVAG_474980	thioredoxin reductase	-151	-147				
TVAG_009450	conserved hypothetical protein	-155	-151				
TVAG_193770	Ras family protein	-155	-151				
TVAG_147860	conserved hypothetical protein	-162	-158				
TVAG_196270	tubulin beta chain	-166	-162				
TVAG_110300	conserved hypothetical protein	-169	-165				
TVAG_111510	conserved hypothetical protein	-171	-167				
TVAG_491150	conserved hypothetical protein	-178	-174			-180	-174
TVAG_046120	ankyrin repeat protein	-180	-176				
TVAG_493290	pyridoxal kinase family protein	-191	-187				
TVAG_491130	conserved hypothetical protein	-195	-191				
TVAG_196700	glutamate dehydrogenase	-197	-193				
TVAG_483750	conserved hypothetical protein	-204	-200				
TVAG_123670	conserved hypothetical protein	-211	-207				
TVAG_110950	polyadenylate-binding protein	-215	-211				
TVAG_319320	transcriptional regulator Sir2 family pro	-220	-216				

TVAG_013870	60S ribosomal protein L35	-223	-219				
TVAG_222490	conserved hypothetical protein	-234	-230				
TVAG_331060	conserved hypothetical protein	-241	-237				
TVAG_359090	tubulin beta chain	-262	-258				
TVAG_323540	conserved hypothetical protein	-274	-270			-276	-270
TVAG_070850	ankyrin repeat protein	-290	-286				
TVAG_525930	conserved hypothetical protein	-290	-286				
<b>MRE1 TAACGA - Myb 3 binding site</b>		<b>-110</b>	<b>-105</b>				
<b>+Fe</b>							
TVAG_183510	conserved hypothetical protein	-32	-27				
TVAG_072570	conserved hypothetical protein	-36	-31				
TVAG_370340	CAMK family protein kinase	-40	-35				
TVAG_000810	conserved hypothetical protein	-60	-55				
TVAG_054130	60S ribosomal protein L7	-71	-66				
TVAG_402260	coactosin	-76	-71				
TVAG_178580	alpha-amylase	-82	-77				
TVAG_440840	conserved hypothetical protein	-132	-127				
TVAG_076610	conserved hypothetical protein	-161	-156				
TVAG_072120	conserved hypothetical protein	-173	-168				
TVAG_205910	phosphoglucosyltransferase	-174	-169				
TVAG_159160	profilin	-201	-196				
TVAG_429360	WD domain containing protein	-224	-219				
TVAG_398480	chaperonin-60kD	-244	-239				
TVAG_467850	conserved hypothetical protein	-263	-258				
<b>-Fe</b>							
TVAG_402660	conserved hypothetical protein	-56	-50				
TVAG_045970	conserved hypothetical protein	-154	-148				
TVAG_491150	conserved hypothetical protein	-160	-154				
TVAG_176480	structural maintenance of chromosome	-179	-173				
TVAG_405240	S-adenosyl homocysteine hydrolase	-220	-214				
TVAG_053720	conserved hypothetical protein	-223	-217				
TVAG_222490	conserved hypothetical protein	-231	-225				
TVAG_441960	polyubiquitin	-255	-249				
TVAG_204060	conserved hypothetical protein	-289	-283				
<b>MRE2f TATCGT</b>				<b>TATCGTC</b>			
<b>+Fe</b>		<b>-59</b>	<b>-54</b>			<b>-59</b>	<b>-53</b>
TVAG_072120	conserved hypothetical protein	-19	-14			-19	-13
TVAG_455090	conserved hypothetical protein	-26	-21				
TVAG_148040	ADP-ribosylation factor 1	-28	-23				
TVAG_178680	conserved hypothetical protein	-32	-27				
TVAG_192290	conserved hypothetical protein	-34	-29			-34	-28
TVAG_422780	alcohol dehydrogenase (Fe containing)	-37	-32				
TVAG_035180	arp2/3 complex subunit, putative	-45	-40			-45	-39
TVAG_242960	PFO	-64	-59				
TVAG_212020	transketolase	-64	-59				
TVAG_271570	equilibrative nucleoside transporter, putative	-72	-67			-72	-66
TVAG_090470	actin	-82	-77				
TVAG_249200	actin	-92	-87				
TVAG_204360	malate dehydrogenase	-94	-89				
TVAG_160060	actin	-94	-89				
TVAG_218790	conserved hypothetical protein	-110	-105				
TVAG_073810	beta-tubulin	-127	-122				
TVAG_298080	TVCPT	-135	-130				
TVAG_219770	macrophage migration inhibitory factor	-162	-157				
TVAG_226870	conserved hypothetical protein	-174	-169				

TVAG_194770	conserved hypothetical protein	-178	-173			-178	-172
TVAG_200190	actin	-188	-183			-188	-182
TVAG_477640	conserved hypothetical protein	-190	-185				
TVAG_405900	phosphoglucomutase	-199	-194				
TVAG_292710	ferredoxin 4	-219	-214				
TVAG_328940	alcohol dehydrogenase (Fe containing)	-228	-223				
TVAG_424960	conserved hypothetical protein	-233	-228				
TVAG_206890	alpha-tubulin 1	-238	-233				
TVAG_126970	RAB GDP-dissociation inhibitor	-248	-243				
TVAG_569810	conserved hypothetical protein	-248	-243				
TVAG_061930	glucose-6-phosphate isomerase	-251	-246				
TVAG_259190	succinyl-CoA syntahse $\beta$ subunit	-258	-253				
TVAG_259990	calmoduline	-294	-289				
<b>-Fe</b>		<b>1.position</b>		<b>2.position</b>			
TVAG_216210	phosphotyrosyl phosphatase activator	-25	-20			-25	-19
TVAG_114310	peroxiredoxin	-27	-22				
TVAG_204390	thioredoxin	-33	-28			-33	-27
TVAG_386080	Clan MG, family M24, aminopeptidase	-35	-30				
TVAG_239660	IscS-2, cystein desulfurase	-36	-31				
TVAG_359510	conserved hypothetical protein	-38	-33				
TVAG_407170	myo-inositol-1 phosphate synthase	-46	-41				
TVAG_383370	conserved hypothetical protein	-81	-76				
TVAG_381310	lactate dehydrogenase	-83	-78				
TVAG_026290	oxysterol-binding protein	-95	-90			-95	-89
TVAG_471390	40S ribosomal protein S5	-100	-95				
TVAG_436700	glykosyl hydrolase	-101	-96				
TVAG_474980	thioredoxin reductase	-107	-102				
TVAG_496030	conserved hypothetical protein	-148	-143				
TVAG_456770	IscA2	-149	-144				
TVAG_144520	protein phosphatase-1	-155	-150				
TVAG_117200	conserved hypothetical protein	-155	-150				
TVAG_177320	conserved hypothetical protein	-160	-155				
TVAG_441960	ubiquitin, putative	-180	-175				
TVAG_209010	Clan CA, family C40, NlpC/P60 super	-189	-184				
TVAG_335250	conserved hypothetical protein	-190	-185				
TVAG_110540	polyubiquitin	-224	-219				
TVAG_157460	conserved hypothetical protein	-227	-222			-227	-221
TVAG_474660	serine/threonine protein phosphatase	-252	-247				
TVAG_174180	ubiquitin	-261	-256				
TVAG_028320	conserved hypothetical protein	-280	-275	-159	-154	-159	-153
TVAG_259010	conserved hypothetical protein	-297	-292	-176	-171	-176	-170