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Purification of specific mRNP via the nascent polypeptide
The RNA Binding Proteins ZC3H22 and ZC3H38

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A mis madres Susana y Edith

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Summary

The main determinants of the cytoplasmic fate of an mRNA are the interactions between RNA-binding proteins (RBPs) and *cis*-regulatory motifs, present in the untranslated regions (UTRs) of the mRNAs. It is expected that translating mRNAs associate with many RBPs and other proteins that are recruited but do not necessarily interact directly with the mRNA, thus forming the messenger ribonucleo-protein particles (mRNPs). In the present dissertation, a method to detect key factors involved in the regulation of gene expression in trypanosomes was tested. The aim was to affinity purify specific ribosome-associated mRNPs and detect their protein components. The purification relies on three streptavidin binding peptides (3SBPs) at the N-terminus of the nascent polypeptide. These 3SBPs connected to the actively translating mRNAs on polyribosomes will bind to the streptavidin matrix. The average yield of the reporter mRNA was 16% relative to the input polysomal fraction. The reporter was also eight-fold enriched compared to the housekeeping gene.

The method was validated using a known RNA-protein interaction in trypanosomes. A zinc finger protein, ZC3H11, binds to an AU-rich element present in the *HSP70* 3'-UTR and stabilizes this mRNA upon heat shock. Two independent purifications were made, one using a reporter containing the complete *HSP70* 3'-UTR and another without the AU-rich element, as a negative control. Indeed, ZC3H11 was detected in the purification when the AU-rich element was present in the *HSP70* 3'-UTR, and was absent from the control purification. The limitation of the method was the detection by mass spectrometry.

Furthermore, two RBPs were studied, zinc finger proteins ZC3H22 and ZC3H38. ZC3H22 was found by quantitative mass spectrometry when purifying the *EP* 3'-UTR reporter using the affinity purification method described above. This protein seems to be present in the polyribosomes but down regulation of the gene by RNAi was only achieved to 30%, the protein was still produced and no changes in the growth phenotype observed. Only a single conditional knockout was obtained but not the double knockout. The role of this protein in procyclic trypanosomes is still not discovered.

The protein ZC3H38 was previously found in our laboratory by Dr. Erben to increase target mRNA expression. My Tethering assay showed indeed, that the protein stabilized the reporter mRNA approximately 2-fold and increased 1.5-folds the amount of reporter protein produced. Analysing different parts of the protein by tethering assay also indicates that a region containing the HNPY domain might be responsible for the reporter mRNA stabilization. ZC3H38 protein is mainly localized in the cytoplasm. RNAi against *ZC3H38* mRNA presents a change in the growth phenotype after 24 hours of tetracycline induction in bloodstream trypanosomes. Currently, more experiments are being carried out in order to further characterize this protein.

Zusammenfassung

Die wichtigsten Determinanten des cytoplasmatischen Schicksals einer mRNA sind die Interaktionen zwischen RNA-bindenden Proteinen (RBPs) und *cis*-regulatorischen Motiven in den nicht-translatierten Regionen (UTR) dieser mRNA. Es wird erwartet, dass mRNAs mit vielen RBPs und anderen Proteinen, die nicht notwendigerweise direkt mit der mRNA interagieren, assoziieren, wodurch sie die Boten-ribonucleoprotein Partikel (mRNPs) formen. In der vorliegenden Dissertation wurde eine Methode entwickelt, um Regulatoren der Genexpression in Trypanosomen zu identifizieren. Das Ziel war die Affinitätsreinigung spezifischer Ribosomen-assoziierter mRNPs sowie die Detektion ihrer Proteinkomponenten. Die Aufreinigung beruht auf drei Streptavidin-bindenden Peptiden (3SBPs) am N-terminus des naszenten Polypeptids. Diese 3SBPs, die mit der aktiv translatierenden polyribosomalen mRNA verbunden sind, binden an eine Streptavidin-Matrix. Die durchschnittliche Ausbeute an Reporter-mRNA lag bei 16% relativ zum anfänglich eingesetzten Polyribosomen-Material. Die Reporter-mRNA war um ein achtfaches angereichert verglichen mit einem Haushaltsgen.

Die Methode wurde validiert mithilfe einer bekannten RNA-Protein Interaktion in Trypanosomen. Ein zinc finger Protein, ZC3H11, bindet ein AU-reiches Element in der 3'-UTR der *HSP70* mRNA und stabilisiert diese unter Hitzeschock-Bedingungen. Zwei unabhängige Aufreinigungen wurden durchgeführt, eine mit einem Reporter, der die komplette *HSP70 3'-UTR* enthält, und die andere, ohne das AU-reiche Element, als Kontrolle. Tatsächlich wurde ZC3H11 nur detektiert, wenn das AU-reiche Element vorhanden war. Eine Einschränkung der Methode war allerdings die Identifikation von Peptiden mittels Massenspektrometrie.

Überdies, wurden zwei RBPs untersucht, die zinc finger Proteine ZC3H22 und ZC3H38. ZC3H22 wurde mittels quantitativer Massenspektrometrie bei einer Affinitätsaufreinigung der *EP 3'-UTR* Reporter-mRNA identifiziert. Dieses Protein scheint mit Polyribosomen zu assoziieren aber es konnte nur eine 30-prozentige Verringerung der Expression mittels RNAi erreicht werden, das Protein wurde immer noch produziert und keine Veränderung im Wachstumsphänotyp war zu beobachten. Lediglich für ein Allel des Gens konnte ein konditionales Knockout erzeugt werden. Die Rolle dieses Proteins in procyclischen Trypanosomen ist weiterhin unklar.

Bereits zuvor wurde in unserem Labor von Dr. Erben entdeckt, dass das Protein ZC3H38 die Expression einer Ziel-mRNA steigert. Mein tethering-Experiment zeigte dass das Protein die Stabilität einer Reporter-mRNA etwa verdoppelt und die Menge an produziertem Reporter-Protein um ein 1.5-faches erhöht. Die Analyse verschiedener Domänen des Proteins mittels tethering-Assay deutet darauf hin, dass eine Region, die eine HNPY-Domäne enthält, für die Stabilisierung der Reporter-mRNA verantwortlicht ist. ZC3H38 ist ein zytoplasmatisches Protein. RNAi gegen ZC3H38 führt 24 Stunden nach Induktion zu verlangsamtem Wachstum von Blutstromform Trypanosomen. Im Moment werden weitere Experimente durchgeführt, um dieses Protein eingehender zu charakterisieren.

1. Introduction

Trypanosoma brucei, the protozoan pathogen used as model organism in the present work, belongs, among others such as *Trypanosoma cruzi* and *Leishmania species*, to the family *Trypanosomatidae*, order *Kinetoplastida*. *T. brucei* is the causative agent of the neglected tropical disease Human African Trypanosomiasis, also called sleeping sickness [1]. Two subspecies of trypanosomes are infective to mammals. *T. brucei rhodesiense* is endemic in east and southern Africa, infects game animals, cattle and humans and is responsible for producing the more severe and acute disease. In cattle the disease is called Nagana and is caused mainly by *T. congolense* and *T. vivax*. *T.brucei gambiense*, found in west and central Africa, infects humans and a wide range of animals, mostly pigs; leading to the chronic disease that can persist for months or years without major symptoms [2, 3].

African trypanosomes are introduced to the host bloodstream by the bite of infected Tsetse flies of the genus *Glossina*. After the Tsetse fly had a blood meal, the parasite establishes itself in the mid gut of the insect where it expresses the EP/GPEET proline-rich surface proteins. After this, procyclic trypanosomes migrate to the salivary glands, where epimastigotes expressed the brucei alanine-rich proteins (BARP), a stage specific glycosylphosphatidyl inositol-anchored proteins [4]. Later on, the metacyclics change the surface protein for the variant surface glycoprotein (VSG) and are ready to be transmitted to the human host [5]. In the bloodstream of the host, the parasite evades the immune response by antigenic variation, changing its VSG coat in order to avoid antibody recognition. The parasites proliferate in the bloodstream as slender forms and change to stumpy forms, cell cycle arrested forms, when the levels of parasitemia increase (Figure 1) [5-7].

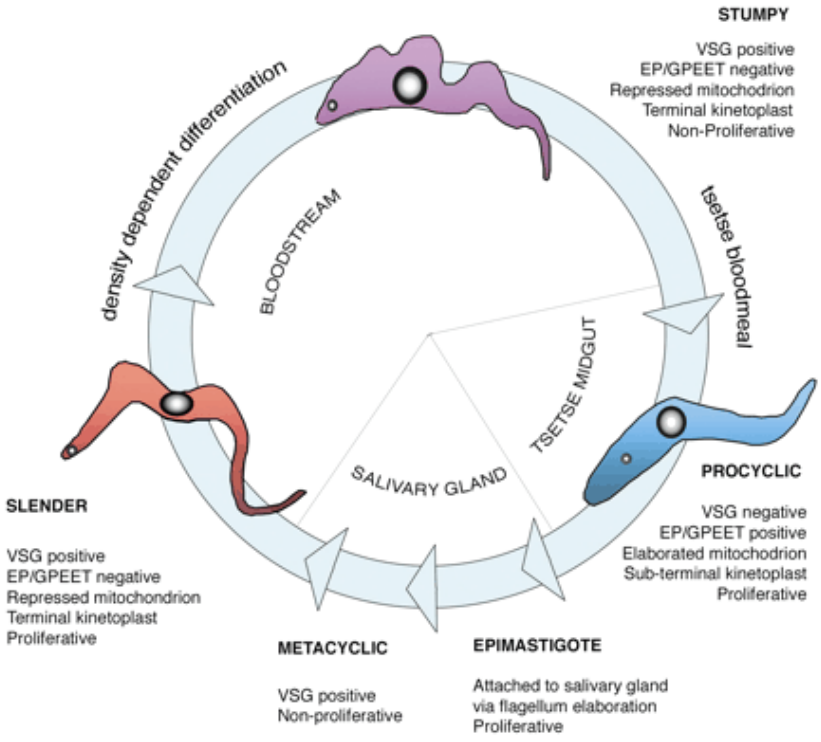


Figure 1. Life cycle of *Trypanosoma brucei*
The figure shows the two major replicative stages of trypanosomes, the bloodstream form trypomastigote, that proliferates in the blood and mammalian tissue fluids and the procyclic form, found in the midgut and salivary glands of Tsetse flies (trypomastigotes, epimastigotes and metacyclic forms). This picture was extracted from reference [5].

Due to the different trypanosome habitats, the mammalian bloodstream, the Tsetse midgut and the salivary gland of Tstse flies, this parasitic protozoa must be able to adapt to the environment. For instance, morphological and biochemical changes must happen in order to evade the mammalian immune response or to program and trigger differentiation. To perform these changes, a stringent developmental program and control of gene expression are required.

1.1 Trypanosome gene arrangement and expression

The unicellular eukaryotic parasite *T. brucei* contains a two unit genome, a nuclear and a mitochondrial (Kinetoplast) one. The total haploid genome has 35 megabases and contains approximately 8100 protein-coding genes. The nuclear genome is distributed over 11 diploid chromosomes (0.9-5.7 Mb), intermediate (300-900 Kb) and more than 100 minichromosomes (50-100 Kb) [8, 9].

One characteristic feature of the trypanosome genome is the organization of genes in large transcription units [9-11]. These units can contain several (100-200) open reading frames (ORFs) and are transcribed into polycistronic mRNA precursors [12-14], which are later processed into mature monocistronic mRNAs by *trans*-splicing and polyadenylation (Figure 2) [15]. The *trans*-splicing reaction consists of the addition of a 39 nucleotide spliced leader sequence [8] to an AG dinucleotide downstream of a polypyrimidine tract [15, 16] and is coupled to the addition of a poly(A) tail to the upstream gene [17, 18].

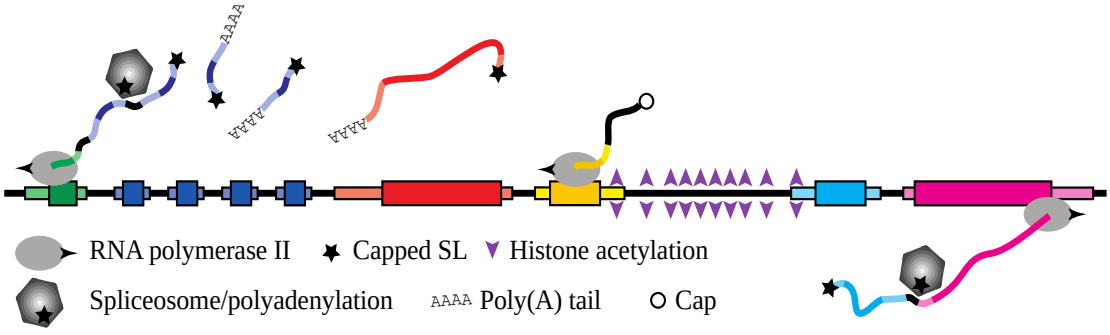


Figure 2. Gene distribution scheme in trypanosomes.
Trypanosomal genes are transcribed in multiple protein coding genes and individual mRNAs are generated by coupled trans-splicing and polyadenylation reaction. The genes that belong to a polycistronic unit do not necessarily have a similar function and transcription can start in each due to epigenetic marks, such as histone acetylation. Each mRNA contains a cap structure called the spliced leader [8]. This picture was kindly provided by C. Clayton (unpublished)

The spliced leader [8] was found first at the 5'-end of the *VSG* mRNA. Interestingly, the splice leader it is not encoded in the VSG polycistron [19, 20]. The precursor of the SL has 141nt and is called *SLRNA* or mini-exon derived RNA, its genes are arranged in tandem and individually transcribed from a well characterized RNA polymerase II (RNApol II) promoter [1, 21-23]. The *SLRNA* is composed of a 39nt SL sequence located upstream of an intron that can fold in two-stem loops (sl II and sl III) and a single stranded region that connects these loops. The SL RNA contains an unusual cap-structure (named cap4), where the first 4 nucleotides after the 7-methylguanosine (m^7G) are methylated [24-26].

In *T. brucei trans*-splicing is a universal mechanism, whereas *cis*-splicing is rare. Polyadenylation occurs 200–500 nucleotides upstream of the SL addition site [27-29]. Although, there are some examples of *cis*-splicing in *T. brucei,* so far only four genes were reported to contain introns and undergo *cis*-splicing in trypanosomatids, these are the poly (A) polymerase genes of *T. brucei* and *T. cruzi* [30] the ATP-dependent DEAD/H RNA helicase, and two conserved hypothetical RNA binding proteins [8, 31].

Only few promoters have been described in trypanosomes that are regulated transcriptionally. Among the genes that presented this type of regulation are genes encoding the major surface glycoproteins of the parasite, the EPs and GPEETs in procyclic forms of the parasite and the bloodstream-form variant surface glycoproteins (VSGs). Genes in these clusters as well as the 18S, 5.8S and 28S rDNA units are transcribed by RNA polymerase I [32-35].

In trypanosomatids most of the mRNAs are transcribed by RNA polymerase II (RNA pol II), which also transcribes the *SLRNA* gene [28]. Although the SL RNA promoter has been well characterized [28, 36] there is a lack of evidence of RNA pol II regulation for protein coding genes. No promoters for this polymerase have been identified [34, 37]. Experimental evidence suggests that transcription by RNA pol II seems to initiate usually bi-directionally and in between two gene clusters (strand-switch regions), diverting or converging from the ends of the chromosome [29]. Transcription usually terminates by the presence of distinct chromatin modifications, such as histone modifications (acetylation and methylation) and histone variations [38].

Due to the fact that the trypanosome genome contains few transcription factors and that the regulation of transcription by RNA pol II is almost absent, the trypanosomes render the regulation of gene expression to the post transcriptional level [34]. Furthermore, tandem arranged genes are transcribed constitutively by RNA pol II. These genes encode usually very abundant proteins such as the heat shock protein 70 (HSP70), cytoskeletal proteins (for instance α-tubulin and β-tubulin) and histones [39-41]. Interestingly, with the exception of the genes mentioned above, genes present in a polycistronic unit usually do not have the same regulatory mechanisms and their proteins do not share a similar function.

RNA polymerase III transcribes tRNAs, 5S RNA, the 7SL RNA (the RNA component of the signal recognition particle) and also most *trans*-spliceosomal uridyl acid-rich small nuclear RNAs (U snRNAs), such as the U6 snRNA and the U-snRNA B (U3 homolog), which are involved in the processing of rRNAs [42, 43]. The transcription of these snRNAs and of the 7SL RNA depends on regulatory A and B box *cis*-elements (promoter elements) present on a tRNA gene that is located upstream and oriented oppositely to these genes [44, 45].

1.2 Regulation of gene expression in trypanosomatids

In order to progress through the different life-cycle stages, trypanosomes must regulate their gene expression, to control mRNA expression and protein abundance. Due to the polycistronic gene distribution of trypanosomatids, the individual regulation of genes is not possible. Therefore, trypanosomes used the combinatory effects of *trans*-actiing factors and *cis*-regulatory motifs for control of mRNA export, localization, and degradation as well as protein translation, modification and stability.

1.2.1 mRNA degradation in trypanosomatids

The degradation of mRNA is one of the ways to regulate gene expression in trypanosomes and it has been widely studied [46, 47]. The average half-life of bloodstream form mRNAs has been estimated to be 12 min and 20 min in procyclics [48], long stable mRNAs can exhibit half-lives of 4 hours and the very unstable ones approximately 10 min. Degradation of an mRNA starts with the removal of the poly(A) tail by the CAF1/NOT complex [49-51].

The major deadenylase for trypanosomes is CAF1. When performing RNAi against this protein, increase on the average lengths of poly(A) tails was observed, causing a delay in the decay of constitutively expressed mRNAs [52]. The removal of the poly(A) tail prevents the binding of the poly(A) binding protein (PABP) and exposes the mRNA to be degraded by other enzymes and complexes, as is the case for the degradation mediated by the 5'-exoribonuclease XRNA. This type of degradation immediately stops translation initiation. Interestingly, the 5'-3' exoribonuclease, XRNA is also involved in the degradation of developmentally regulated mRNAs, some of these unstable mRNAs are decapped and degraded by this exoribonuclease without previous removal of their poly(A) tails [51].

Once the poly(A) tail is removed by the CAF1/NOT complex, the 3'-end of the mRNA is also available for degradation by the RNA exosome protein complex. The *T. brucei* exosome is a 3' to 5' exonucleases complex and its components are similar to the yeast exosome [53]. It is involved in the maturation of 5.8S rRNA in trypanosomes [53, 54] and in the degradation of stage-specific mRNAs that contain U-rich elements (UREs) in their 3'-UTRs. These encoding the major surface protein in procyclic trypanosomes *EP1*, the cytosolic phosphoglycerate kinase *PGKB* and the pyruvate phosphate dikinase *PPDK* in bloodstream form trypanosomes [53, 54].

1.2.2 Developmental regulation in trypanosomatids

The differentiation from bloodstream trypanosomes to procyclic forms, involves the loss of the VSG in bloodstreams and the acquisition of the major surface protein in procyclics, the EP procyclin protein [55, 56]. The procyclin gene has four different isoforms, the highly phosphorylated GPEET and the EP1, EP2 and EP3 which contain glutamic acid-proline dipeptide repeats [57, 58]. EP1 and EP3 contain specific glycosylated residues whereas the EP2 is not modified by N-linked carbohydrates [57]. After 24 hours of triggering differentiation in media containing glycerol, the cells differentiate as early procyclic, expressing GPEET in high levels and traces of EP [59]. If glycerol is not present in the media, the cells proliferate to late phase procyclics in 7 to 9 days, replacing GPEET by EP1 and EP3 [60, 61]. The repression of GPEET is due to the glycerol responsive element, a 25mer element, present on its 3'-UTR [60, 62]. These changes happen in culture as well as in the insect vector [63].

Stage-specific transcripts, such as the *EP* procyclin, are unstable and have short half-lifes specifically in the life-cycle stages that have no use for them (*EP* half-life in bloodstreams is 5 min), but are stable in the stage that requires them (*EP* half-life 30 min in procyclics). The *EP* mRNA is 11-fold more abundant in the procyclic than in bloodstream forms and translation of the EP protein is repressed approximately 10-fold in the mammalian form trypanosome compared to the insect form [64, 65].
The *EP* 3'-UTR contains U-rich elements (UREs) and forms 3 domains (Figure 3), two of stable domains (I and III) and domain II [65, 66]. Domain I is present in the first 40bp of the EP 3'-UTR, contains sequences that decrease translation in procyclics but is not necessary for developmental regulation [64, 65, 67]. The central domain (domain II) contains a 26mer regulatory element that is used in bloodstreams trypanosomes for the rapid degradation and translation repression of the *EP* mRNA [64, 65, 67]. It has also been shown that domain II interactions are very unstable, no stable interactions within itself or other parts of the 3'-UTR were determined by RNase digestion or lead ion hydrolysis [66]. The 3'-most domain, domain III is a conserved stem-loop of 16mer that stabilizes the *EP* mRNA [64, 67]. This element is necessary for the adequate expression of a reporter gene (CAT reporter) and upon deletion of this element, the expression of the reporter protein decreased 10- to 12-fold, without a change in the reporter mRNA levels; therefore, this element has an effect in translation. Furthermore, not only the sequence but also the secondary structure of the 16mer are necessary for its effects [68].

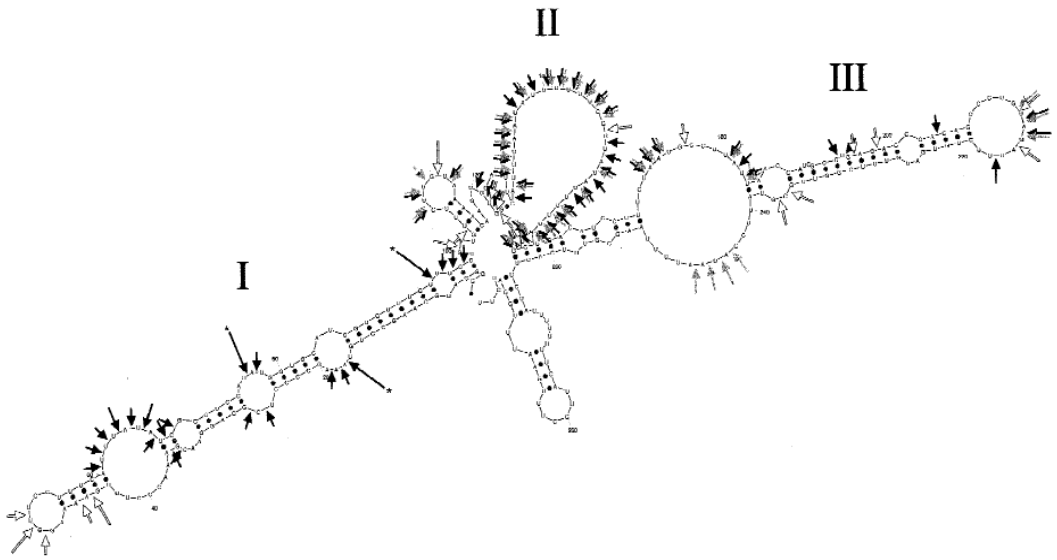


Figure 3. Predicted secondary structure of the *EP1* 3'-UTR
Predicted structure showing the three domains (I to III). Domains I and III are more stable and form hairpins with internal loops. Digestion with RNase T1 and T2 are shown as white and grey arrows respectively. Lead ions promote the cleavage of unstructured RNA. Therefore to further determine the structure of domain II, lead ions were used to hydrolyse this RNA. Black arrows show the sites of lead hydrolysis, the black ones with an asterisk at the end are sites of strong catalytic cleavage also produced by lead ions. This picture was extracted from reference [66].

Post-transcriptional regulation of gene expression is not as simple as one mRNA interacting with a specific set of RNA binding proteins (RBPs). Genome-wide screens indicate that distinct groups of RNAs can interact with several RBPs and that different RBPs can compete for the target mRNA; these dynamic interactions regulate the fate of mRNAs at different levels, such as export, localization, degradation, stability and translation; increasing the complexity of post-transcriptional regulation [69-71].

1.3 RNA binding proteins

The RNA binding proteins (RBPs) are key regulatory factors that can interact not only with sequences present on target mRNAs but also interact with other proteins that help them determine the fate of their target mRNAs [72]. For instance, if an RBP interacts with or is able to recruit XRNA, a member of the degradation machinery, then this mRNA will undergo 5′-3′ degradation; or if another RBP is able to recruit the CAF1/NOT complex, then the target mRNA will be deadenylated and rapidly degraded. In an opposite case, if a *trans*-acting factor binds to the PABP, this will stabilize its target mRNAs and if it interacts with translation factors then translation will be increased. Furthermore, regulatory proteins can also render their effects by interfering with the binding of proteins that enhance degradation or translation.

The RBPs are usually classified according to their structural domains, which allow the binding to the mRNA [73]. For instance, proteins containing CCCH

zinc finger domains (zinc finger proteins ZFPs), RNA recognition domains (RRM proteins), PUF proteins and K homology domain (KH proteins, two predicted in *T.brucei* genome) among others, such as the arginine glycine rich motif (RGG proteins) and the cold shock domain (CSD proteins).

More than 125 proteins containing RNA binding domains are encoded in the trypanosome genome. A genome-wide screen in order to find potential mRNA regulators using a tethering system, performed in our laboratory, identified 300 proteins that can be involved in the regulation of mRNA; among these proteins 39 were RBPs, 16 were shown to inhibit mRNA expression and 23 were implicated in the stabilization of the reporter mRNAs [74]. Complete information about and characterization of most of these regulatory proteins is still missing. In the pages below, a summary of what is known about relevant proteins for this work is provided.

1.3.1 The RRM Proteins

Proteins that contain an RNA recognition motif (RRM) are among the most abundant in eukaryotes and have a great diversity of functions, mainly due to the presence and structure of the RRM motif [75]. The RRM motif is 90 amino-acid long, containing a RNP1 (K/R)G(F/Y)(G/A)FVX(F/Y) octapeptide and a RNP2 sequence-motif [76]. The structure of this motif contains two α-helixes and a four-stranded β-sheet (βαββαβ topology) [75]. The β-sheet structures, composed of the RNP1 and RNP2 motifs, are directly responsible for RNA binding; they can bind single stranded RNA (2-8 nucleotides) as well as other RRM proteins [77, 78].

In trypanosomes approximately 75 RRM proteins were identified [76]. An example of RRM proteins that perform different functions is the case of the polypyrimidine track binding proteins (PTBs) DRBD3/PTB1 (two RRM domains) and DRBD4/PTB2 (4 RRM domains), which can bind directly to the mRNA and are involved in the stabilization of their target mRNAs [79, 80], as well as in translation initiation [81], processing of the 3'-end [82] and splicing regulation [83, 84].

The most known RRM proteins involved in translation are the poly(A) binding proteins PABP1 and PABP2, in charge of stabilizing the mRNA via binding to the poly(A) tail and interaction with the translation initiation complex [85]. There are many RRM proteins involved in a variety of functions such as splicing regulators, like U2 snRNP (UAF2), an heterodimer involved in pre-mRNA processing [78] and RBP24, a component of the spliceosome [86]. Other RRM proteins are nuclear processors, for instance NRBD1 and NRBD2 involved in the import and assembly of rRNAs [87, 88]. There are also proteins that might be involved in the regulation of mRNA turnover like UBP1, UBP2 and RBP3, which contain a single RRM domain [76].

1.3.2 Pumilio-Domain Proteins

The Pumilio-Fem3 proteins (PUF proteins) are composed of multiple copies of a tri-helical PUF repeat, each of which binds to a nucleotide sequence

(consensus UGUA) in the mRNA via three amino-acid residues [89, 90]. The mRNA targets of PUF proteins are often functionally related. The T. brucei genome encodes for 11 PUF proteins that are involved in very different aspects of mRNA metabolism, playing key roles in the degradation as well as in the stabilization of mRNAs. For instance PUF9, composed of 6 copies of the tri-helical PUF repeat acts in the mid-to-late S-phase of cell cycle neutralizing a destabilization motif on its target mRNAs. PUF9 target mRNAs are a kDNA ligase, histone H4 and two unknown proteins named PUF nine target 1 and 2 (PNT1 and PNT2) [91]. It could be that some of these mRNAs encode proteins that function in the S-phase of the cell cycle and therefore their regulation by PUF9 is essential. In contrast, other pumilio-domain proteins like PUF2 may be involved in targeting a subset of mRNAs to degradation (results of tethering experiments) [92]. Other proteins such as PUF7 and PUF8 localize in the nucleolus and are involved in rRNA processing [93].

1.3.3 The Zinc Finger Proteins

The CCCH zinc finger domain proteins are composed of a well conserved cysteine-histidine motif, Cx_{4-15}-Cx_{4-6}-Cx_3-H which is often present in two copies. These proteins bind with higher affinity to single-stranded RNA and are involved in most processes of mRNA metabolism [94, 95]. *T. brucei* genome encodes for 49 CCCH proteins that can be divided in two families, the cell cycle sequence binding proteins (CSBP) and the ZFP proteins [96].
The CSBPs that were identified first in *Crithidia fasciculata*, CSBPA (*T. brucei* ZC3H39) and CSBPB (*T. brucei* ZC3H40), bind to their target mRNAs and regulate them during S-phase of the cell cycle. The target mRNAs reported so far are the dihydrofolate reductase-thymidylate synthase DHFR-TS, the kinetoplast associated type II DNA topoisomerase (TOP2), the large sub-unit of the nuclear replication protein-A (RPA1) and the kinetoplast histone H1-like DNA binding protein KAP3 [97]. In the case of *TOP2* and *RPA1* mRNAs, the sequences responsible for the CSBPA dimer binding were found in the 5'-UTR, whereas for *KAP3* in the 3'-UTR. The motifs present in the UTRs were two or more octamers (CAUAGAAG or similar) that are necessary but not always sufficient for the cycling of these transcripts [98, 99].

The ZFP proteins containing only one CCCH domain involved in *T. brucei* differentiation are ZFP1, ZFP2 and ZFP3. ZFP1 contains two additional proline-rich domains and is up-regulated during differentiation [100, 101]. ZFP2 has a WW domain and is able to bind to proline-rich regions. Down-regulation of this protein delays the expression of EP procyclin [101-103]. ZFP3 also contains a WW domain and possesses additionally an RGG motif that is involved in the assembly of RNP complexes. ZFP3 binds to two cis-regulatory elements present in loop II of the *EP1* and *GPEET* mRNAs via its CCCH domain and acts as a translation regulator [104, 105].

The zinc finger protein ZC3H20 is necessary for procyclic forms to grow and stabilizes two developmentally regulated mRNAs, the mitochondrial carrier protein (MCP12) and trans-sialidase (TS-like E) [106].

Procyclic trypanosomes grow in normal culture conditions at 27°C and heat shock response can be induced by 37 to 41°C. The protein ZC3H11 is part of the heat shock response for both life cycle stages. This protein is essential in bloodstream forms and is necessary to maintain appropriate levels of chaperone mRNAs (such as *HSP70* mRNA) during heat shock in procyclic forms. ZC3H11 recognizes AU-rich elements present in the 3'-UTRs of its target mRNAs. It has been proposed that this protein acts as a dimer and that phosphorylation could be implicated on its function. The protein also binds other protein transcripts involved in the heat shock response, such as *HSP83*, *HSP100* and *HSP110* as well as transcripts necessary for stress recovery [107]. In order to stabilize transcripts during heat shock ZC3H11 binds to other regulatory proteins, such as MKT1 and poly(A) binding protein-binding protein (PBP1), these interactions are necessary for the stabilization of its target mRNAs and for the heat shock response in *T. brucei*.
The proposed mechanism is as follows, the N-terminal part of ZC3H11, where the CCCH domain is, binds to the mRNA and the C-terminal part interacts with MKT1 and PBP1. Then PBP1 recruits other proteins such as LSM12 and PABP1or PABP2. This last protein binds to the poly(A) tail of the mRNA and to the initiation factor eIF4G making the mRNP stable [108].

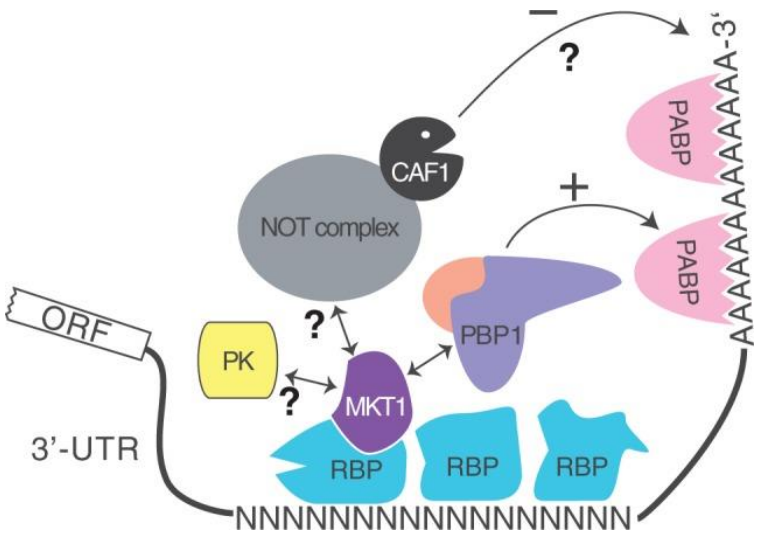


Figure 4. Proposed model of mRNA stabilization
This mechanism for mRNA stabilization involves an RBP (in blue) that can be ZC3H11 recognizing its target mRNA (*HSP70*) via a specific *cis*-regulatory motif (UAUU for ZC3H11). The interactions between ZC3H11 (in blue), MKT1 (purple), PBP1 and PABP stabilize this mRNP. This stabilization is only achieved by the combinatory effects of all these regulatory proteins. The model is dynamic because it shows the possibility to have interactions with proteins of the degradation machinery, such as CAF1/NOT complex, that can target the mRNA for degradation. This picture belongs to reference [108].

In the present work, the interaction between ZC3H11 and the AU-rich element present in the 3'-UTR of *HSP70* mRNA was used as proof of concept for my method.

1.4 Searching for transcriptional regulators in *Trypanosome brucei*

The function of RBPs can be assessed by a genome-wide tethering screen [74]. In this method target RNA bearing the 19 nucleotide boxB sequence is co-transfected with the protein of interest fused to the lambda N peptide (λN-peptide). Due to the presence of the boxB, the tagged protein binds to the reporter. This way the potential effect of the tethered protein on bound mRNA can be measured [109].

The aim of the genome-wide tethering screen was to identify post-transcriptional regulators in *T. brucei*. Using random fragments of the *T. brucei* genome, a library of fusion proteins was generated. Since there is a lack of introns in trypanosomes, one can expect that one of twelve plasmids cloned encoded a fusion protein in frame with the λN-peptide. This method has two steps, one is the generation of the fusion proteins and the other one is the creation of the tethering reporters, both have to be transfected in the same cell lines [74].
The fusion proteins consist of the λN-peptide at the N-terminus of the genomic fragment and a myc-tag at the C-terminus. The expression of the fusion proteins is induced upon tetracycline addition, whereas the tethering reporters are transcribed constitutively [74].
Three different kinds of reporters were generated (Figure 5).

1. To identify proteins that increase expression upon tethering. The reporter contains a GFP and blasticidin resistance (BLA) gene, both ORFs have a 5x boxB element on their 3'-ends and are transcribed as a single transcript.
2. The control reporter consists of a GFP ORF upstream of the 5x boxB element and a BLA gene without the downstream 5x boxB element.
3. To detect proteins that decrease expression. The reporter has a PGKB ORF with a 5x boxB element on its 3'-end and also the BLA construct (without boxB). Both ORFs are separated by independent promoters, meaning that two transcripts will be generated.

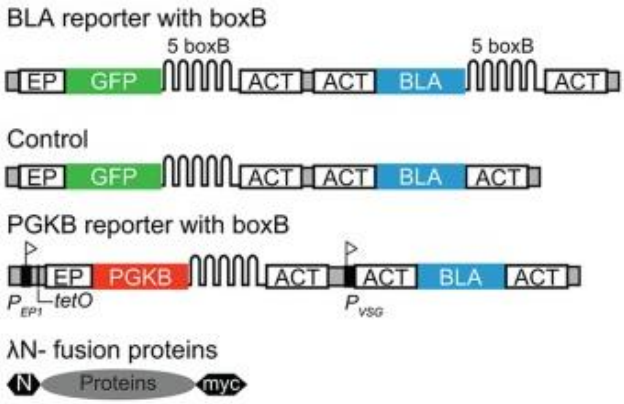


Figure 5. Genome-wide tethering screen
This approach searches for regulators of the mRNA fate in the model organism *Trypanosoma brucei*. The figure shows a schematic representation of the reporters used in this screen. This picture was taken form reference [74].

The blasticidin library: To detect possible expression repressors, cells were grown in 1x-2x blasticidin and to identify expression activator candidates, cells were grown in increasing concentrations of blasticidin (6x-20x). The cells that recover express proteins, which might be associated with the increase of the *BLA* reporter or enhanced translation [74].
It has previously been shown that inducible expression of cytosolic phosphoglycerate kinase B (*PGKB*) is lethal in bloodstream trypanosomes [110]. Therefore the *PGKB* construct contains an inducible promoter in order to regulate its expression. Using the PGKB library, one can detect repressors of expression, because these proteins will impair *PGKB* expression and allow the cells to grow [74].
The genomic fragments (cloned as fusion proteins) were extracted from the cells and PCR amplified, using specific primers in the λN-peptide and at the 3'-end of the cloning site and send for high-throughput sequencing.
This method allowed the confirmation of regulatory proteins that determine the fate of mRNAs. For instance, eIF4E1, ZC3H13, RBP9 and RBP12 that reduce mRNA stability, on the other hand, proteins such as ZC3H37, PUF9 and eIF4G4 that are implicated in mRNA stabilization. Furthermore, it also identified several proteins that have not been associated to mRNA regulation [74].

1.5 Methods to characterize the composition of mRNPs

There have been many approaches previously reported that tried to identify the composition of messenger ribonucleoprotein particles (mRNPs). In a general way, one can divide them in two main groups, the approaches designed to identify the RNA components of the mRNPs (CLIP and variants) and the ones that try to detect the protein components.

1.5.1 Methods to identify the RNA components of mRNPs.

1.5.1.1 Early methods

The study of mRNP composition started with biochemical *in vitro* approaches, for instance, proteins that bind to specific sequences were detected by electrophoretic mobility shift assay (EMSA, also called gel shift assay) [111]. Formaldehyde or UV cross-linking (at 254nm) of direct RNA and protein interactions represented an alternative for *in vivo* studies. In some cases the complexes were cross-linked and immune-purified using epitope-tags [112, 113]. In other cases, the mRNPs were subjected to two rounds of purifications, for instance, first using oligo (dT)-cellulose and then with antibodies raised against a protein component of the mRNP [114].

1.5.1.2 RNA immunoprecipitation on chip (RIP-chip)

Some approaches used the purification of a specific RBP in order to identify its potential targets using microarrays. This is achieved by tagging the RBP of interest or raising antibodies against it, that will immunoprecipitate the target RNAs, and subsequently labelling the samples and hybridizing them to DNA microarrays [115]. Alternatively, protein microarrays containing the complete proteome of the organism of interest can be hybridized with different RNAs (total RNA labelled with Cy3 and oligo-dT purified RNA labelled with Cy5) [116]. This method is limited to the use of stable RNPs for the purification and the site of interaction in the RNA was unresolved.

1.5.1.3 Cross-linking and immunoprecipitation (CLIP)

The method is used to map RNA-protein interactions *in vivo*, for example it can be used to discover new binding sites for a specific RBP, to determine small RNA-RNA interactions, for miRNA target detection etc.
The samples used for CLIP, which can be organisms or cells, are irradiated with UV at 254nm to covalently cross-linked RNA-protein complexes (Figure 6 #1). Once cross-link, the RNA is partially digested (using RNases) into fragments between 30-50 nucleotides (Figure 6 #2). Later on, the complexes are purified by immunoprecipitation of the known RBP. After stringent washings the 3'-end of the RNAs is dephosphorylated and ligated to an adaptor (Figure 6 #3-4). Then the RNAs are radioactively-labelled at the 5'-end (Figure 6 #5). The non-covalently associated RNA is removed using denaturing gel electrophoresis and nitrocellulose membrane transfer. The membrane is exposed to an X-ray film where the radioactively labelled RNA determines the position of the complex on the membrane (Figure 6 #6). After the complex is excised from the membrane, it is treated with proteinase K, which degrades the protein components, leaving only a small peptide at the cross-linked site (Figure 6 #7). In the next step, an adaptor is added to the 5'-end of the purified RNA (Figure 6 #8) and the products are amplified by reverse transcription (RT-PCR) using primers complementary to the linkers (Figure 6 #9). Then the cDNAs of individual clones are send to Sanger sequencing. In the final step the sequences obtained are mapped to the reference genome (Figure 6) [117-119].

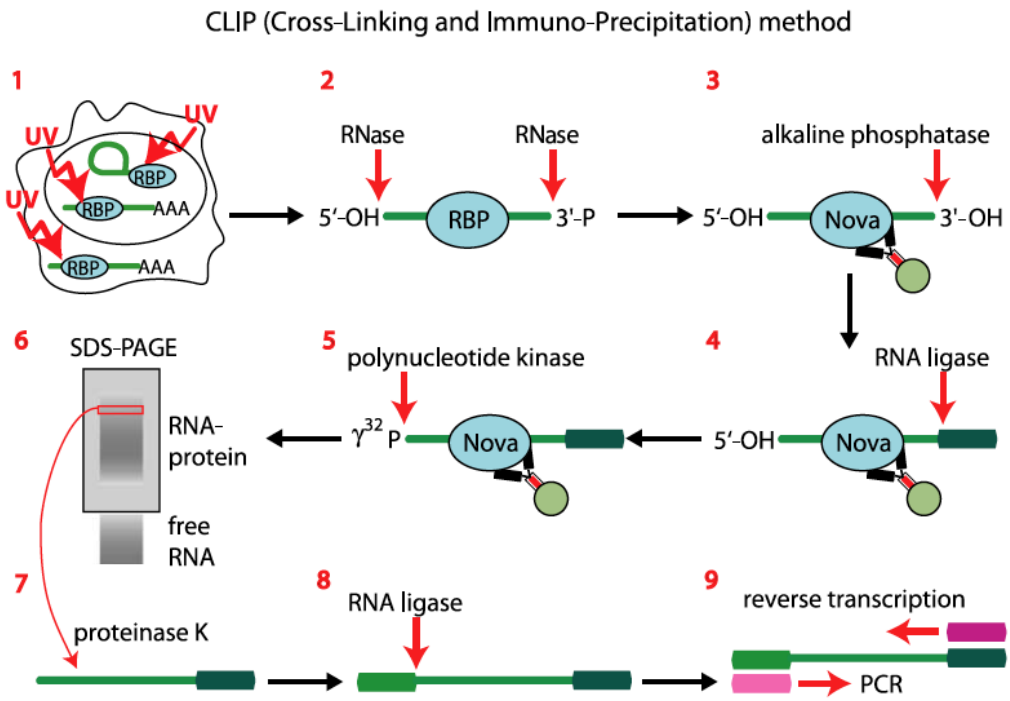


Figure 6. Cross-linking and Immunoprecipitaion method
The figure provides a general view of the principle used by CLIP. RNA and protein complexes are cross-linked (UV or formaldehyde). The cell lysate is then digested with RNases before the immunoprecipitation of RBPs. The next step is to gel purify the complexes and treat them with Proteinase K in order to obtain only the RNA component. After the RNA linker ligation RT-PCR is performed and the cDNA samples are used for microarray analysis or sent for RNA sequencing. This figure was taken from reference [118].

1.5.1.4 High-throughput methods

1.5.1.4.1 Affinity purification tags and high-throughput analysis

Tags are widely used in the purification of mRNPs. For instance, the tandem affinity purification (TAP-tagging), where two different tags are used to purify a known RBP (two-step purification). This purification allows the determination of the proteins that co-purify with the tagged protein. Some approaches used the TAP purification in combination with high-throughput sequencing to determine the target mRNAs of the purified RBP. These purifications have contributed to the characterization of mRNPs. In yeast for example, TAP-tagging of the poly(A) binding protein Nab2 revealed the co-purification of mRNA-export factors as well as the interaction of the tagged protein with different classes of mRNAs [120].

An alternative method to purify mRNPs is by ribosome affinity purification [121] [121]. This method is base on tagging a ribosomal protein and purify the complexes bound to it. RAP can be done with different tags. For instance using the protein A and the tobacco-etch virus protease tag (A-TEV-tag). The

cells are lysed and the tagged-ribosomal protein is affinity purified via the binding of protein A to the IgG matrix. The mRNP complexes are later released by cleavage with TEV protease. The mRNA is later on purified and the translatome assessed by microarrays or RNA sequencing. RAP allows the study of translational regulation in different conditions without the use of sucrose gradient centrifugation [122].

1.5.1.4.2 Cross-linking and immunoprecipitation (CLIP) with high-throughput analysis and its variants

The main problem with CLIP was that this method required specific antibodies for the purification. As an alternative, the cross-linking and analysis of cDNAs (CRAC) approach was designed. This method relies on the generation of a His_6-Tev-Protein A tag (HTP-tag). The HTP tag is similar to the TAP-tag but the calmodulin binding peptide (CBP) is replaced by a six histidine tag (Figure 7B) [123].

In the CRAC strategy, the RNA-protein complexes are UV cross-linked at 254nm (like in CLIP) followed by a first purification step using IgG beads and a partial digestion with RNases. The second step uses a denaturing affinity purification on a nickel matrix that binds the His_6 part of the HTP-tag. Then the linkers are added to the RNA and the product eluted with imidazole. This eluate is run in a gel and transferred to a nitrocellulose membrane. After proteinase K digestion the RNAs are purified, amplified by RT-PCR and sequenced (Figure 7) [123]. The main difference of this method when comparing to CLIP is the cross-linking strategy and the specific use of the His_6-tag. Using the CRAC method pinpoint protein-RNA interactions can be assessed *in vivo* as well as *in-vitro*. To map precisely cross-linking sites this method used Sanger sequencing of multiple cloned fragments as well as high-throughput RNASeq.

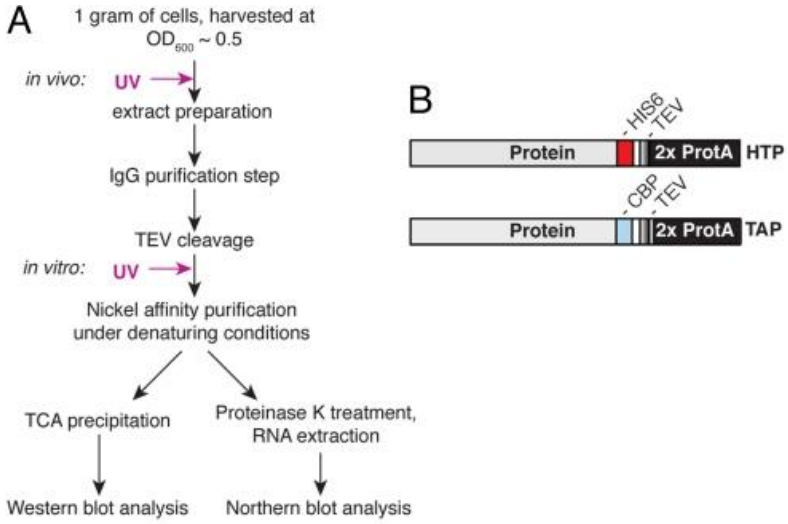


Figure 7. Schematic representation of CRAC method
This method is based on CLIP but it was developed in order to purify complexes via a His_6-Tev-Protein A tag (HTP-tag) in two steps.

A. Flow of the method: The samples are UV cross-linked and then purified on an IgG matrix. After partial RNase digestion the second purification is done (nickel beads), then the adaptors are incorporated and the RNA amplified by RT-PCR and sent for sequencing.
B. Schematic representation comparing the HTP-tag and the TAP-tag. The main difference is that the in the HTP-tag the calmodulin binding protein (CBP) is replaced by a six histidine tag. This figure was taken from reference [123].

High-throughput sequencing (HITS) in combination with cross-linking and immunoprecipitation (CLIP) was developed to identify RBP targets on a genome-wide scale and to contribute to the analysis of RNA-protein interactions that were not seen by other methods (Figure 8 middle). The only difference between CLIP and HITS CLIP is that the first one uses Sanger sequencing and the second one RNASeq to obtain the sequenced data. For instance, using HITS CLIP it was discovered that in alternative splicing, the position at which Nova (an RBP involved in neuronal synapsis) binds has a direct implication on the result of splicing, meaning, it determines if to exclude or include an exon [124]. This type of study allows the generation of regulatory maps for RBPs.

The resolution of the binding site in the traditional CLIP protocol is limited by the length of the fragmented RNAs. Therefore, two strategies improved the CLIP protocol to achieve single-nucleotide resolution. One of them is the photoactivatable-ribonucleotide-enhanced crosslinking (PAR-CLIP), which uses the deletions and point mutations caused by cross-linking to identify the cross-linked site of a specific RBP within its target RNA. This approach incorporates photoreactive ribonucleoside analogues, such as 4-thiouridine (4-SU) and 6-thioguanosine (6-SG) in the transcripts during cell culture. The cells are UV irradiated at 365nm to efficiently cross-link the photoreactive-labelled RNA to the RBPs. The RBP-binding sites are detected by the change from thymidine to cytosine and guanosine to adenosine in the cDNA sequences (Figure 8 left) [125].

The individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP), is an alternative approach to increase the specificity and obtain nucleotide resolution of the transcript-RBP interaction.
One problem during the preparation of cDNA libraries for the traditional CLIP method is that reverse transcription can stop due to the presence of peptides that remain cross-linked to specific nucleotides after proteinase K digestion. This truncated cDNA will not contain the 5'-linker and will not be included in the library. To solve this problem, iCLIP captures these truncated cDNAs by adding an oligonucleotide that contains a 3' and a 5' adaptor region allowing the circularization of the cDNAs. After reverse transcription the 5'-linker is added and the samples sent to high-throughput sequencing (Figure 8 right). Due to this strategy, iCLIP has the advantage that it can identify sites of cross-linking independently of mutations generated in the cDNA by the reverse transcription [126].

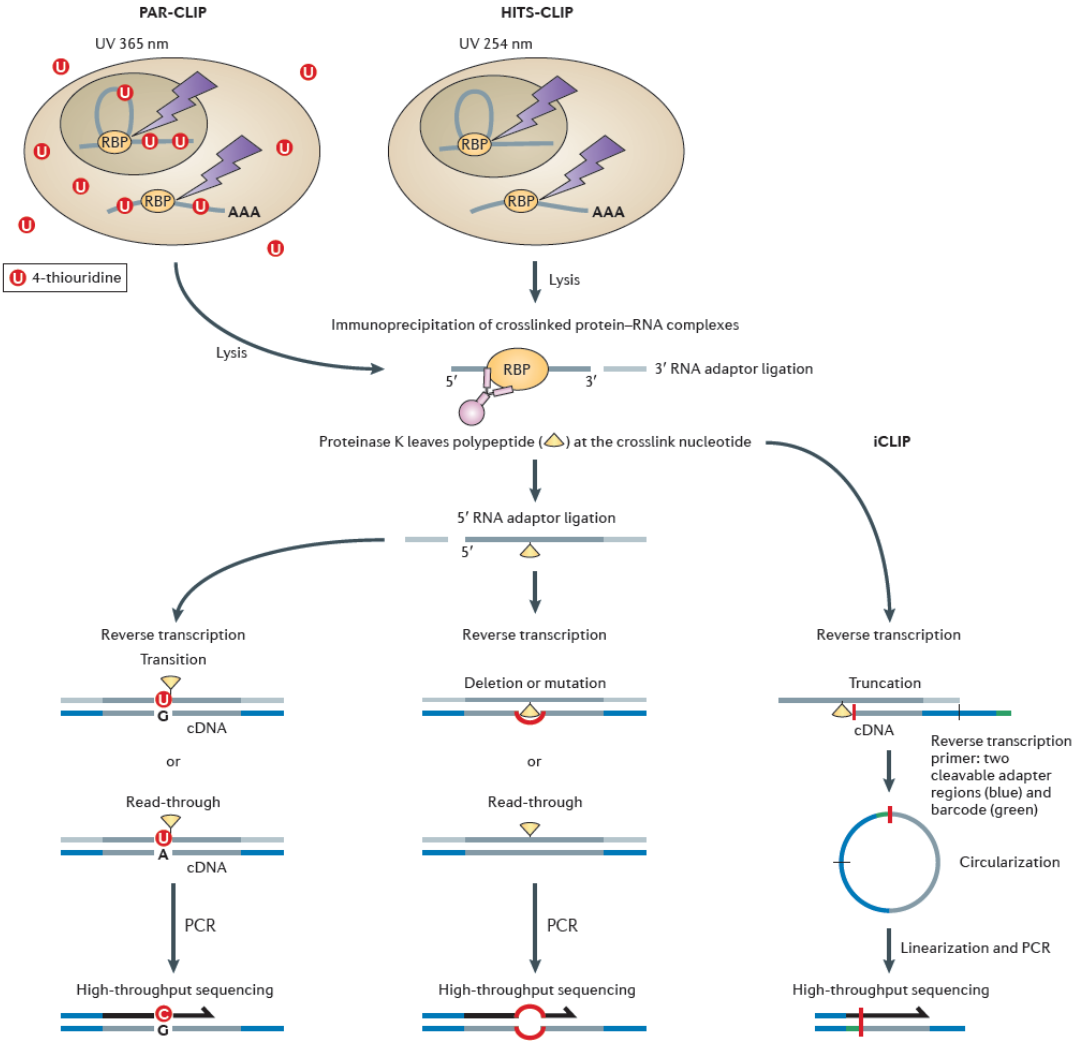


Figure 8. Scheme of high-throughput methods using CLIP

The right panel shows the photoactivatable-ribonucleotide-enhanced crosslinking (PAR-CLIP) strategy. In this method cells incorporate ribonucleoside analogues such as 4-thiouridine in the newly transcribed RNAs. These analogues are efficiently cross-linked with UV light at 365nm. During the reverse transcription the nucleotide analogues lead to a base transition at the cross-linking site allowing the detection of cross-linking sites at a nucleotide resolution.

The middle panel represents the high-throughput sequencing with cross-linking and immunoprecipitation (HITS-CLIP). This method is like the CLIP strategy described above with the only difference that the final cDNAs are submitted to RNASeq and not to Sanger sequencing.

The left panel is a scheme of the individual-nucleotide resolution cross-linking and immunoprecipitation (iCLIP) method. The main difference of this protocol when comparing it to HITS-CLIP is that truncated cDNAs can be capture by the addition of a 3'-linker that contains a 3' and a 5' region followed by the circularization of the cDNA. The 5'-linker is added after reverse transcription. This way the exact position where the reverse transcription stopped can be mapped.

This picture was obtained from reference [127].

1.5.2 Methods to detect the protein components of mRNPs.

1.5.2.1 Early methods

The first methods used in the detection and purification of mRNP protein components were developed in the 70s-80s. The development of these methods was encouraged by the discovery that the polyribosomal mRNA is associated to different proteins in different cell compartments (nucleus and cytosol) and that these complexes were not simply a lysis artefact [128]. The protein components of *globin* mRNPs were dissociated using reagents such as DTT and urea [129], puromycin or EDTA and also low or high salt treatments were assessed [130]. These experiments contributed with the finding of the poly(A) binding protein and confirmed that the mRNA is associated with proteins different than the ribosomes [130, 131].

1.5.2.2 Density gradient centrifugation and immunoprecipitation

The characterization of mRNAs was possible due to the combination of the sucrose gradient centrifugation with immunoprecipitation. An example is the purification of *myosin* mRNA from embryonic cells. After linear sucrose gradient centrifugation the fractions corresponding to the polyribosomes are collected and their RNA purified using phenol-chloroform. These RNAs are later added to muscle ribosomes in a cell free system to generate *de novo* proteins. Finally, *myosin* mRNA was purified using specific antibodies against the myosin protein [132, 133]. Another example is the myeloma *light-chain* mRNA purification. Linear sucrose gradient centrifugation and oligothymidylate (oligo-dT) chromatography were used to purify all mRNAs. The purified mRNAs were then used to produce proteins in a cell-free system and finally the *light-chain* mRNA obtained by immunoprecipitation using an antibody specific to the light-chain myeloma protein [134].
Other mRNAs were also obtained in a similar way, such as the *hemoglobin* mRNA [135, 136] and *ovalbumin* mRNA from rabbit reticulocytes [137]. For the purification of the *heavy-chain immunoglobulin* mRNA in a mouse myeloma model, the mRNA was immunoprecipitated using an antibody against the myeloma protein H_2L_2 that binds specifically to the *heavy-chain immunoglobulin* mRNA [138]. Another example, is the purification of the *ovalbumin* from Hen oviduct, the translating polyribosomes were separated first by sucrose gradient centrifugation, then immunopurified using an antibody against ovalbumin and later the mRNA extracted using poly(U) sepharose chromatography [139].
In trypanosomes, the *VSG* mRNA was also purified using sucrose gradient centrifugation and oligo (dT)-cellulose. The purified mRNAs were used in a reticulocyte cell-free system to produced VSG protein. As in the other cases, the VSG mRNA was purified using antiserum raised against a previously purified VSG [140-142].

It is important to mention that in these early studies highly specialized cells were used, producing predominantly one protein, where the purified mRNA represents approximately 10-5% of all mRNAs in the cell. The abundance of these mRNAs was the key determinant for a successful purification.

The method developed in the present dissertation is based on a previously published method [143] that used a matrix-bound antibody against the ovalbumin nascent peptide to isolate specific mRNPs from total polysomes (Figure 9).
Prior to the purification, antibodies against the ovalbumin protein of hen oviduct are raised. There are three main steps in the purification of *ovalbumin* mRNA from hen oviduct. The first is the separation of polyribosomes from hen oviduct via discontinuous sucrose gradient centrifugation, and then the polyribosomes are incubated with the first antibody, in order to allow the recognition of the nascent peptide (Figure 9 step I). In the second step, the antibody-polyribosome complex is recognized by the secondary antibody that is covalently cross-linked to the para-aminobenzyl cellulose (PAB cellulose) matrix (Figure 9 step II). In the final step, the complexes are dissociated by EDTA and the cellulose is separated by centrifugation (Figure 9 step III) [143].

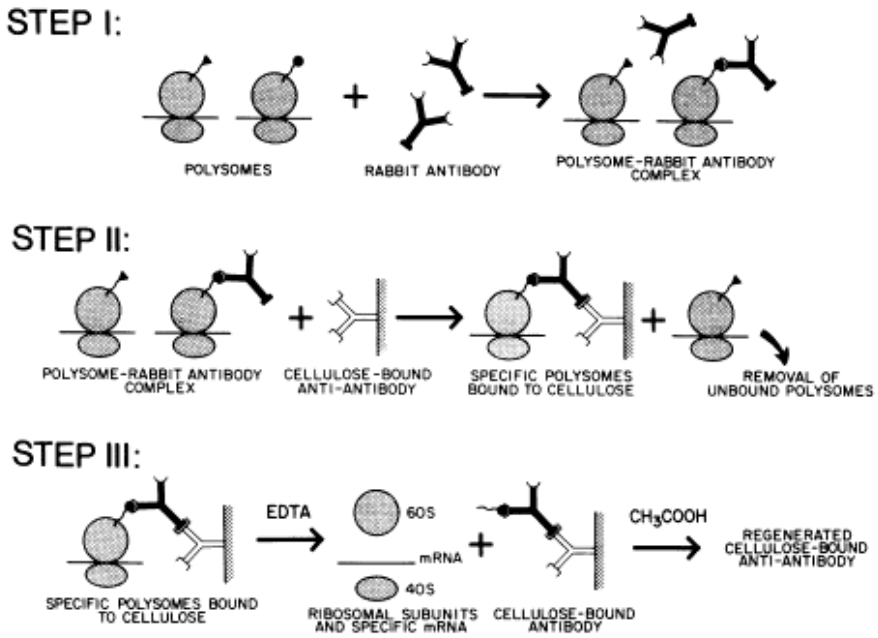


Figure 9. Isolation of specific mRNPs
Schematic representation of a method used to isolate specific mRNPs described in 1977. An antibody against the ovalbumin nascent peptide was used to purify the ovalbumin mRNP complexes (step I). These purified complexes are later recognized by a secondary antibody that is immobilized on the PAB cellulose matrix (step II). In the final step the complexes are released from the cellulose and the matrix can be regenerated for a second use (step III). This picture was obtained from reference [143] and is the base of the method used in the present dissertation.

The main reason for the purification of a specific mRNA was to produce cDNAs and clone them to generate libraries for further mRNP characterization studies. For instance, one study purified *VSG* mRNA using an oligo (dT)-

cellulose from four different VSG variants, each of them expected to expressed a different type of VSG. These mRNAs were used for cDNA preparations, cloned into a recombinant plasmid and transformed in *Escherichia coli*. Positive clones were selected by clone hybridization [144].

An interesting approach was developed 30 years later by Oeffinger *et al [145]*. In this mRNP purification method, cells are frozen in liquid nitrogen and lysed with a planetary ball that generates particles of 1-2 µm. The lysate is cleared by filtration and the complexes are separated from the rest of the lysate with an antibody coupled to magnetic beads. With the use of a magnet the beads containing the mRNPs are purified and later on the complexes are eluted under denaturing conditions [145].

1.5.2.3 Purification using affinity-tags

Most of these methods were developed from the 90s on, the simplest approaches purified mRNPs by immunoprecipitation, using antibodies against a tagged-protein component of the mRNP of interest. Different tags can be used for this purpose, such as the FLAG-tag, a small protein tag that is recognized by the M2 antibody. For instance, FLAG-tagging the insulin-like growth factor II protein (3xFLAG-IMP1) of human embryonic kidney cells allows to purify this mRNP from total lysates and to detect its protein components by mass spectrometry [146].

The homotetrameric protein streptavidin (SA) is produced by *Streptomyces avidinii* and when used as a tag its main advantage is that the purified complexes can be eluted with biotin. Also each monomer of streptavidin can bind one molecule of biotin with femtomolar affinity. This non-covalent biotin-streptavidin interaction is one of the strongest in nature (biotin dissociation constant K_d 10^{-14} M) [147, 148]. A variation of this system was done by the design of a Strep-tag composed of nine amino acids (AWRHPQFGG) that binds streptavidin using the same binding site as biotin. The limitation of this tag was that it could only be used in the C-terminus of a protein, because the free carboxy-terminus of the tag is required for binding [149]. The Strep-tag was later on modified in order to be cloned also in the N-terminus and it was called StrepII-tag (AWRHPQFGL) [150]. The conserved HPQ motif present in both tags is involved in the interaction with SA, the main disadvantage of these tags was that they bind SA with low affinity (37 µM for Strep-tag and 72 µM in the case of StrepII-tag) [149]. Many optimizations have been assayed in order to improve the Strep-tag for protein detection, for instance, the possibility of having two StrepII-tags separated by 12 aminoacids (StrepIII-tag) improved the detection of protein complexes [151]. In a later study, the use of multivalent StrepII-tags was assessed, either one or up to 5 StrepII-tags were fused to the N-terminus of the Sefin A Quadruple Mutant (SQM) scaffold protein, each tag was separated by 3 glycine linkers. The results of this study show that when using 2 StrepII-tags the relative binding capacity of the tagged protein is increased 5-fold compared to the monomeric tag and more importantly, there is a significant increase when using 3 StrepII-tags (12-fold increase compared to a single-tag) [152].

In the present dissertation I make use of the StrepII-tag and it is referred to as streptavidin binding peptide (SBP). The first studies were done using only one StrepII-tag and later on, the size of the tag was increased to three tags, separated by three glycine linkers (GGGS) as in reference [152].

1.5.2.4 Purification using aptamers

RNA affinity tags are short sequence tags, called aptamers, which were created to allow the purification of tagged-RNAs. Among the first aptamers designed were the ones that bind streptavidin (S1 aptamer) and Sephadex (D8 aptamer) [153]. One of the most used aptamers was discovered in the *E. coli* bacteriophage MS2 coat protein (CP) that binds to a 21 nucleotide stem-loop structure on its genomic RNA [154]. For the use as a purification system, the RNA of interest containing the stem-loop binds to the coat protein that can be covalently linked to a solid matrix. The advantage of this system is that a specific RNA containing the stem-loop can be purified from a mixture of RNAs in a single step [155].

Another method that uses the MS2 aptamer system is the RNA binding protein purification and identification (RaPID) technique. RaPID uses a fluorescent fusion protein composed of the MS2 coat protein fused with the green fluorescent protein (MS2-CP-GFP) to bind RNAs containing the MS2 stem-loop. This method can be used for studying localization of specific mRNAs by fluorescence microscopy. The fusion protein (MS2-CP-GFP) is coupled to a streptavidin binding protein that later on allows the purification of the mRNPs via streptavidin conjugated beads. Samples for RNA and protein isolation can be taken. Detection of bound proteins can be done by Western blot and mass-spectrometry [156]. This method involves the reversal of *in vivo* cross-linking with formaldehyde and two independent blocking steps. One step is to block biotinylated proteins present in the lysate with avidin, a biotin binding protein. The other step is to block the streptavidin beads with yeast tRNA to eliminate nonspecific binding [156]. These two blocking steps were included in the method developed in the present dissertation (see material and methods 2.4).

The streptavidin-binding aptamer (S1) is a 44 nucleotide long RNA sequence and consists of two double-stranded RNA helices connected by an internal loop of 13 nucleotides and a terminal loop of 9 nucleotides [148, 153]. It is also one of the most commonly used RNA affinity tags and it has been used to purify different RNP complexes. For instance, this tag was used in the RNA subunit of the human nuclear RNase P in HeLa cells, which was found to be part of the active RNase P enzyme. The tagged RNA was able to bind to the protein components of the enzyme and was later on purified using a streptavidin matrix whereas the mutant version could not form the active enzyme [157]. Another example is the purification of RBPs that interact with the intron2 or the 3′-UTR of the polarity protein *Crumbs3* transcript. For this purification, a tRNA was added to the streptavidin aptamer (tRSA) increasing its binding efficiency 10-fold [158].

A later method optimized the interaction of the SA-binding aptamer (S1m modified) to streptavidin. The modification of the SA-binding aptamer was done by introduction of a strand complementary structure in the terminal loop of the S1, generating a more stable structure of a 15 bp perfect stem. The final modification was the introduction of multiple copies of the modified aptamers. Using four stem-loop conformations (4xS1m) is 15-fold more efficient than using S1 to bind to the streptavidin matrix [159].

This method has the advantage that different RNA sequences can be used for the purification of regulatory proteins. The approach is based on the in-vitro transcription of the 4xS1m aptamer placed downstream of the AU-rich element (35 nucleotides long ARE) of TNFα. The in-vitro transcribed sequences are later on coupled to streptavidin sepharose beads and incubated with total cell lysates. After this incubation period and several washing steps, the specific RBPs that bind to the ARE element are eluted with RNaseA and identified by mass-spectrometry (Figure 9) [159].

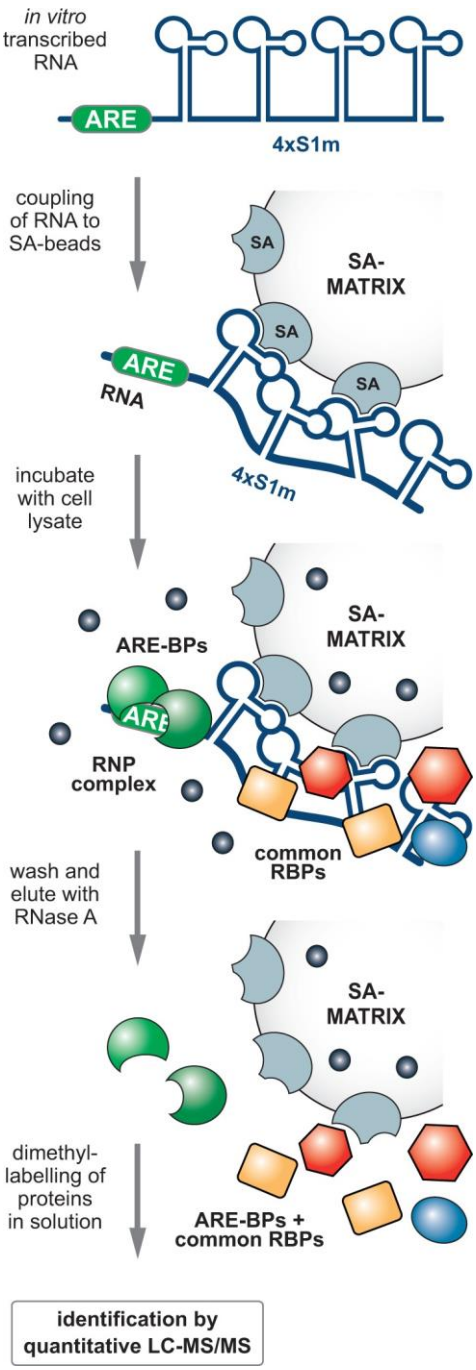


Figure 9. Schematic representation of the *in-vitro* 4xS1-mRNP purification.
A modified 4xSm aptamer placed upstream of an ARE element is *in-vitro* transcribed and coupled to a streptavidin matrix (SA). After incubation of the matrix with concentrated cellular extracts, the RBPs that bind specifically to this ARE and other common RBPs are eluted using RNases. In the final step the bound proteins are detected by mass-spectrometry. This figure was taken from reference [159].

To gather information on the RBP targets as well as on the proteins interacting with a specific mRNA it is a key step to characterize and understand the composition, and later on dynamic, of mRNPs, which are part of the elements involved in post-transcriptional regulation of gene expression in trypanosomes. In order to contribute to the characterization of mRNPs in trypanosomes, the method described in the present dissertation tries to purify actively translating mRNPs and to detect their protein components.

2. Materials and Methods

Some of the standard procedures described below were taken and modified from previous dissertations.

2.1 Cell culture

2.1.1 Procyclic form trypanosome culture

Monomorphic Lister 427 procyclic trypanosomes were cultured in supplemented MEM-Pros medium in tightly closed cell culture flasks at 27^{o}C at densities between 1-2x10^{6} cells/ml. All work was done under sterile conditions in a laminar flow hood.

Supplemented MEM-Pros medium, 500mL

MEM-PROS mixture was mixed with MEM vitamins, MEM non-essential amino acid solution and 100mg phenol red (pH 7.4). The media was sterilized by filtration and stored at 4°C.

Additional components to be added to 450ml MEM-Pros before use:

Heat-inactivated FBS	10% (v/v) (50ml)
Hemin	7.5mg/l (1.5ml of stock solution)
Penicillin/Streptomycin	50U/ml (5ml of stock (5000U/ml), Sigma)

Heat inactivation of FBS was done at 55°C for 30 min.

2.1.2 Bloodstream-form trypanosome culture

Monomorphic Lister 427 bloodstream form trypanosomes were cultured on incubators, in a humid atmosphere, at 37°C with 5% CO_2 in supplemented HMI-9 medium. In order to allow gaseous exchange the lid of the culture flasks were slightly loose. Cell densities (determined using Neubauer counting chamber) did not exceed 1.5 x 10^{6} cells/ml for experiments. Cell culture work was done in sterile conditions under a laminar flow hood.

Supplemented HMI-9 medium, 500ml

HMI-9 medium contained; 17.66g/l Iscove's modified Dulbecco's medium, 3.024g/l NaHCO3, 136mg/l hypoxanthine, 110mg/l sodium pyruvate, 39mg/l thymidine and 28mg/l bathocuprono disulfonic acid disodium salt at (pH 6.3). The media was sterilized by filtration and stored at -20°C.

Additional components to be added to 450ml HMI-9 before use

Heat-inactivated FBS	10% (v/v) (50ml)
Penicillin/Streptomycin	50U/l (5ml of Penicillin-Streptomycin mix)
L-Cysteine	1.5mM (5ml of stock solution)
β-mercaptoethanol	0.14% (7µl in 5ml frozen stock)

2.1.3 Antibiotics

For selection of transgenic trypanosomes antibiotic resistance genes were used and antibiotics added in the given concentrations:

Antibiotic	Bloodstream	Procyclic
Phleomycin	1µg/ml	1µg/ml
G418	5µg/ml	15µg/ml
Hygromycin	15µg/ml	50µg/ml
Puromycin	0.2µg/ml	1µg/ml
Blasticidin	5µg/ml	10µg/ml

All growth experiments were performed in the absence of selective drugs. For inducible expression, tetracycline was added to a final concentration of 200 ng/ml

2.1.4 Transfection of bloodstream / procyclic trypanosomes

A total of 1-2x10^7 cells were used per transfection. Cells were washed twice in the appropriate transfection buffer (see below) and resuspended in a final volume of 0.5ml. 6-10µg of digested plasmid was mixed with the cells and transferred to a 2mm gap cuvette for electroporation. When transfecting bloodstream trypanosomes the electroporation was performed using the program X-001 of the Amaxa Nucleofactor (Lonza Cologne AG, Germany). In the case of procyclic trypanosomes the settings used were 1.5 kV and resistance R2 in the BTX, Harvard apparatus. On the next day, the selection antibiotic was added and the cells were plated in serial dilution on a 24 well plate. Proliferating clones were picked from the plate days later and checked for expression of the transgene. Aliquots of the generated cell lines were frozen in medium with 10% glycerol and stored in liquid nitrogen.

Transfection buffers:

Zimmerman's Post Fusion Medium (ZPFM) Used for transfection in procyclic trypanosomes; the buffer was composed of 132mM NaCl, 8mM KCl, 8mM Na_2HPO_4, 1.5mM KH_2PO_4,1.5mM MgAc x 4 H_2O, 90µM $Ca(OAc)_2$ (pH 7.0), filter-sterilized and stored at 4°C

Cytomix Used for procyclic and/or bloodstream trypanosomes. Components: 2mM EGTA, 120mM KCl, 0.15mM $CaCl_2$, 10mM K_2HPO_4/KH_2PO_4 (pH 7.6), 25mM HEPES, 5mM $MgCl_2$, 0.5% Glucose, 100µg/ml BSA, 1mM Hypoxanthine (pH 7.6), filter-sterilized and stored at 4°C

Amaxa Used for bloodstream trypanosomes. Tb-BSF buffer: 90mM sodium phosphate, 5mM potassium chloride, 0.15mM calcium chloride, 50mM HEPES (pH7.3) [160]

2.2 Cloning

Cloning experiments were done using the supplied buffers and as stated in the manufacturer instructions. Enzymes from NEB or fast digest from Fermentas were used for the restriction reactions. In the case of dephosphorylation of linearized plasmids Antarctic Phosphatase (NEB) was

used. For standard PCR reactions or colony PCRs GoTaq® DNA Polymerase from Promega was used. Only when cloning ZC3H22 or ZC3H38 the Phusion® High-Fidelity DNA Polymerase from Finnzymes was used. PCR products and digests were confirmed on 1% agarose gels prepared with 1xTAE buffer and addition of ethidium bromide to the gel. Gel extraction kits from Macherey-Nagel were used in the purification of plasmid DNA and PCR products. The illustr™ tissue & cells genomicPrep Mini Spin Kit from GE Healthcare was used to extract genomic DNA. DNA and RNA concentrations were measured using the NanoDrop machine (peqlab)

2.3 Digitonin fractionation

Digitonin fractionation was used to determine the localization of proteins within the cell. Digitonin interacts with sterols, the more sterols a membrane contains, the less digitonin is needed to permeabilise it. For instance, the plasma membranes are broken first, followed by mitochondrial and then glycosomal. In the present dissertation, digitonin was used as part of the affinity purification of cytoplasmic polyribosomes in order to determine if the mature CAT protein (3SBPs-CAT-SKL) was indeed being targeted to the glycosomes. It was also used to confirm that the glycosomes remain intact after SiC treatment.

Trypanosome Homogenization Buffer (THB buffer): 25mM Tris-Cl (pH 7.8), 1mM EDTA, 150mM NaCl and 0.3M Sucrose.
The buffer was supplemented with protease inhibitors such as 2µg/ml leupeptin and 100µM TLCK, also 1mM DTT were added when required.
Digitonin stock solution 1mg/ml dissolved in THB buffer and heat 10 min at 95ºC.

Digitonin fractionation: $2.5x10^6$ cells were pelleted for 10 min at 3500 *g*, followed by a washing step with 1X PBS and spun down for one min at 11,600 *g*. A second step of washing was done with THB buffer without protease inhibitors. Digitonin was added at different concentrations (0, 25 or 50, 100, 150 and 200µg/ml) and the samples were filled up to 250µl with THB buffer plus inhibitors and 1mM DTT. Mild lysis with digitonin was achieved by 1 hour of incubation on ice followed by 5 min at 25ºC. After lysis, the pellets were resuspended in 10µl laemmli buffer and the supernatant precipitated by TCA.

TCA precipitation: Add ¼ volumes of 100% TCA to the samples and incubate for 2 hours or overnight at -20°C. The precipitates were washed twice with 1ml of ice-cold acetone and the pellet solved in 10µl laemmli buffer.

2.4 Affinity purification of cytosolic polyribosomes

This method was used in order to purify cytosolic polyribosomes from membrane bound polyribosomes and it was adapted from protocols for glycosome purification [161] and [162]. Lysis was performed in the absence of detergents, to maintain the organelles intact.

Glycosome buffer (2x): 40mM Tris-Cl (pH 7.8), 20mM $MgCl_2$ and 600mM KCl. At the moment of the experiment add: 2mM DTT, 2µg/ml leupeptin, 100µM TLCK, 40U/µl rRNasin (Promega 100U/ml), 100µg/ml cycloheximide and 200mM sucrose.
Leupeptin and TLCK can replace a pill of Protease Inhibitors Cocktail EDTA free (Roche Diagostics GmbH).

Biotin Buffer: Make 10mM from 50mM stock (Invitrogen) diluted in glycosome buffer. Samples were eluted in 50µl of buffer.

Lysis: The lysis was done using carbamide, an abrasive substance, in an amount that was roughly equal to the pellet volume (for 10^9 cells, 100µl). The carbamide was pre-washed twice with glycosome buffer and spun at 900 *g* for 2 min.
For short scale purifications, 10^8 cells were centrifuged at 900 *g* for 3 min, then washed twice with glycosome buffer and resuspended in 300µl of glycosome buffer. The cells were then transferred to the washed carbamide and centrifuged at 900 *g* for 2 min to remove all the supernatant. Cells were ground on ice for 5 to 8 min using a pellet pestle. For control breakage, before the lysis, a 1:100 dilution in glycosomal buffer was made. After the grinding, again a 1:100 dilution of the sample was taken and loaded (10µl each) in a Newbauer chamber (90 to 95% breakage was ideal). To remove the carbamide and collect the lysate, 200µl of glycosome buffer was added to the the pellet pestle, spun down for 2 min at 400 *g* and transferred to a new tube. The pestle was washed once more with 100µl of glycosome buffer and the lysate collected in a final volume of 300µl. The lysate was transferred to the pre-washed Avidin-agarose beads for blocking.

Blocking step: To block free biotinylated proteins present in the sample, the lysate was incubated with Avidin-agarose beads (Thermo-Pierce). 25µl of 50% Avidin slurry were pre-wash trice with glycosome buffer and spun at 20 *g* for 1 min at 4°C. For the blocking step, the mixture lysate and Avidin beads were tumbled for 10 min at 4°C and spun down at 400 *g* for 5 min [159]. The supernatant was collected in a new Eppendorf.

Sucrose density gradient fractionation: After the blocking step, the lysate was centrifuged at 33,000 *g* for 15 min to pellet the glycosomes (first step of ultracentrifugation). Later on the supernatant was loaded into a sucrose gradient and ultracentrifuged at 164,326 *g* for 2 hours in a SW60 rotor. The samples were fractionated by time (22 seconds Program #4) using the ISCO160 fractionator. The polyribosome profile was recorded at 454nm.

Affinity purification with SA-sepharose beads: This step was performed after the polyribosomal fractionation, all the polysomal fractions were pooled. 50µl of Streptavidin-sepharose (SA, GE Healthcare) slurry were taken and washed 3 times with glycosome buffer. The pooled polysomal fractions were tumbled for 1 to 1.5 hours at 4°C. The unbound fraction was collected by centrifugation at 2400 *g* for 3 min. The beads were washed once with glycosome buffer. The supernatant (wash fraction) was removed into a new

tube and the RNA present in the beads extracted using peqGold TriFast (peqLab) as stated in the manufacturer's instructions.
Alternatively, after the washing step, mRNP complexes were eluted from the beads by adding 50µl of 10mM biotin-containing buffer at 4°C as detailed in the Strp-tagged Protein Purification Handbook (Strep-Tactin Magnetic Beads, Qiagen).

2.5 Nascent peptide affinity purification method

This method was designed to purify non-abundant mRNPs from the cell and is based on a method that purified polyribosomes with a matrix-bound antibodies [143]. The purification of mRNPs could help to identify *trans* activating factors that might influence the cytoplasmic fate of the mRNA to which they bind. Therefore, the main goal was to detect the protein components of the specific mRNPs purified.

Polyribosome buffer: Two different polyribosomal buffers were tested, both buffers allow the specific purification of the multi-tag reporter mRNA.
Polyribosome buffer Kramers' modified: This buffer gave a better resolution of the polyribosomal fractions and is composed of 20mM Tris (pH 7.5), 20mM KCl and 2mM $MgCl_2$ [163].
Polyribosome buffer Schutzs' modified: Alternative polyribosomal buffer, composed of 20mM Hepes (pH 7.5), 25mM NaCl and 5mM $MgCl_2$ [143].
Both buffers were supplemented with the following regents, at the moment of the experiment; 5µg/ml leupeptin, 0.5mg/ml heparin, 2mM DTT, 100µg/ml cycloheximide, 40U/µl rRNasin, 200mM Sucrose, 1pill/10mL of Protease Inhibitors EDTA-free and 0.2% NP40 (the detergent was only added at the moment of lysis).

Affinity Purification buffer (AP buffer): This buffer is based in the polyribosomal buffer.
Affinity Purification buffer Kramers' modified: 20mM Tris (pH 7.5), 120mM KCl, 2mM $MgCl_2$ and 0.4 mg/ml of heparin, added at the moment of the experiment [163].
Affinity Purification buffer Schutzs' modified: Alternative affinity purification buffer, composed of 20mM Hepes (pH 7.5), 280mM NaCl, 5mM $MgCl_2$ and 0.4 mg/ml of heparin, added at the moment of the experiment [143].

Blocking buffer: Based on the polyribosomal buffer.
Blocking buffer Kramers' modified: 20mM Tris (pH 7.5), 20mM KCl and 2mM $MgCl_2$ [163].
Blocking buffer Schutzs' modified: Alternative to blocking buffer, composed of 20mM Hepes (pH 7.5), 25mM NaCl and 5mM $MgCl_2$ [143].
For both buffers, add at the moment of the experiment 0.1mg/ml tRNA from *E.coli* (Roche Diagostics GmbH) and 0.4mg/ml heparin.

Blocking Step: 50µl of Streptavidin Sepharose High Performance beads (GE healthcare) were used for $3x10^8$ cells, in the case of heat shock experiments; the same amount of beads was used for $6x10^8$ cells. The beads

were washed once with polyribosome buffer without detergent, spun at 900 *g* for 2 min and blocked for 2 hours with 1ml of blocking buffer per 25µl beads. After the blocking step, the beads were washed twice with polyribosome buffer without detergent before and once with the AP buffer.

Pre-lysis treatments: Cells were collected by centrifuging at 500 g for 10 min and the pellet was washed with 20ml MEM Pros media without serum. In order to avoid ribosome disasembly, 100µg/ml of cycloheximide (100 mg/ml) were added to the media and incubated for 5 min at room temperature. After cycloheximide treatment, the cells were centrifuged at 500 g for 8 min, the pellet resuspended in 1ml of polyribosome buffer without detergent and transferred to a 1.5ml LoBind eppendorf tube. Then the cells were spun at 900 g for 3 min and the supernatant removed. In this step, the cell pellets could be frozen in liquid nitrogen and store at -80°C or continue with the purification.
In special cases, depending on the protein of interest, the RNA-protein complexes were cross-linked before cycloheximide treatment (as was the case of V5-ZC3H11 or V5-MKT1). The cell pellet, corresponding to approximately $3x10^8$ cells, was washed in 10ml of MEM Pros media without FBS and distributed in a Petri dish (145mm radius). The RNA-protein complexes were cross-linked at 254nm in the UV StratalinkerTM 2400, Stratagene ($3\text{-}6x10^8$ cells in 10ml of cells per petri dish). The treated cells were collected in a 50ml falcon and the petri dish washed, to collect the rest of the cells, with 10ml more of MEM Pros media without FBS.

Lysis: The cell pellets were resuspended in 200µl of polyribosome lysis buffer supplemented with RNase inhibitors. Cell lysis was carried out by passing the cells 15 to 30 times through a 21G 1$_{1/2}$ needle (BD Microlance). To clear the lysate, the samples were centrifuged at 16,000 *g* for 10 min and the salt concentration adjusted to 120mM KCl. The supernatant was loaded carefully in a sucrose gradient.

Sucrose gradients: Gradients were prepared by adding the AP buffer supplemented with 5µg/ml leupeptin, 0.5mg/ml heparin and 2mM DTT to 15% and 50% sucrose mixtures. The sucrose layers were made by leaving the gradients in a 90° position for 2 hours. Gradients were stored at -80°C.

Sucrose density gradient fractionation: The gradients were ultra-centrifuged at 164,326 *g* for 2 hours in the SW60 rotor. The samples were fractionated by time (22 seconds, Program #4) using the ISCO160 fractionator. The polyribosome profile was recorded at 454nm.

Affinity Purification: Pooled polyribosome fractions were added in an equal volume to the pre-blocked Streptavidin Sepharose beads and tumbled for 1 hour. Afterwards, the beads were spun down at 900 *g* for 3 min and left on ice to settle, due to the high concentration of sucrose, this step can take approximately 20 min. The unbound fraction was taken and the beads washed 3 times with AP buffer. Protein elution for Western blot or mass spectrometry was achieved by adding SDS laemmli buffer and boiling the

samples at 95°C for 10 min. Samples for Northern blot were resuspended in peqGold TriFast (peqLab).

Proteinase K treatment: In case cross-linked samples, proteinase K treatment previous to TriFast was performed as follows; 400µg/µl of Proteinase K, 8mM of EDTA (4M) and 0.2% of SDS (10%) per 50µl beads and incubated for 20 min at 42°C with.

2.6 CAT Assay

Chloramphenicol acetyl transferase [124] assay was use in tethering experiments to determine the effect of a protein on translation. Also it was used to measure the activity of the mature 3SBP-CAT-SKL protein in the polyribosome fractions and to ensure intact glycosomes.
The CAT enzyme transfers an acetyl group from radioactivetively ^{14}C labeled butryl CoA to chloramphenicol. As chloramphenicol takes up an acetyl group, it becomes water insoluble and therefore move from the aqueous phase of the reaction solution to the upper non-aqueous phase of the scintillation solution.
For CAT assay, 10^7 cells were spun down 900 *g* for 5 min, washed once with 5ml cold 1x PBS and centrifuged at 900 *g* for 5 min. The pellets were resuspended in 300µl of CAT assay buffer (200µl 100mM Tris-HCl (pH 7.8), 2µl chloramphenicol 40mg/ml) and transferred to new Eppendorf tube. The samples were lysed by freezing-thawing thrice on liquid nitrogen and centrifuged at 16,200 *g* at 4°C for 3 min. The supernatant was transferred to a new tube and stored at -80°C. In order to determine the protein concentration of the samples, the Bradford assay was performed.
Bradford's Standard curve:
0, 5, 10, 15, 20µg of BSA (0.5µg/µl stock) in 800µl.
50µl of each sample was taken and 750µl of sterile water added.
To all the samples, 200µl of Bradford reagent (Biorad) was added and incubated for 5 min. The protein concentration was measured at 595nm.
The relative CAT activity was determined by taken 0.5 to 1µg of total protein from each sample and scaled up to a total volume of 50 µl using 100mM Tris-HCl, pH7.8. In the final step, CAT assay buffer containing 10µl butryl CoA per sample and 4ml scintillation cocktail (Econofluor-2) were added. The counts were measured in a scintillation counter (Beckman LS6000IC) using program #7 (0.50 seconds per sample measuring ^{14}C).

2.7 Tethering Assay

For the tethering experiment, two stable cell lines were used, one expressing a reporter containing the *5 box B* element in the 3' –UTR of the CAT gene (pHD2277) and the control cell line, expressing a reporter which does not contain the *box B* element (pHD1991). Both cell lines were transfected with a fusion protein composed of the λN peptide at the N-terminal of the protein of interest and a myc-tagged at the C-terminal. This approach was tested in both life cycle forms, for λN-ZC3H22-myc in procyclic form trypanosomes and λN-ZC3H38-myc in bloodstream forms. The effect of the protein on the tethered reporter mRNA was determined by CAT assay (material and methods 2.6).

2.8 RNA extraction and Northern Blot

Total RNA and RNA from sucrose gradients was extracted using peqGold

Trifast (peqLab) for liquid and solid samples. The extraction was performed following the manufacturer instructions. Formaldehyde gels were used in order to separate the RNA and the gel blotted onto Nytran membranes (GE Healthcare). Northern blots were hybridized with radioactively labelled DNA (Prime-IT RmT Random Primer Labeling Kit, Stratagene) or RNA (MAXIscript, Ambion) probes. Measurement of the signals was done using phosphorimager (Fuji, FLA7000). For normalization purposes the signal for *HSP70* mRNA, *tubulin* mRNA or the structural RNA SRP were used.

2.9 RNA-Immunoprecipitation (RNA-IP)

$2x10^8$ procyclic trypanosomes or $4x10^8$ bloodstream forms were used per experiment. Only for the RNA-IP using the cell line that express V5-ZC3H22, RNA-protein complexes were UV cross-linked at 254nm (UV StratalinkerTM 2400, Stratagene). Cells were concentrated in 10ml of media and plated on a 145mm radius Petri dish. Then, the cells were collected in 1ml PBS, pelleted by centrifugation at 900 *g* for 3 min and the pellet stored at -80C.

Immunoprecipitation buffer IPP150: 150mM NaCl, 10mM Tris-HCl (pH 7.5) and 0.1% IGEPAL.

Lysis buffer: 10mM NaCl, 10mM Tris-HCl (pH 7.5) and 0.1% IGEPAL.
For 5ml of lysis buffer add a tablet of Protease Inhibitors Cocktail EDTA free (Roche Diagostics GmbH)

Washing step: 50µl of anti-V5-bead or anti-myc-bead slurry (Biomol) were used per immunoprecipitation. The beads were spun for 1 min at 400 *g* and washed twice with IPP150.

Lysis: Cells were lyzed in 450µl of lysis buffer supplemented with 200µg/ml of RNasin® Ribonuclease inhibitor (Promega) and 20µl VRCs. Lysis was performed by passing the cells 15 times through a 27G 1$^{1/2}$ needle (BD Microlance). The lysate was cleared by centrifugation at 3,500 *g* for 5 min and the supernatant added to the pre-washed beads. At the moment of the IP, the concentration of NaCl was adjusted to 150mM and the samples tumbled for 1 hour. Afterwards, the beads were washed 5-10 times with IPP150. Samples for Western blot and Northern blot were taken.
In case of cross-linked samples, proteinase K treatment was necessary prior to RNA extraction by Trifast (see material and methods 2.5).

Preparation of samples for RNA sequencing: Approximately, $1x10^9$ bloodstream form trypanosomes were harvested for RNA-IP. A Northern blot was performed in order to determine the quality of the mRNA, the samples with visible ribosomal RNA (by methylene blue staining) were depleted of it using oligos against the ribosomal RNA [164].
The samples were sent to the Bioquant facility to perform the Illumina sequencing.
This protocol is currently under optimization in our laboratory.

2.10 Tandem affinity purification [140]

To determine the interaction partners of ZC3H38, TAP-tag purification was performed. The ZC3H38 protein was fused to a C-terminal TAP-tag. TheTAP-tag is compose of a calmodulin binding peptide (CBP), a tobacco etch virus protease (TEV protease) cleavage site and two IgG binding domains from *Staphylococcus aureus* Protein A [121].

Lysis buffer: 20mM Tris (pH 7.8), 10mM NaCl and 0.1% IGEPAL

IPP150 IP buffer: 150mM NaCl, 20mM Tris (pH 7.8) and 0.1% IGEPAL

TEV cleaveage buffer: 150mM NaCl, 20mM Tris (pH 7.8), 0.5mM EDTA, 1mM DTT and 0.1% IGEPAL

Calmodulin binding buffer: 150mM NaCl, 20mM Tris (pH 7.8), 10mM β-mercaptoethanol, 1mM magnesium acetate, 1mM imidazole, 2mM $CaCl_2$ and 0.1% IGEPAL

Calmodulin elution buffer: 150mM NaCl, 20mM Tris (pH 7.8), 10mM β–mercaptoethanol, 1mM magnesium acetate, 1mM imidazole, 2mM EGTA and 0.1% IGEPAL

Lysis: Approximately, $1x10^{10}$ bloodstream form trypanosomes were lysed in 4ml of buffer supplemented with 1 tablet of Protease Inhibitors Cocktail EDTA free (Roche Diagnostics GmbH). Lysis was done by passage of the cells through a 21G 1$_{1/2}$ needle (BD Microlance) for 20 times. Cell debris was pelleted by centrifugation at 10,000 *g* for 15 min. The clear lysate was transferred to a new tube and the concentration of NaCl adjusted to 150mM (sample for input after lysis, equivalent to $6x10^8$ cells was taken).

IgG purification: The IgG sepahrose beads (approximately 200µl) were transferred to a Bio-Rad column and pre washed with 10ml of IPP150 buffer. The clear lysate was then transferred to the column and tumbled for 1 hour at 4°C. The unbound sample was collected by gravity flow and an equivalent number of $6x10^8$ cells was taken for further analysis. The beads were washed 3 times with 10ml of IPP150 and once with 10ml TEV cleavage buffer. The cleavage was done with 100 units of TEV protease (Gibco) in 1ml of TEV cleavage buffer added directly to the column and incubated overnight at 4°C in slow rotation. The elution was obtained by gravity flow and a sample, equivalent to $1.5x10^7$ cells was taken as the eluate after TEV cleavage.

Calmodulin purification: Approximately, 200µl of calmodulin bead suspension were transferred to a column (BioRad) and pre washed with 10ml of calmodulin buffer. The eluate recovered from the IgG sepharose beads (after the TEV cleavage) was approximately 1ml, to this sample, 3ml of calmodulin binding buffer and 3µl of 1M $CaCl_2$ were added. Then this solution was transferred to the calmodulin beads and tumbled for 1 hour at 4°C. The column was washed 3 times with calmodulin binding buffer. In the final step, the bound proteins were eluted with 1ml of calmodulin elution buffer and concentrated by TCA precipitation. Samples were loaded in a SDS-PAGE gel and sent to mass spectrometry.

2.11 Yeast two hybrid high-throughput screen

Yeast two-hybrid analysis: The Matchmaker Yeast Two-Hybrid System (Clontech) was used according to the manufacturer's instructions. The ZC3H38 ORF was PCR-amplified and cloned into pBD-gate2 [165] used as bait. As prey, the pGADT7 vector was used. As controls we used the pGBKT7 bait plasmid containing murine p53, which interacts with the SV40 large T-antigen (positive control) and the pGBKT7 bait plasmid containing Lamin, which does not interact with the SV40 large T-antigen (negative control). The bait plasmid contains an N-terminal-GAL4 DNA binding domain and myc-tag. The prey has an N-terminal fused GAL4 activation domain and HA-tag. The pairwise co-transformation of the bait and prey plasmids was done into AH109 yeast strains (Matchmaker 3 System, Clontech). Positive clones were selected on quadruple drop-out medium (minimal SD media lacking tryptophan, leucine, histidine and adenine) and positive interactions were indicated by growth on SD-QDO and by the change to blue color due to the presence of X-α-gal in the medium. Protein expression was confirmed by Western blotting, using the myc and HA tags for detection for further reference see [108].

High-throughput screen**:** The positive cell line containing the ZC3H38 ORF was used as bait (cloned into pGBKT7 plasmid) and mated with Y187 MAT α strain, which carried a random genomic fragment library of *T. brucei* made by Dr. Esteban Erben in our laboratory (prey, in pGADT7). Selection of transformants was performed on SD-QDO medium after 5 days of incubation at 30°C. After selection, yeast cells were harvested and plasmid DNA isolated by cell wall digestion with lyticase, followed by NucleoSpin Plasmid alkaline lysis spin kit (Macherey Nagel). To identify putative interactors, PCR amplification was carried out using pGADT7 vector-specific primers [108].
The conditions necessary for the amplifications were as described below.
Using GoTaq polymerase 94°C for 5 min, 94°C for 40 sec, 63°C for 40 sec, 72°C for 4 min, 22 cycles and a final step of 72°C for 5 min.
In case of amplification with Q5 polymerase use 98°C for 10 sec, 98°C for 10 sec, 65°C for 20 sec, 72°C for 3 min, 22 cycles and a final step of 72°C for 5 min.
Quality of the amplified products was checked on an agarose gel. Samples were prepared for Illumina sequencing using standard Illumina kits and sequenced for 50 cycles on an Illumina MiSeq.

Bioinformatic analysis: Custom scripts designed by Dr. Abeer Fadda in our laboratory, in order to identify only mRNA sequences that were fused in-frame to the pGADT7 activation domain will be use in the analysis.
Currently, the samples are in the Bioquant sequencing facility, but when the results be available, the sequences containing the terminal junction of the insert-vector sequence will be identify and remove. The remaining sequences will be map to the *T. brucei* 927 genome using Bowtie and allowing one base mismatch. Sequences that are in frame with an annotated ATG start codon and in-frame sequences in 5' -UTRs will be selected and analyzed using customize SAMtools and Perl scripts.

2.12 List of plasmid generated for this study

Plasmid No	Description
PHD2174	EP 5'UTR backbone + 1SBPat N-term of CAT ORF and SKL at C-term + EP 3'UTR
PHD2306	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + EP 3'UTR
PHD2319	EP 5'UTR backbone + No SBPs on CAT ORF and SKL at C-term + EP 3'UTR
PHD2320	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + VSG117 3'UTR
PHD2376	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + HSP70 3'UTR
PHD2392	ZC3H22 N-term ORF (in p2T7)
PHD2414	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + AATP11 3'UTR (BamHI/MluI)
PHD2415	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + HSP70 3'UTR delAUU
PHD2416	in situ V5-tagged ZC3H22
PHD2417	ZC3H22 middle ORF (in p2T7)
PHD2440	EP 5'UTR backbone + 3SBPs at N-term of CAT ORF and SKL at C-term + AATP11 3'UTR del328nt
PHD2517	ZC3H22-myc, in pHD1700 (C-term tags)
PHD2526	ZC3H22 KO plasmid, Puromycin (in pHD1747)
PHD2528	ZC3H22 for tethering (in pHD2451)
PHD2604	ZC3H22 KO plasmid, Blasticidin
PHD2614	Exogenous V5-ZC3H38 (in EP locus)
PHD2616	ZC3H38 for tethering (in pHD2451)
PHD2620	P2T7 RNAi *Tb*927.10.12790
PHD2621	CAT SKL no SBP in T7 plasmid for in-vitro transcription
PHD2629	*in situ* V5-tagged ZC3H38
PHD2651	p2T7 RNAi ZC3H37ORF
PHD2652	ZC3H38 without HNPY domain for tethering (1-813bp)
PHD2653	ZC3H38-myc, in pHD1700 (C-term tags)
PHD2654	ZC3H38 without 1st CCCH for tethering (193-847bp)
PHD2656	ZC3H38 without both CCCH for tethering, only HNPY (475-843bp)
PHD2666	ZC3H38 KO plasmid, Blasticidin (in pHD1748)
PHD2668	ZC3H38 C-terminal TAP-tag (in PHD918)
PHD2681	ZC3H37 and ZC3H38 KO plasmid, Blasticidin (in pHD1748)
PHD2702	ZC3H37 and ZC3H38 KO plasmid, Puromycin (in pHD1747)
PHD2759	ZC3H38 N-terminal TAP-tag (in pHD2643)

2.13 List of primers designed

Numbers	Sequence	Restriction site	Description
CZ 3848	agcttgccaccatggctagctggagccacccgcagttcgagaagggtggcggcagcctcgagatctt		FW with 1SBP
CZ 3849	agctaagatctcgaggctgccgccacccttctcgaactgcgggtggctccagctagccatggtggca		RV with 1SBP
CZ 3932	gctcaagcttgccaccatggctagctggag		FW with SKL signal
CZ 3933	ctatggatcctcagagtttagaccatctattcgccccgccctgccactcatcgcagtac		RV with SKL signal
CZ 4494	gatctggtggcggcagctggagccacccgcagttcgagaagggtggcggcagca	BglII	FW with 2 SBPs
CZ 4495	agcttgctgccgccacccttctcgaactgcgggtggctccagctgccgccacca	BglII	RV with 2 SBPs
CZ 4496	agcttaccatggcgtggagccacccgcagttcgagaagggtggcggcagcggtggcg	BamHI	FW with 2 SBPs
CZ 4497	gatccgccaccgctgccgccacccttctcgaactgcgggtggctccacgccatggta	BamHI	RV with 2 SBPs
CZ 4550	cacaagcttcgacgagattttcagg		FW upstream CAT ORF for cloning the control without 3SBPs
CZ 4556	cacaagcttatgactagtgtgagcaagg	HindIII	FW for eGFP ORF instead of CAT
CZ 4557	gatcggatcctcagagtttagaccatctattcttgtacagctcgtccatgccgag	BamHI	RV for eGFP ORF instead of CAT+SKL
CZ 4615	atggagaaaaaaatcactggatat		FW for CAT ORF
CZ 4847	gacccgcggttgttacacccctttccctc	SacII	FW ZC3H22 5'-UTR
CZ 4848	gctctagaatccgtgaaaattcaaaagt	XbaI	RV ZC3H22 5'-UTR
CZ 4849	gatgtcgacttccttcccgaacaatcgaa	SalI	FW ZC3H22 ORF
CZ 4850	atgggcccagacttccatccactccctc	ApaI	RV ZC3H22 ORF
CZ 4874	atgaccacagcaactgatgtg		FW on N-term ZC3H38 ORF
CZ 4875	caaagaacacgtttggagctg		RV middle of ZC3H38 ORF
CZ 4884	gctctcgagatgaccacagcaactgatgtg	XhoI	FW ZC3H38 ORF for V5 in situ tag

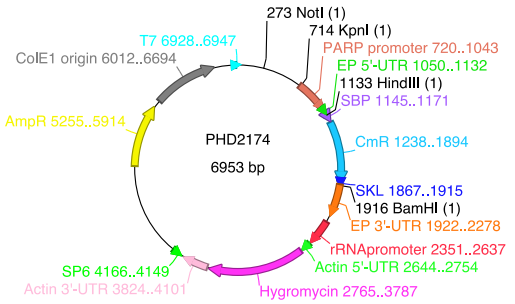
CZ 4885	actgggccctcggtctccggcgaggaataa	ApaI	RV ZC3H38 ORF for V5 in situ tag
CZ 4886	tgcccgcggtgacatcagcggaaaacattc	SacII	FW 5'-UTR ZC3H38 for V5 in situ tag
CZ 4887	acgactagtcttgactttgtttggttcttc	SpeI	RV 5'-UTR ZC3H38 for V5 in situ tag
CZ 4892	gatccgaggggaggcggcgtagcagg	BamHI	FW 3'-UTR AATP11
CZ 4913	agtacgcgttttgcgcgcttgcgcatagttttg	MluI	RV 3'-UTR AATP11
CZ 4916	tctcagattacgccgagctt		FW middle ZC3H22 ORF
CZ 4917	acataccaacagtccgctcc		RV middle ZC3H22 ORF
CZ 4920	tggctcgagttccttcccgaacaatcgaa	XhoI	FW N-term ZC3H22 without ATG
CZ 4931	ccgccgcgccctttattaagat		RV middle ZC3H22 ORF
CZ 4947	cttattttcgttctgtttgttgtc		FW 3'-UTR AATP11 deletion
CZ 4948	ttgatttatagagacactgagctg		RV 3'-UTR AATP11 deletion
CZ 5017	gatagcgtaatgaatgtccacacttgtttta	MluI	RV 3'-UTR EP 26mer deletion
CZ 5018	aggacgcgtgtgacatcctttctaatgcct	MluI	FW 3'-UTR EP 26mer deletion
CZ 5019	tggaaactcgagcgaacccaaaaga	PspXI	RV 3'-UTR EP 26mer deletion
CZ 5020	tgaggatccgcggatgcaagcgtgt	BamHI	FW 3'-UTR EP 26mer deletion
CZ 5037	gatcgttaactgtgatgtttcggttgcgtattc	HpaI	FW 3'-UTR EP 16mer deletion
CZ 5038	atccgttaactgggtctcaggcgatggttaata	HpaI	RV 3'-UTR EP 16mer deletion
CZ 5066	agctctcgagatccgtgaaaattcaaaagtactgg	XhoI	RV 5'-UTR ZC3H22 knockout
CZ 5067	actcgaattcggtaagaaaagaaaaaaagtaacaa	EcoRI	FW 3'-UTR ZC3H22 knockout
CZ 5068	cgtggatccccccttcatttcgccgctcatttctc	BamHI	RV 3'-UTR ZC3H22 knockout
CZ 5078	acggtaccttctcaccgttttgccaattttgaa	KpnI	FW 5'-UTR ZC3H22 knockout
CZ 5091	gatcctcgaggggcctttccaagattgctgttt	XhoI	FW HSP70 3'-UTR
CZ 5092	atagggcccgccattccattttagacgctatg	ApaI	RV HSP70 3'-UTR
CZ 5093	agagcggccgcaccatggcgtggagccaccc	NotI	FW 3SBP-CAT-SKL
CZ 5094	tcagctcgagtcagagtttagaccatctatt	XhoI	RV 3SBP-CAT-SKL
CZ 5150	aaagcaggctccatgttccttcccgaacaatcgaa		FW ZC3H22 for tethering

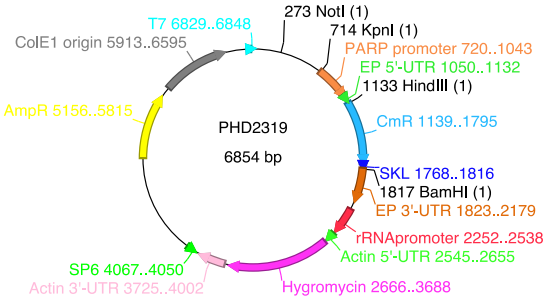
CZ 5151	gtacaagaaagctgggttcaaaagggtacgtgtaggggaa		RV ZC3H22 for tethering
CZ 5152	gatctgcatcctgggatcaa		FW blasticidin ORF for sequencing
CZ 5153	cattgacaccagtgaagatgc		RV blasticidin ORF for sequencing
CZ 5154	caccagggcaagggtctg		FW puromycin ORF for sequencing
CZ 5155	agttcttgcagctcggtgac		RV puromycin ORF for sequencing
CZ 5173	gcataagcttatgttccttcccgaacaatc	HindIII	FW ZC3H22 for over-expression (PHD1700)
CZ 5174	gacggatccaaagggtacgtgtagggga	BamHI	RV ZC3H22 for over-expression (PHD1700)
CZ 5231	ccccgatgagcaatgctttttataatgccaactttgtacaaaaaagcaggctccatg		attL1 for tethering (Ph.D. Esteban Erben)
CZ 5232	gggggataagcaatgctttcttataatgccaactttgtacaagaaagctgggt		attL2 or tethering (Ph.D. Esteban Erben)
CZ 5528	taatcacaatacgctggtacggtga		FW Tb927.7.2690
CZ 5558	gatcggatcctcagtgattcatcaggaaaaca	BamHI	RV Tb927.10.12770 ORF
CZ 5559	gatcgtcgacaaaaaataattatatatatata	SalI	FW Tb927.10.12770 ORF
CZ 5560	gatcgtcgactgaatggaacgggttacgccat	SalI	FW Tb927.10.12790 ORF for RNAi
CZ 5561	gatcggatcccatatattgactgagcgaataa	BamHI	RV Tb927.10.12790 ORF for RNAi
CZ 5562	gatcgtcgacaacccgtgtgctgcaggagtac	SalI	FW ZC3H38 ORF unique region for RNAi
CZ 5563	gatcggatccatattgcatacttccaactgca	BamHI	RV ZC3H38 ORF unique region for RNAi
CZ 5588	tcagtgtatcatcaggaaaacaataaaaa		RV Tb927.10.12770 ORF for RNAi
CZ 5589	cacttccttttttctttactcctcatg		FW Tb927.10.12770 ORF for RNAi
CZ 5629	gatcgtcgaccggttcatttcccgatgg	SalI	FW ZC3H37 ORF unique region for RNAi
CZ 5630	gatcggatccgagatgcccgttatcgc	BamHI	RV ZC3H37 ORF unique region for RNAi
CZ 5654	accactttgtacaagaaagctgggtttacagctgctggtaag		RV ZC3H38 for 1st and/or 2nd CCCH deletion
CZ 5655	gatcgggcccgatttacgtatcgccccattt	ApaI	FW_5'-UTR_ApaI
CZ 5656	gatcctcgagcttgactttgtttggttcttctt	XhoI	RV_5'-UTR_XhoI
CZ 5657	gatcggatccatgtggggaatcccttct	BamHI	FW ZC3H38 3'-UTR for knockout
CZ 5658	gatcactagtcgaacgaaaattaaaacggc	SpeI	RV ZC3H33 3'-UTR for knockout
CZ 5682	aaagcaggctccatgaatgttgattacagcacgc		FW ZC3H38 for 1st CCCH deletion

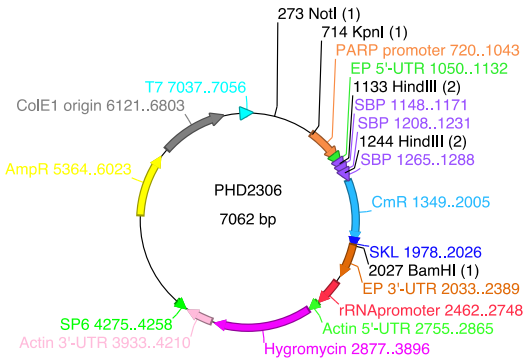
CZ 5683	aaagcaggctccatggttgccgtcgaccctga		FW ZC3H38 for 2nd CCCH and/or HNPY deletion
CZ 5684	gtacaagaaagctgggttcagaaacgtgggcgc		RV ZC3H38 for HNPY deletion
CZ 5685	gatcggatccttcgctgagtttaattgtaacc	BamHI	FW ZC3H37 3'-UTR for knockout
CZ 5686	gatcactagtcgaaatgacgcataaaagaagt	SpeI	RV ZC3H37 3'-UTR for knockout
CZ 5697	gatcaagcttatgaccacagcaactga	HindIII	FW ZC3H38 ORF for over-expression (PHD1700) and TAP-tag (PHD918)
CZ 5698	gatcggatcccagctgctggtaaggatta	BamHI	RV ZC3H38 ORF for over-expression (PHD1700)
CZ 5718	gatcgttaaccagctgctggtaaggatt	HpaI	RV ZC3H38 ORF for TAP-tag (PHD918)
CZ 5741	gatcgaattcatgaccacagcaactgatgtg	EcoRI	FW ZC3H38 ORF for yeast two hybrid
CZ 5742	gatcggatccttacagctgctggtaaggat	BamHI	RV ZC3H38 + Stop odon for yeast two hybrid
CZ 5860	gatcgagctcgatttacgtatcgccccattt	SacI	FW 5'-UTR ZC3H38 N-term TAP (in situ)
CZ5900	gatcaagctttgaccacagcaactga	HindIII	FW ZC3H38 ORF N-term TAP-tag (PHD2643) no ATG!
CZ4874	atgaccacagcaactgatgtg		FW ZC3H38 and ZC3H37 homologous part of the ORF
CZ4875	caaagaacacgtttggagctg		RV ZC3H38 and ZC3H38 homologous part of the ORF

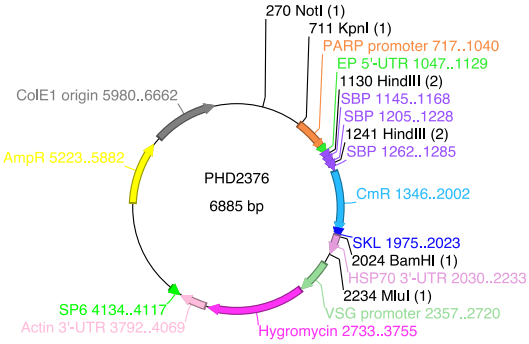
2.14 Plasmids Maps

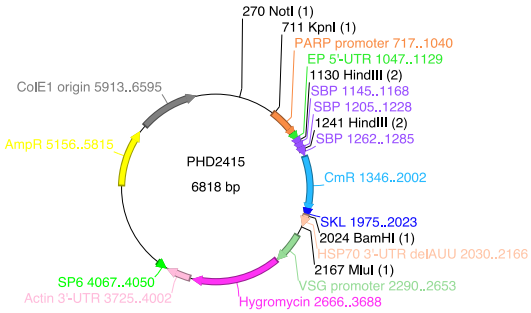
2.14.1 Affinity Purification Plasmids

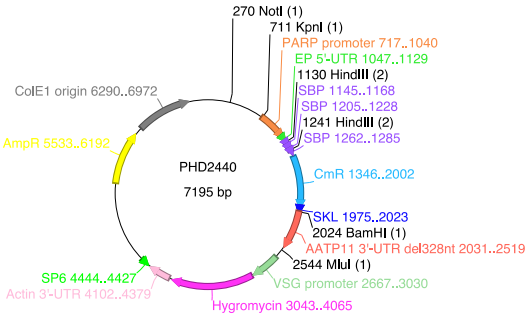


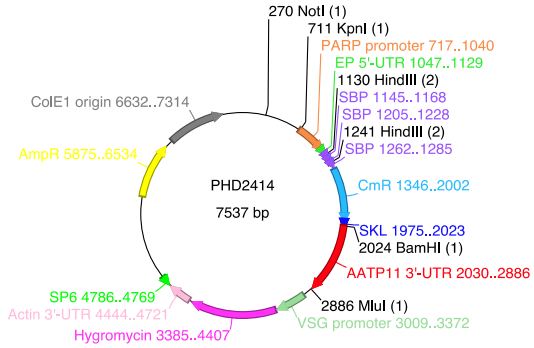


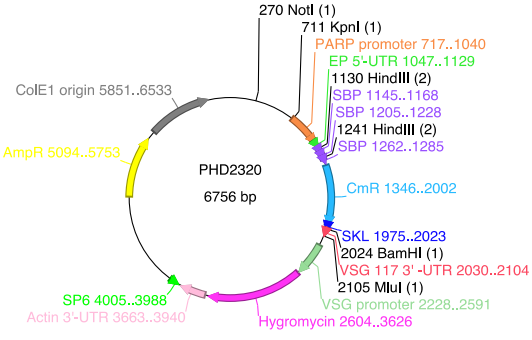


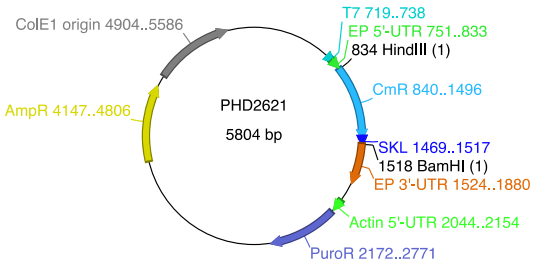


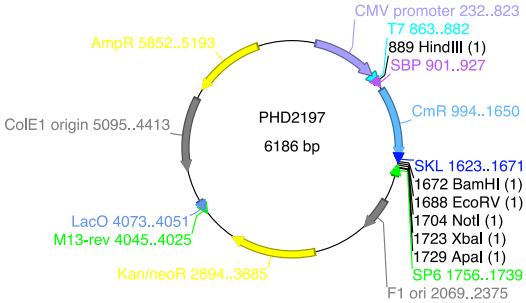




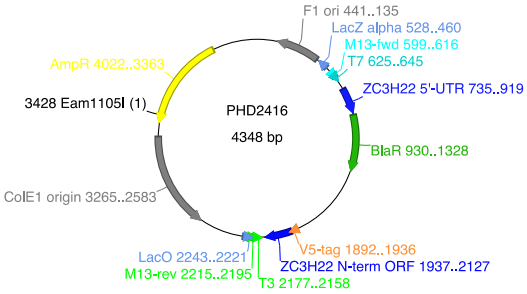


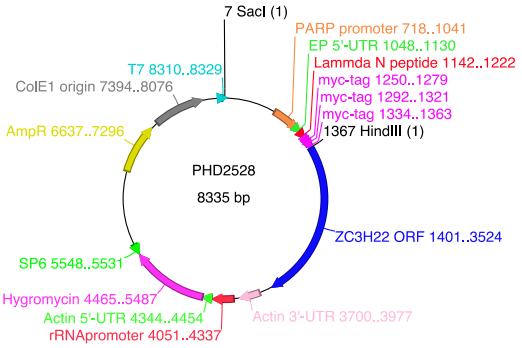


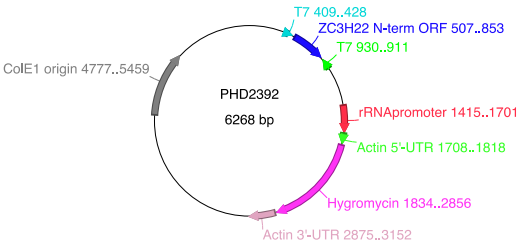


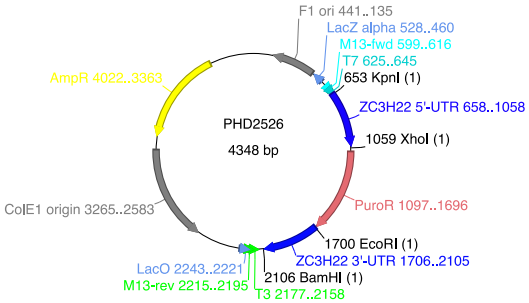


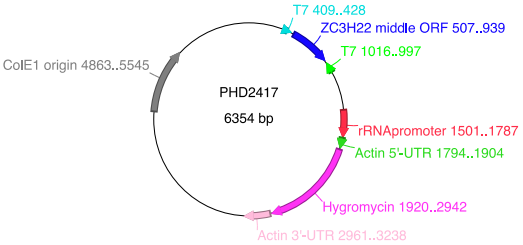
2.14.2 Plasmids for ZC3H22

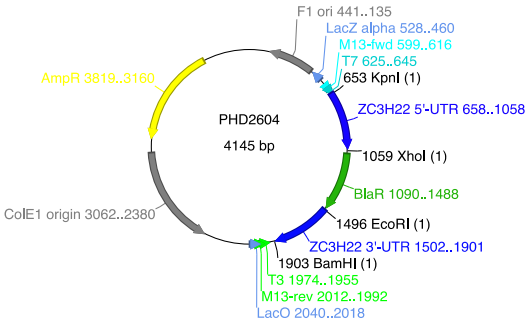


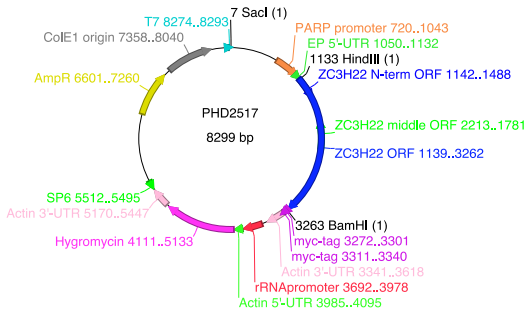




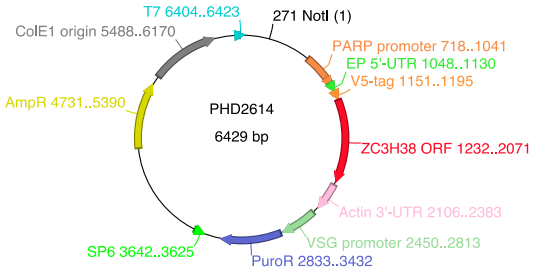


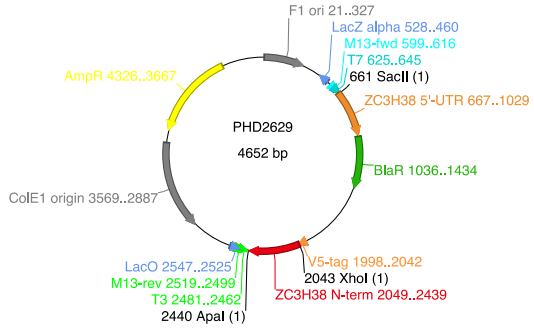


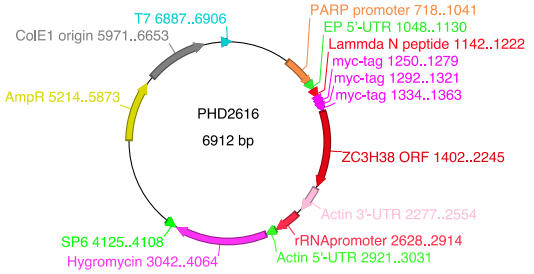


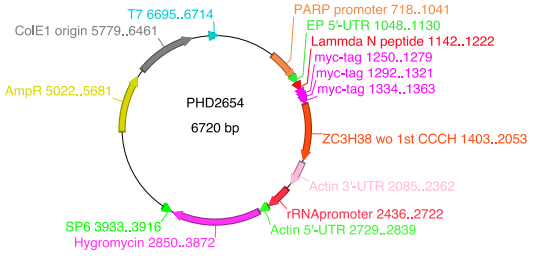


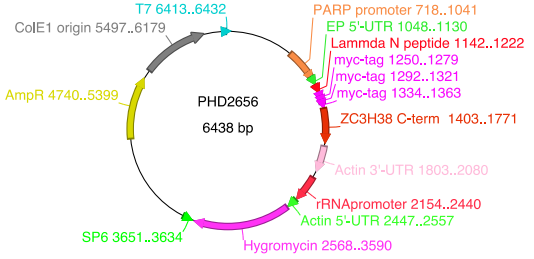
2.14.3 Plasmids for ZC3H38

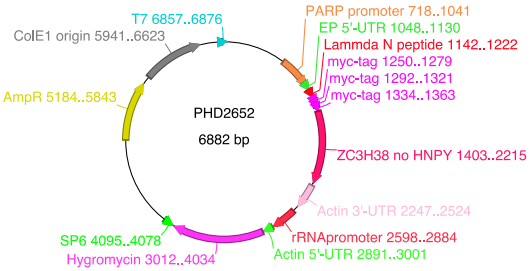


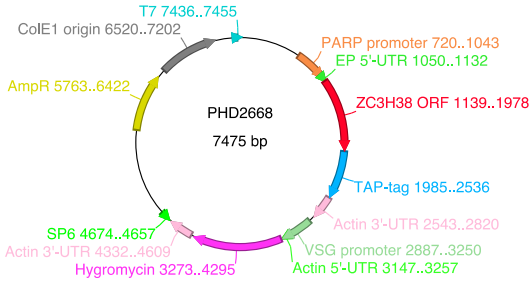


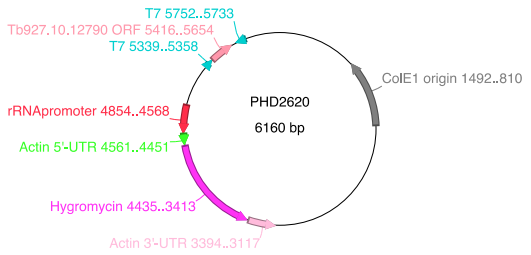


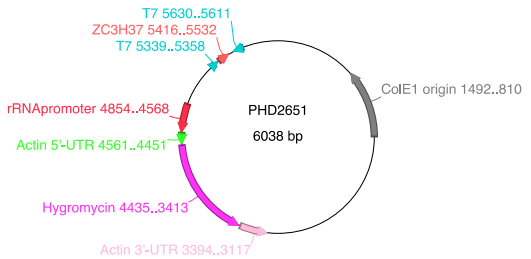


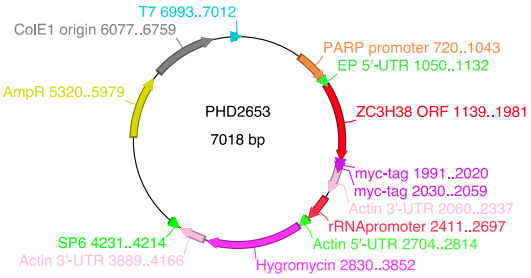


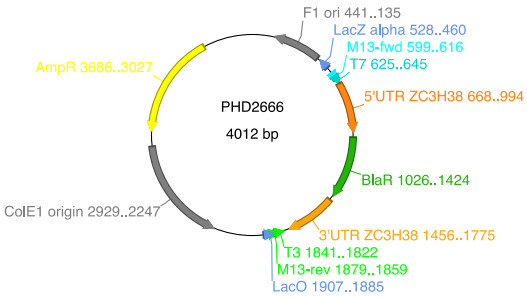








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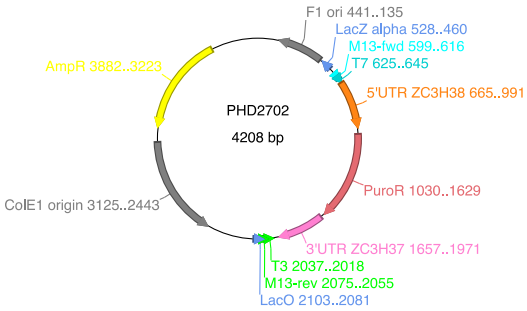
LacZ alpha 528..460
M13-fwd 599..616
T7 625..645
AmpR 3686..3027
5'UTR ZC3H38 668..994
PHD2666
4012 bp
BlaR 1026..1424
ColE1 origin 2929..2247
3'UTR ZC3H38 1456..1775
T3 1841..1822
M13-rev 1879..1859
LacO 1907..1885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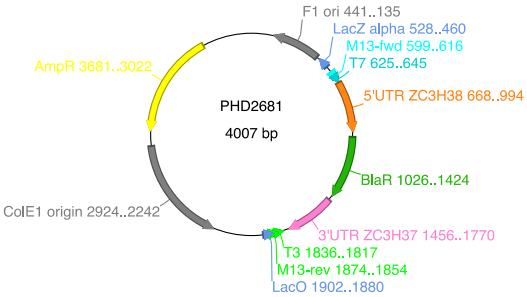
LacZ alpha 528..460
M13-fwd 599..616
T7 625..645
AmpR 3882..3223
5'UTR ZC3H38 665..991
PHD2702
4208 bp
PuroR 1030..1629
ColE1 origin 3125..2443
3'UTR ZC3H37 1657..1971
T3 2037..2018
M13-rev 2075..2055
LacO 2103..2081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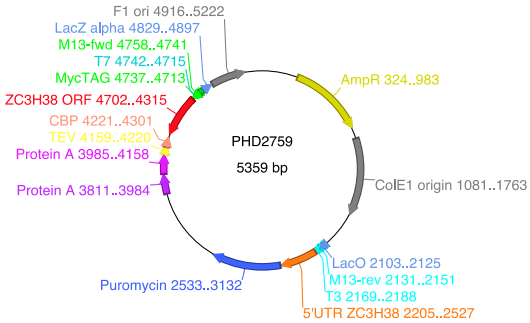
LacZ alpha 528..460
M13-fwd 599..616
T7 625..645
AmpR 3681..3022
5'UTR ZC3H38 668..994
PHD2681
4007 bp
BlaR 1026..1424
ColE1 origin 2924..2242
3'UTR ZC3H37 1456..1770
T3 1836..1817
M13-rev 1874..1854
LacO 1902..1880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LacZ alpha 4829..4897
M13-fwd 4758..4741
T7 4742..4715
MycTAG 4737..4713
AmpR 324..983
ZC3H38 ORF 4702..4315
CBP 4221..4301
TEV 4159..4220
PHD2759
5359 bp
Protein A 3985..4158
Protein A 3811..3984
ColE1 origin 1081..1763
LacO 2103..2125
M13-rev 2131..2151
Puromycin 2533..3132
T3 2169..2188
5'UTR ZC3H38 2205..2527

3. Results

3.1 Principle of the nascent polypeptide affinity purification

The method described in the present dissertation is based on a previously published method that purified polyribosome complexes using an antibody against the nascent peptide of hen oviduct. The immunoprecipitation was achieved using an antibody covalently cross-linked to para-aminobenzyl-cellulose matrix [143]. Others had also succeeded in the purification of polyribosome complexes before, but most of them had purified abundant mRNAs such as those encoding ovalbumin [166, 167], histone F2c *[168],* histone H5 [169] and globin [170] among others.

The method described below was designed to purify a specific mRNP from translating polyribosomes using the strong interaction of a streptavidin binding peptide (SBP) and a streptavidin matrix [149, 171, 172]. The main goal of this method is to detect *trans*-activating factors, that are usually of low abundance, but influence the cytoplasmic fate of an mRNA, either by binding directly to the target mRNA or in association with other proteins present in the mRNP.

A graphic representation of the reporters used in the present study is shown below (Figure 10). The reporter mRNA of interest contained a sequence encoding three streptavidin binding peptides (3SBPs) at the 5'-end of the open reading frame (ORF, Figure 10a). As control, a reporter mRNA without the 3 SBPs was used (Figure 10b). Both reporters contained the same untranslated regions (5'-UTR and 3'-UTR).

With this technique, the relevance of motifs or specific sequences present in the UTRs can be tested. For instance, by comparing a reporter containing an intact 3'-UTR (Figure 10c) to a reporter with a deletion on the 3'-UTR (Figure 10d). This will allow the identification of proteins that bind to this specific region, as both reporters contain the same ORF and the 3SBPs and differ only in this specific region of the UTR.

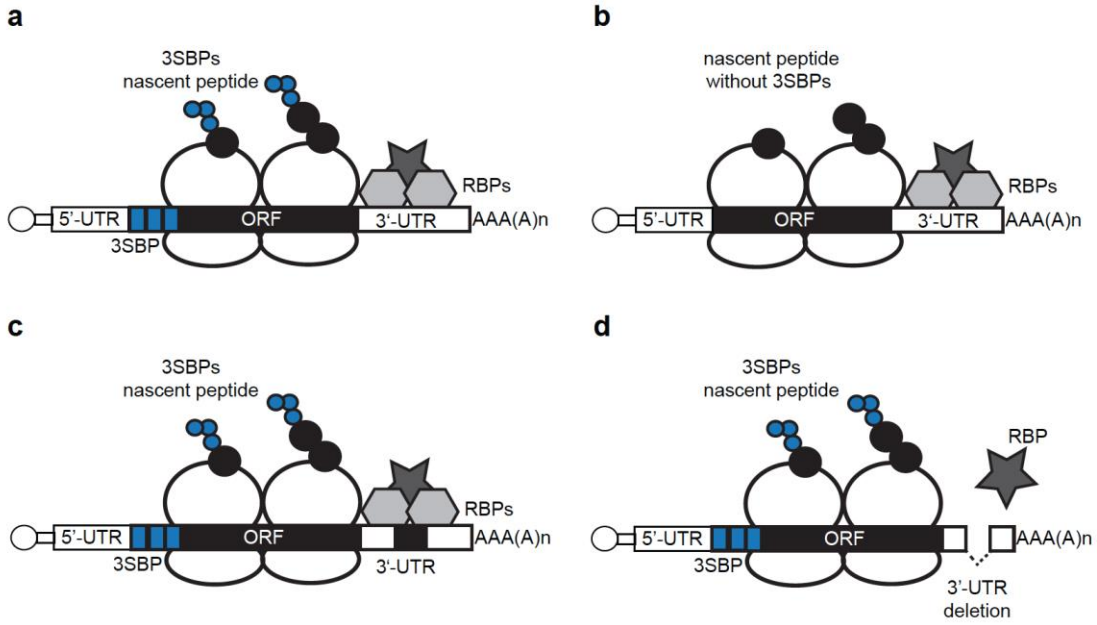


Figure 10. Reporters used in the affinity purification method
(**a**) Multi-tag reporter composed of three streptavidin binding peptides (3SBPs) at the 5'-end of the open reading frame [173] and the RNA binding proteins (RBPs) bound to the 3'-untranslated region (3'-UTR). The blue circles represent the translated SBPs that came out of the ribosomal tunnel as nascent peptide and the black ones the CAT protein.
(**b**) Control reporter, same as in (**a**) but without the 3SBPs.
(**c**) Multi-tag reporter with a complete 3'-UTR, containing the *cis*-regulatory element of interest.
(**d**) Multi-tag reporter without the *cis*-regulatory element, shown as two squares connected by dashed lines.

The strategy relies on the purification of translating polyribosomes via the affinity of the 3SBP multi-tag to a streptavidin matrix. Due to the fact that the multi-tag reporter mRNAs designed, contained the 3SBPs at the 5'-end of the ORF, the first part of the reporter mRNA to be translated and to exit the ribosomal tunnel, as a nascent peptide, were the 3SBPs-tag. The complexes expressing the multi-tag were pooled and affinity purified using streptavidin beads (Figure 11).

In the first step of the purification, RNA-protein complexes were UV cross-linked at 254nm. Using this approach, covalent bonds are formed between RNA and proteins that reside in close proximity, maintaining theses direct interactions is essential to proceed further with this method, allowing the purification of mRNP complexes under stringent conditions (Figure 11a).

In the next step, cycloheximide was added prior to cell lysis with detergents, in order to avoid polyribosome disassembly (Figure 11b). Then, the lysate was cleared by centrifugation and the translating mRNAs obtained by performing a polyribosomal fractionation. In this type of fractionation, the translating polyribosomes, present in the higher sucrose fractions were separated from the lower sucrose fractions containing ribosomal complexes (40S, 60S and 80S) and other proteins that did not bind to the translating mRNPs (Figure

11c). The fractions containing the actively translating polyribosomes were pooled (fractions 6-10 of the polyribosome gradient in Figure 11c) and incubated with the streptavidin matrix (affinity purification step), allowing the interaction between the 3SBPs-tag (nascent peptide) and the streptavidin matrix (Figure 11d). In the final step, the specificity of the pull down was tested by Northern blot and the proteins bound to the purified mRNPs were detected by Western blot and mass spectrometry (Figure 11e).

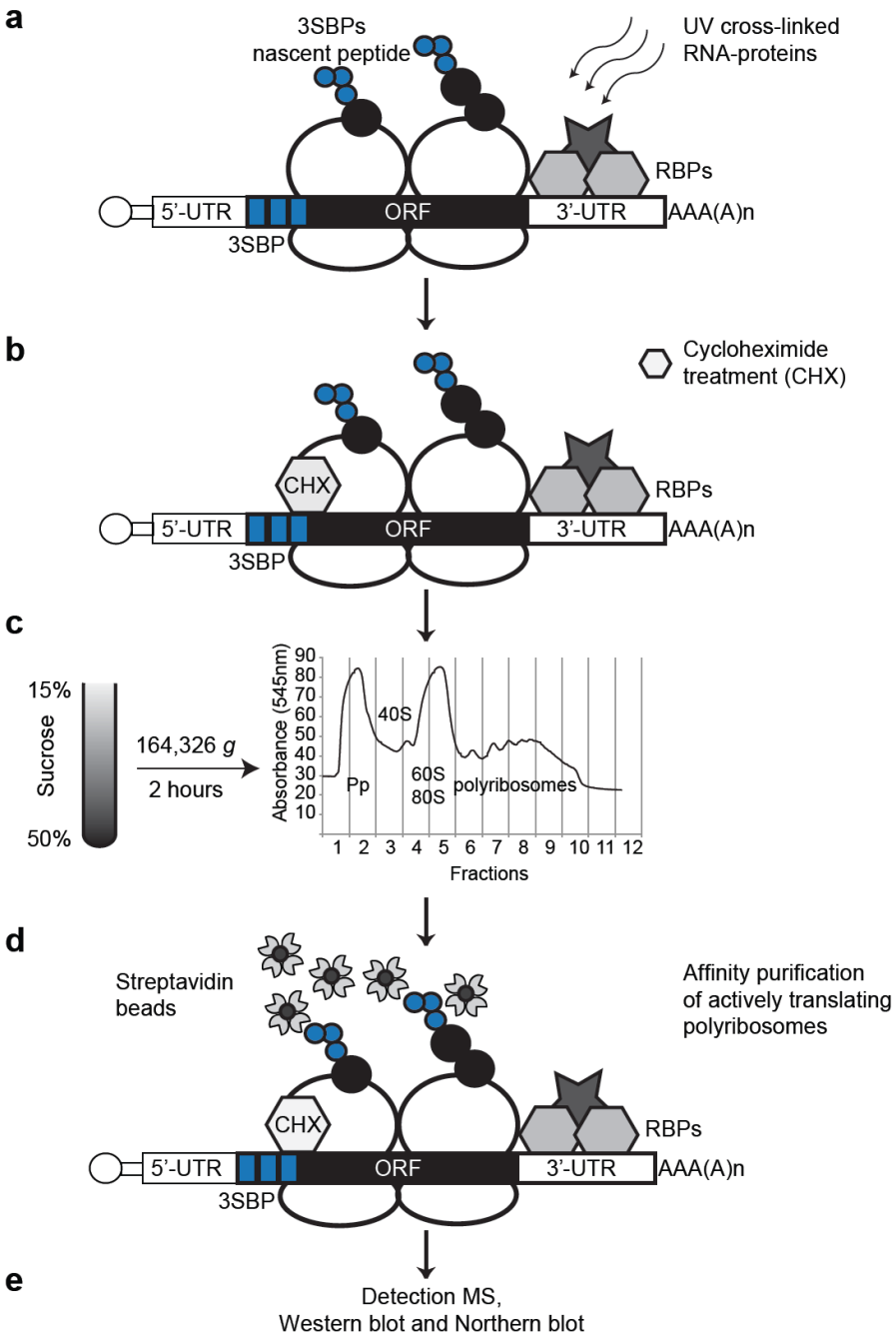


Figure 11. Workflow of the mRNP affinity purification.
(**a**) RNA and proteins were UV-cross linked at 254nm followed by (**b**) 5 min of incubation with cycloheximide, CHX. (**c**) Cells were lysed with detergent and the clear lysate loaded onto a sucrose gradient. (**d**) The fractions containing actively translating polyribosomes were pooled and incubated with streptavidin beads for 1 h.

(**e**) Proteins present in the purified mRNPs were detected by mass spectrometry (MS), Western blot and the pull down was assessed by Northern blot.
SBP, streptavidin binding peptide; RBPs RNA binding proteins; UTR, untranslated regions; ORF, open reading frame.

Table 1. Summary of problems found in the affinity purification method
The table shows the expected problems in the purification, the measures taken to overcome these problems and the outcome.

Problem 1. Mature SBP-CAT protein contains the tag and could represent a major contaminant.	
Possible solution	**Outcome**
Target the mature protein to the glycosomes via a glycosome targeting signal (SKL) at the 3'-end of the CAT open reading frame.	Successful, the SKL signal targets the mature protein to the glycosomes, this was measured using CAT assay (Figure 7b).
Separation of cytosol from organellar fraction (where glycosomes are present).	Successful, the glycosomes are separated by an initial ultra-centrifugation step. This was measured by CAT assay (Results Figures 4b and 7b).
Trial of different lysis methods without detergents (carbamide and glass beads), to avoid lysis of glycosomes.	Both methods gave similar results, carbamide method was chosen because it requires less time to partially lyse the cells (Figure 3).
Problem 2. SBP-tag not binding to the streptavidin beads	
Possible solution	**Outcome**
Increase the number of SBP-tags	Successful, *CAT* mRNA was detected by Northern blot (Figure 8).
Use of different matrixes (magnetic or sepharose beads)	Sepharose beads were chosen because less unspecific mRNAs bind (Figure 6). Also the amount of sepharose beads to use was tested (Figure 9).
Problem 3. Poor yield	
Possible solution	**Outcome**
Compare lysis with and without detergents.	Lysis with detergents gave a higher yield; represented by the *CAT* mRNA being detected by Northern blot (Figure 8).
Optimization of the cell number.	3-6x10^8 cells were used when lysing with detergent.
Problem 4. Higher background; abundant mRNAs binding unspecifically to the matrix	
Possible solution	**Outcome**
Block the lysate with Avidin-agarose beads to avoid the binding of biotinylated proteins to the matrix.	Not sure if it makes a difference
Block streptavidin beads with tRNA and heparin.	Not sure if it makes a difference

Problem 5. Not enough reporter mRNA produced	
Possible solution	**Outcome**
Calculate the number of reporter mRNA per cell.	Approximately, 400 molecules of *CAT* mRNA were produced in our cell lines (Figure 10).
Change the promoter to T7 polymerase.	Not used in the present work
Problem 6. Few peptides identified by mass spectrometry	
Possible solution	**Outcome**
Perform MS or DL-MS	Using DL-MS the number of proteins identified was less then when using only MS (compared Tables 3 and 4).
UV cross-link the samples.	This is protein dependent and it was successful for the purification of ZC3H11 (Figure 14).
If known, perform the MS search using data of modified peptides of the protein of interest	One modified peptide form ZC3H11 was found by MS

The following sections describe the standardization of the affinity purification method (results sections 3.2 to 3.8), the validation of the technique (results section 3.9 and 3.10) and the results obtained using this purification (results section 3.11 to 3.13).

3.2 Affinity purification of cytosolic polyribosomes

In order to overcome one of the main problems of this method (the mature protein containing the SBP-tag, see Table 1), it was decided to separate the cytosol from the organellar fraction.

The chloramphenicol acetyltransferase [124] gene was chosen as the ORF for the SBP-reporters because it allows the quantitative detection of the *CAT* mRNA by Northern blot and the mature protein detected by CAT assay or Western blot. Unfortunately, the currently available antibodies for the CAT protein do not work in trypanosomes, giving no specific signal in Western blots using total lysate (data not shown). Nevertheless, there is a strong CAT activity detected by CAT assay, so the protein was detected using this assay only. Furthermore, the inclusion of a serine-lysine-leucine signal (SKL) targeted the mature SBP-CAT-SKL proteins to the glycosomes, peroxisomal-like organelles, which were separated from the sample by an initial ultra-centrigugation step (separation of cytosol from organelles).

Initially, the purification of cytosolic mRNPs was performed using Strep-Tactin magnetic beads (Qiagen). In order to obtain enough cytosolic polyribosomes for the pull down and to detect proteins by mass spectrometry, different conditions were tried; such as different lysis methods (Figure 12), different initial ultracentrifugation conditions (Figure 13) and variations in the cell number.

3.2.1 Carbamide lysis was more efficient than glass beads lysis

Different lysis methods were tried, except for the use of detergents in the lysis buffer, this was not an option when purifying cytosol because the organelles, especially the glycosomes, had to remain intact.

Lysis by vortexing with glass beads was one of the tested methods; the other one was the carbamide method. Carbamide is an abrasive substance and lysis was accomplished by grinding the cells with a pellet pestle on ice (materials and methods 2.4). When using glass beads, the lysis was performed by vortexing for 25 min, 5 min vortex and 2 min pause on ice.
For both methods, the lysate was cleared by centrifugation and the supernatant was transferred to a new tube. A sample of input before lysis (In), pellet (P) and cleared lysate (C) were taken in order to determine which method was better to obtain *CAT* mRNA without degradation. Both experiments were done in parallel. For the quantification, the input from lysis was taken as 1.00 and the intensity of the other signals calculated with respect to 1.00 (Figure 12).

Lysis using the glass beads method rendered 25% of the *CAT* mRNA present in the lysate (Figure 12b), whereas using carbamide, 34% of *CAT* mRNA present in the input was obtained (Figure 12a). The other 70% could still be bound to the carbamide, degraded or precipitated with the nuclear and debris fraction. Taken together these results, it was concluded that both methods are similar. It was decided to use the carbamide method because it requires less time to achieve lysis, therefore reducing the possibility of *CAT* mRNA degradation (Figure 12).

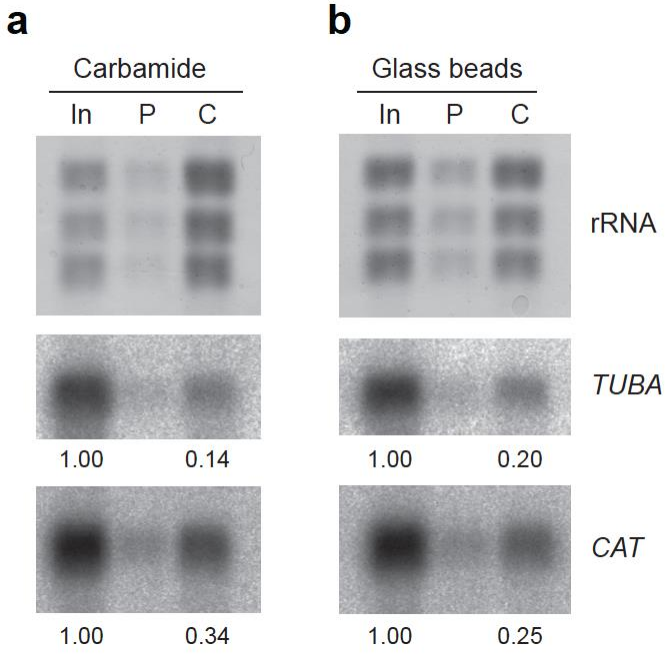


Figure 12. Different lysis methods
(**a**) Carbamide lysis ($3x10^7$ cells used).
(b) Lysis with glass beads ($3x10^7$ cells used).

(**a**) and (**b**) are Northern blots
rRNA, ribosomal RNA; *CAT*, chloramphenicol acetyltransferase mRNA; *TUB*, tubulin mRNA; In, input from lysis; P, pellet after the initial centrifugation; C, cleared lysate; *TUB, tubulin* mRNA; an abundant mRNA of approximately 2kb used as cytosolic loading control.

Alternatively, lysis with an electric pistol was also tried. The results showed that lysis with a pistol was similar to lysing with detergents, i.e. no preservation of the glycosomal membranes. Using this method, the cells were lysed completely, represented by higher CAT activity in the supernatant than in the pellet, measured by CAT assay.

3.2.2 Ultracentrifugation at 33,000 *g* separates cytosolic polyribosomes from the organellar fraction

To further continue with the standardization of the method, different centrifugation conditions were assessed. Cells were lysed using carbamide as described above (results 3.2.1). Additionally, an initial centrifugation step was added to pellet the glycosomes, this pellet was then lysed with Triton-X to release the content of the glycosomes. The integrity of the glycosomes was measured by CAT assay, which determined the relative CAT activity in the samples.

Initial ultracentrifugation at 17,000 *g* proved not to be enough to pellet all glycosomes. The relative CAT activity measured in the supernatant was higher than in the pellet, suggesting that there was more CAT protein in the supernatant (Figure 13a). Using 33,000 *g* was more effective in separating intact glycosomes, represented by a higher relative CAT activity in the pellet fraction, presumably containing the intact glycosomes (Figure 13b). Interestingly, it seemed to be more relative CAT activity in the pellet fraction than in the input in Figure 4b, this might be due to incomplete cell lysis in the input fraction, or perhaps the accumulation of CAT protein in the glycosomes (pellet) increases the signal.

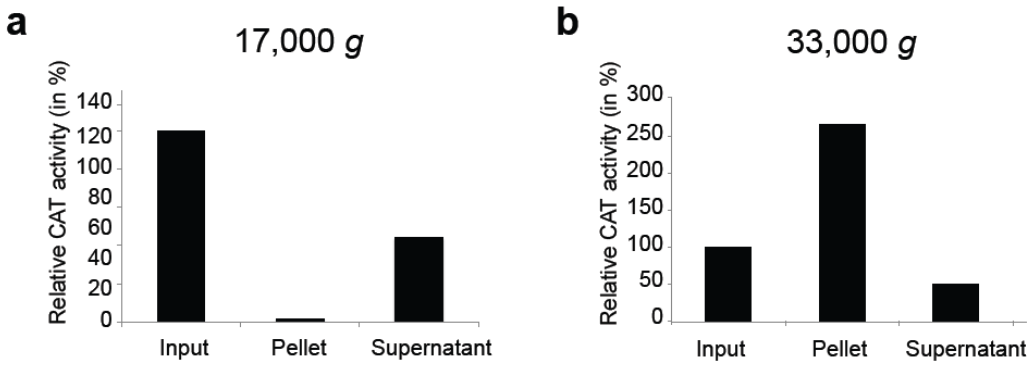


Figure 13. Centrifugation conditions tested
(**a**) Centrifugation using 17,000 *g*.
(**b**) Centrifugation using 33,000 *g*.
The presence of CAT protein was detected by measuring the activity of the CAT enzyme present in the samples via CAT assay.

In, Input of lysis; P, pellet (organellar fraction); S, supernatant (cytosolic fraction, without organelles).

Additionally, it was noticed that 6-8x10^8 cells were needed to obtain a detectable signal for the *CAT* mRNA in the cytosolic purification, using less cells made the detection by Northern blot difficult.

Once the conditions for lysis (carbamide lysis), cell number (8x10^8 cells) and initial centrifugation (33,000 *g*) were decided, the next step was to determine the number of tags to use. In previous experiments, the purification of cytosolic polyribosomes was performed with cells expressing reporters that contained only one SBP. Despite the standardization, no *CAT* mRNA was detected in the beads by Northern blot. The experiment was repeated using different incubation times but there was no change in the results, maybe because the nascent peptide tag and the streptavidin beads were not interacting properly.

3.3 Multi-tag purification of cytosolic polyribosomes

The idea to change the number of tags was taken from a previously published article, where it was shown that the use of one SBP was not sufficient for protein detection using a streptavidin matrix [152]. N-terminal strep tags were fused to a model protein using the SQM protein (Stefin A Quadruple Mutant). Fusion proteins containing from 1 to 5 SBPs, separated by glycine linkers were tested. Busby *et al* showed that adding 3SBPs increased the binding to streptavidin 12-fold compared to a single strep-tag (Busby, Stadler et al. 2010). Taking these results into consideration, it was decided to increase the amount of SBPs to three with glycine linkers separating the tags. With this strategy the multi-tag present at the 3'-end of the *CAT* when translated was 30 amino acids long.

3.3.1 Purification using 3SBPs was more efficient than using a single tag

For these purifications only the number of SBPs was changed, the rest of the procedure remained the same. When using 3SBPs at the 3'-end of the *CAT*, less cells were needed for the detection of *CAT* by Northern blot, thus half the amount of cells was taken to purify cytosolic polyribosomes translating the reporters containing 3SBPs.

3 x 10^8 cells were lysed with carbamide and the *3SBPs-CAT* mRNA purified using magnetic beads. Samples of the input from polyribosomes (In pol), unbound fraction (UB) and beads (B) were taken for Northern blot. In case of In pol and UB, the same amount of sample was used for RNA extraction and loaded in the Northern blot. For the beads, 5 times more sample was taken. To quantify the amount of *CAT* mRNA obtained in the purification, the signal of input from polyribosomes was taken as 1,00 and the other signals calculated in respect to this one. Approximately 20% of *CAT* present in the polyribosomes was purified, although a signal for α-tubulin, *TUBA* was also detected, representing the presence of contaminants. With this purification, *CAT* was enriched 1.6-fold when compared with *TUB* (Figure 14).

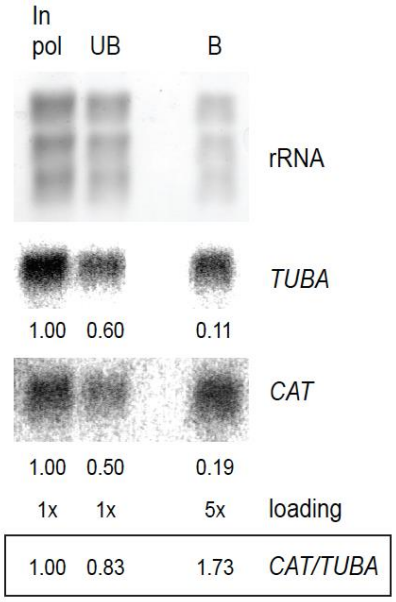


Figure 14. Multi-tag purification of cytosolic polyribosomes
Northern blot showing the amount of *3SBPs-CAT* mRNA obtained from the cytosolic purification. Five times more sample was loaded for the beads (5x) and the same equivalent cell number taken for Input and unbound (1x). The ratio *CAT* versus *TUB* represents the enrichment of *CAT* in the samples. This experiment was repeated at least three times giving similar results.
rRNA, ribosomal RNA; *CAT*, *chloramphenicol acetyltransferase* mRNA; *TUB*, *tubulin* mRNA; In pol, input from polyribosomes; UB, unbound; B, beads.

3.4 Biotin was not sufficient to elute the purified cytosolic mRNPs

As shown in Figure 14, there was still *TUBA* mRNA present in the purification. To reduce unspecific interactions, different types of streptavidin beads and biotin elution were tested (Figure 15). *TUBA*, was used as control for unspecifically purified contaminants.

An experiment using sepharose and magnetic streptavidin coated beads was performed in parallel. In both cases, $6x10^8$ cells were used for the purification. Elution with biotin was performed using 10mM biotin in the elution buffer (elution was done according to Qiagen protocol). To quantify the samples, the intensity of the signals were measured and compared to the signal of the input from polyribosomes (taken as 1.00). The ratio *CAT* versus *TUB* was used to determine the enrichment of *CAT* over *TUB* in the samples.

Neither *CAT* nor *TUB* signals could be measured by Northern blot in the eluates of either magnetic or sepharose beads. When measuring the signal in the magnetic beads, 7% of the *CAT* present in the polyribosomes still remains in this fraction (Figure 15a). Using sepharose beads, stronger signal intensity for *CAT* in the beads was measured when compared to the magnetic beads (Figure 15b).

It appears that most of the *CAT* mRNA was still bound to the beads; the signal in both eluates could not be quantified. The elution with biotin was not efficient for any of the matrixes used.

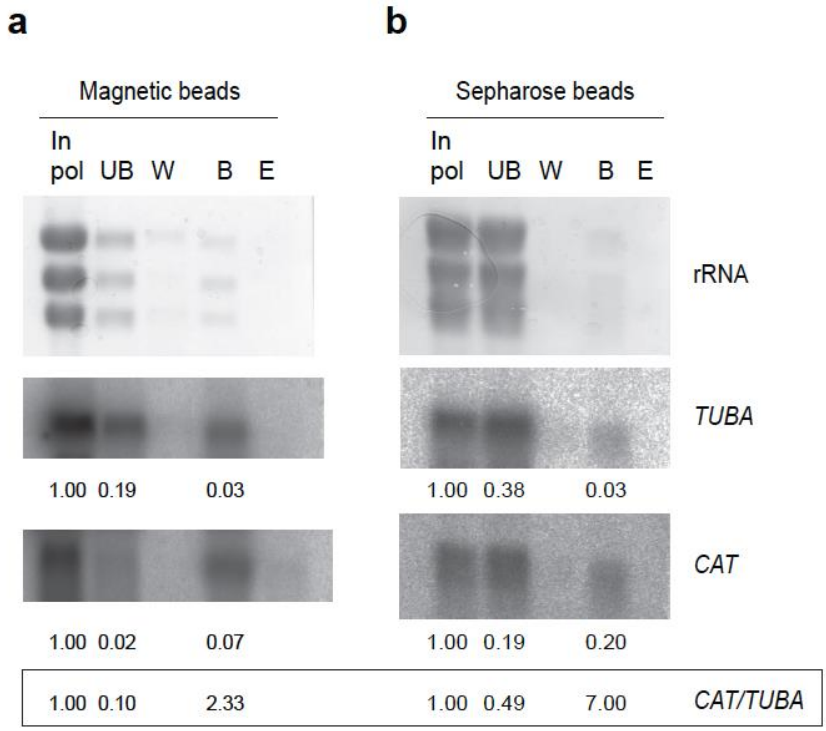


Figure 15. Elution using biotin
(**a**) Purification using magnetic beads.
(**b**) Purification using Sepharose beads.
For quantification purposes, the input from polyribosomes was assumed to be the 1.00. The ratio *CAT*/*TUB* determined the enrichment of *CAT* in the samples.
rRNA, ribosomal RNA; *CAT*, chloramphenicol acetyltransferase mRNA; *TUB*, tubulin mRNA; In, input from polyribosomes; UB, unbound; W, wash; B, magnetic or sepharose beads; E, eluate.

This experiment was repeated at least once more giving similar results. Additionally, the purification using sepharose beads was repeated and the eluate and beads were loaded onto a SDS-PAGE gel and silver staining was performed. In both cases, no bands were observed in the eluted fraction, only in the beads, corroborating the inefficiency of the elution with biotin maybe because of many proteins or other RNAs binding to the beads, blocking the interaction between biotin and the streptavidin present in the beads.

3.5 CAT protein was mainly detected in the organellar fraction

In order to measure the amount of CAT protein present in each step of the cytosolic purification a CAT assay was performed. In this assay $8x10^8$ cells were lysed with carbamaide and the cleared lysate loaded onto a sucrose gradient. The polyribosome profile obtained is shown in Figure 16a representing a profile of cytosolic polyribosomes (not membrane bound polyribosomes). The polyribosomes, fractions 6-9, were pooled and the affinity purification using sepharose beads performed (Figure 16a). The

protein amount in each sample was measured by Bradford assay. For CAT assay, 0.5 µg of total protein was used. Samples from each step of the purification were taken (Figure 16b).

Almost half of the CAT activity was detected in the pellet (P), which represents the intact glycosomes (organellar fraction). 25% was detected in the input from polyribosomes (In pol), corresponding to fractions 6 to 9 of the polyribosome profile and approximately, 7% of the CAT activity was detected in the eluate. This demonstrates that the mature CAT protein did not really represent a main contaminant in the samples and that this protein was partially separated from the cytosolic polyribosomes by the initial ultracentrifugation. Also by performing a polyribosomal fractionation the mature CAT protein was further separated from the sample, most of it was detected to be present in the protein peak fraction (Figure 16b).

The results of the CAT assay showed that only a small percentage of the activity of the protein was still present in the eluate (Figure 16b), meaning that the step of sucrose gradient centrifugation was necessary to separate the mature CAT protein from the purification. Nevertheless, the amount of polyribosomes obtained did not allow the detection of *CAT* mRNA via Northern blot.

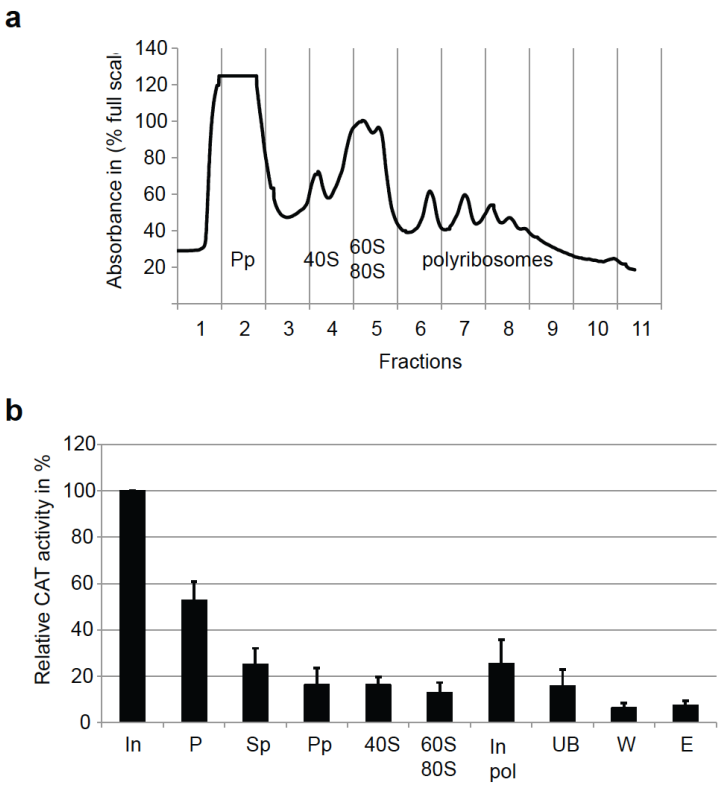


Figure 16. Cytosolic polyribosome fractionation and CAT activity
(**a**) Cytosolic polyribosome profile. Cells lysed with carbamide.
(**b**) Relative CAT activity measure throughout the purification.
This experiment was repeated twice with the same results.
CAT, chloramphenicol acetyltransferase; In, input from lysis; P, pellet; Sp, supernatant (cytosolic polyribosomes); 40S and 60S, ribosomal subunits; 80S,

monosomes; In pol, input from polyribosomes (fractions 6 to 9); UB, unbound; W, wash; E, eluate.

The cytosolic purification was repeated and the entire sample from the beads loaded onto an SDS-PAGE and stained with Colloidal Coomassie. Unfortunately, almost no proteins were observed with the Coomassie blue or silver staining. The main problem of the cytosolic purification was the lower yield obtained compared to other purifications (such as V5-pull down or TAP purification). The amount of material needed for mass spectrometry would have to be higher, at least to allow the visualization of stained proteins by Coomassie blue. For this reason, the step of separating cytosolic and membrane bound polyribosomes was omitted from further purifications. Instead, lysis with detergents was chosen although this implied the disruption of glycosomal membranes.

3.6 Lysis with NP40 allowed the detection of the *3SBPs-CAT* reporter

To further standardize the technique, another purification was tried by lysing the cells with detergents. When lysing with detergents, the amount of mRNAs containing polyribosomes increased in comparison with the ones obtained in the cytosolic purification (compare figures 16a and 17a). The amount of highly translated mRNAs, represented as mRNAs containing several ribosomes, increased; noticed by the higher absorbance in the last sucrose fractions (compare figures 16a and 17a).

In this case the affinity purification was performed using sepharose beads and cells were lysed with 0.01% of NP40. After performing the affinity purification, approximately 20% of the *CAT* present in the polyribosomes was purified; also 8-fold enrichment of *CAT* mRNA in comparison to other abundant mRNAs such as *TUBA* was measured (Figure 17b). The amount of abundant mRNAs such as *TUBA,* which bind unspecifically to the beads, was slightly reduced (compare Figure 15b and 17b). This purification was repeated and beads were loaded into an SDS-PAGE, this time there were protein bands visible after Coomassie blue staining and the samples were send to dimethyl-labelled quantitative mass spectrometry (DL-MS).

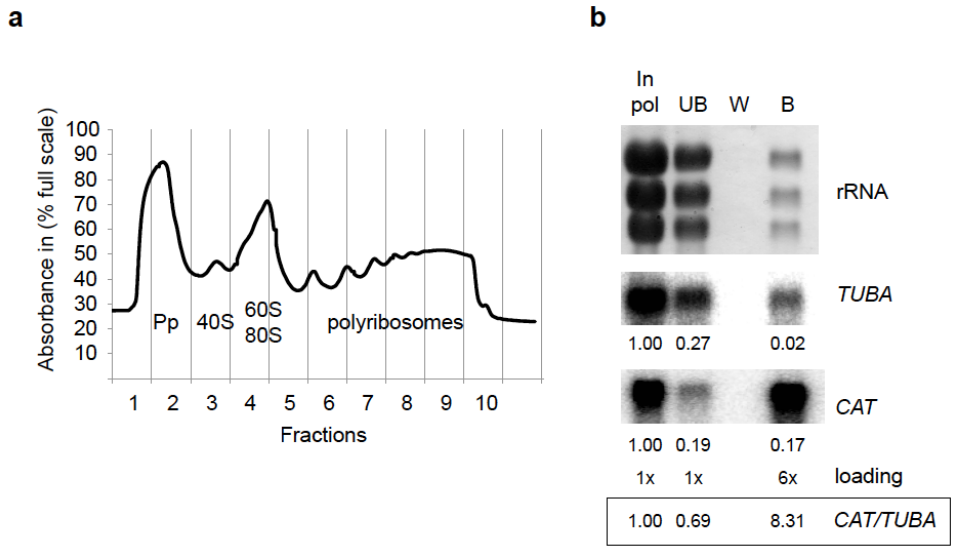


Figure 17. Affinity purification using detergent
(**a**) Polyribosome profile of cells lysed with 0.01% of NP40.
(**b**) Affinity purification of pooled polyribosomes (fractions 6 to 9).
The intensity of the signals was measured by taking the input from polyribosomes as 1,00. The ratio *CAT* versus *TUB* was used to determine the enrichment of *CAT* over *TUB* present in the samples.
rRNA, ribosomal RNA; *CAT*, chloramphenicol acetyltransferase mRNA; *TUB*, tubulin mRNA; In pol, input from polyribosomes; UB, unbound; W, wash and B, beads

Since total polyribosomes were used for the affinity purification, the number of washes with high salt buffer (AP buffer) was increased to five; this seemed to reduce the unspecific binding of abundant mRNAs.

Furthermore, two different polyribosomal buffers were tested (see material and methods section 2.5), one of them used 280mM NaCl (Schutz modified) and the second one used 120mM KCl (Kramer modified). Similar results were obtained using both buffers, meaning, that when performing the polyribosomal purification in both cases the *3SBP-CAT* mRNA was detected to be bound to the matrix whereas the signal of the control reporter was not detectable by Northern blot. The main difference was that Kramers' modified buffer seems to give a better resolution of the polyribosomal fractions, for this reason, it was chosen to work with the KCl buffer.

3.7 Optimization of the amount of beads per purification

In order to improve the purification efficiency, different amounts of beads were tested.

The polyribosomes of three independent sucrose gradient centrifugations were pooled together, then separated into three affinity purifications and purified using different amount of beads (Figure 18a).

The amount of *CAT* purified from the polyribosomes was similar using 50µl and 75µl of beads. Half of the *CAT* signal was detected by purifying with 25µl of beads compared to 50µl. Furthermore, when looking for abundant mRNAs

that can bind unspecifically to the beads, the signal for *TUBA* was lower with 25µl of beads in comparison to 50µl or 75 µl of streptavidin sepharose beads.

The ratio *CAT* versus *TUB*, representing the enrichment of *CAT* mRNA, was the same using 25µl and 50µl of beads, 30% of the *CAT* mRNA present in the polyribosomes was purified. In the case of the purification using 75 µl 25% of *CAT* was purified (Figure 18b).

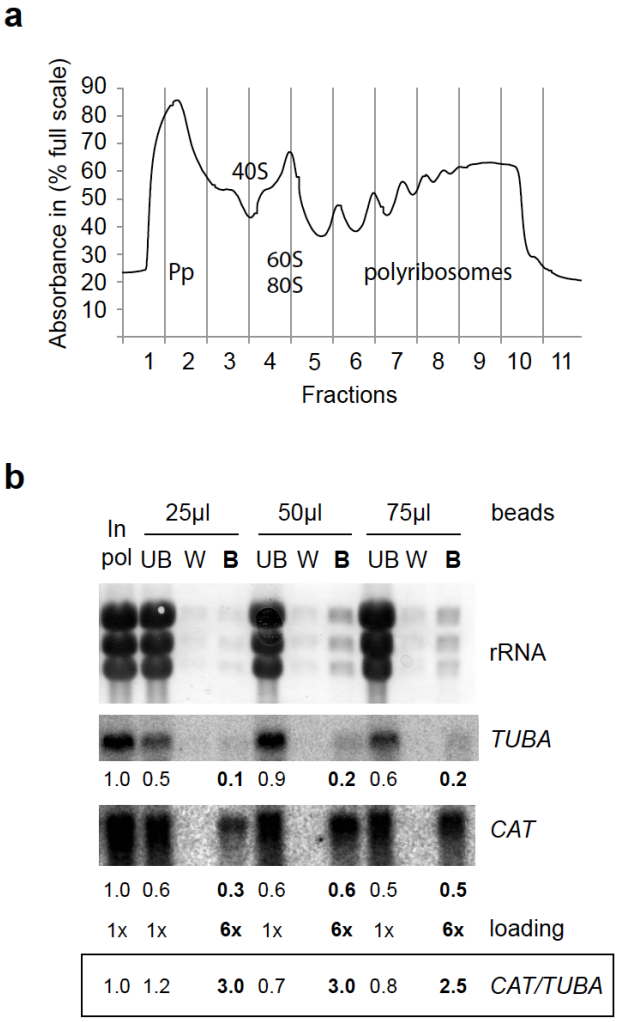


Figure 18. Different amounts of sepharose beads tested
(**a**) Representative polyribosome profile. The polyribosomes of three independent gradients (approximately 7x10^8 each) were pooled to test the adequate number of beads necessary for the purification.
(**b**) Northern blot of the three parallel affinity purifications. The signal of input from polyribosomes was taken as 100% and the intensity of the other signals compared to it.
rRNA, ribosomal RNA; *CAT*, chloramphenicol acetyltransferase mRNA; *TUB*, tubulin mRNA; In pol, input from polyribosomes; UB, unbound; W, wash and B, beads (in bold).

The conditions used for further purifications were as followed: 3-6x10^8 cells were collected for lysis with detergents and the clear lysate loaded onto a

sucrose gradient. The polyribosome fractions were pooled and specific mRNPs purified using 25µl of streptavidin coated sepharose beads.

3.8 Measurement of *3SBPs-CAT* mRNA molecules per cell

After the optimization steps being done, the last step was to calculate the number of *CAT* mRNA molecules produced per cell because it gave an idea of the amount of mRNA available for translation, pull-down and purification.

To determine the number of the *3SBPs-CAT* molecules per cell, an *in vitro* transcription reaction was performed using a plasmid that encoded the *CAT* reporter gene under the control of a T7 promoter. The *in vitro* transcribed *CAT* was compared to the lysate of the *in vivo 3SBPs-CAT* reporter.

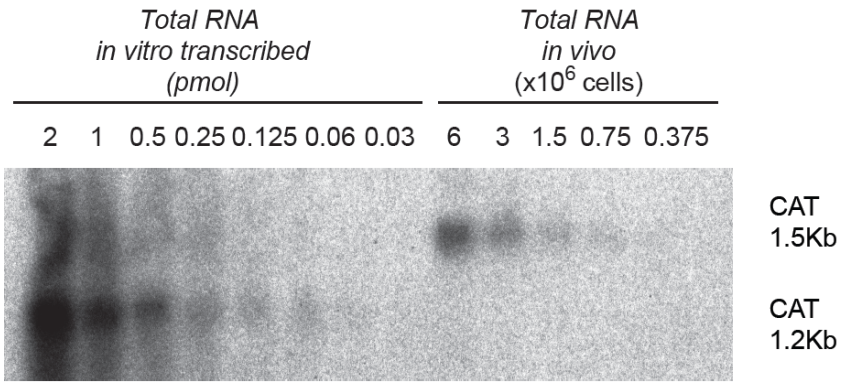
For the quantification, serial dilutions of defined amounts of *in vitro* transcribed *CAT* mRNA in picomol were loaded (2-0.03pmol) in the formaldehyde gel and compared with distinct equivalent cell numbers of total RNA extracted from distinct equivalent numbers of cells expressing the *3SBPs-CAT* mRNA. The difference in sizes of the *CAT* mRNAs were due to the lack of 5'-cap and the 3'-poly(A) tail in the *in vitro* transcribed *CAT* (Figure19a).

The intensity of the signals for the *in vitro* transcribed *CAT* gave the values of the standard curve and were compared to the quantification of the signals for an equivalent cell number of $6x10^6$, $3x10^6$, $1.5x10^6$, $7.5x10^5$ and $3.75x10^5$ (Figure 19a). This allowed the determination of 5.7fmol/µl of *3SBPs-CAT* reporter mRNA in the sample, approximately $34.2x10^8$ molecules.

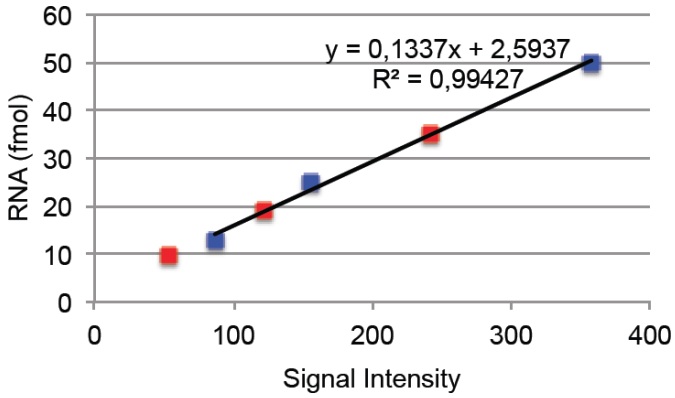
A total of $3x10^8$ cells were lysed for this experiment, obtaining 17.4µg of total RNA from this amount of cells. In the Northern blot an equivalent of $6x10^6$ cells were loaded, corresponding to 8.7µg of total RNA.

The calculations indicate that $3.4x10^9$ molecules of *3SBP-CAT* mRNA were present in 8.7µg of total RNA. Assuming that one trypanosome cell contains 1.1pg of total RNA, it was calculated that the cells used for the affinity purification produced 429 molecules of *3SBPs-CAT* mRNA per cell (Figure 19b). This number is comparable to other multi-copy genes that produce abundant mRNAs, such as *TUBA* [48]. The reason behind this was that RNAPI transcribes the single copy 3SBP-CAT gene, whereas RNAPII transcribes *TUBA* and as it was previously been published, transcription by RNAPI produces 10-fold more protein than RNAPII [32].

a



b



Calculating the number of *3SBPs-CAT* per cell

5.7 fmol/µl *CAT* mRNA in moles 5.7×10^{-15}

$5.7 \times 10^{-15} * 6 \times 10^{23} = 34.2 \times 10^{8}$ molecules

3×10^{8} cells yield 17.4 µg of total RNA

8.7 µg of RNA loaded is equivalent to 6×10^{6} cells

If 1.1 pg of total RNA per cell

$(3.4 \times 10^{9} / 8.7 \times 10^{-6}) * 1.110 \times 10^{-12}$

429 molecules of *3SBPs-CAT* per cell

Figure 19. Number of *3SBPs-CAT* mRNA per cell

(**a**) Northern blot comparing the *in vitro* transcribed *CAT* mRNA, under the control of a T7 promoter (dilution series in picomol), with the in vivo *3SBPs-CAT* mRNA, under the control of the polymerase I promoter (different equivalent cell numbers).

(**b**) Calculation of the number of *3SBPs-CAT* mRNA per cell. The *in vitro CAT* was used as standard. The standard curve was calculated from three samples of the serial dilutions and the signals detected from the in vivo experiment included. The calculation of RNA molecules was done taking in consideration that a single trypanosome cell contains 1.1pg of total RNA [48].

3.9 Purification of the *HSP70* 3'-UTR reporter

Validation of the method was done using a known RNA-protein interaction. It has previously been shown [107] that in trypanosomes, the zinc finger protein, ZC3H11 binds to an AU-rich element present in the *HSP70* 3'-UTR. This interaction stabilizes the mRNA upon heat shock.

Therefore, stable cell lines expressing an endogenous V5-tagged ZC3H11 were transfected with reporters containing the *HSP70* 3'-UTR or the reporter containing the *HSP70* 3'-UTR without the AU-rich element.

3.9.1 Heat shock at 39°C allows the purification of the *HSP70* 3'-UTR reporter and detection of ZC3H11

As a first step it was necessary to determine the heat shock conditions for these purifications. Heat shock was necessary because the ZC3H11 protein is only expressed in detectable levels under stress. Another important criteria was to heat shock the cells using a temperature that allows the purification, meaning when polyribosomes do not disassemble completely.

For this experiment, an exponentially growing culture of procyclic trypanosomes was divided in four parts to test the different heat shock conditions. It is important to mention that, procyclic form trypanosomes are grown in culture conditions at 27°C. The polyribosome profiles show the effect that a change of only two degree Celsius has on translation (Figure 20).

According to the heat shock experiments, it appears that at 37°C (Figure 20b) the polyribosomes of procyclic trypanosomes were similar to cells without treatment (27°C), only a slight reduction of polyribosomes was observed when comparing to standard culture conditions. Furthermore, when using 37°C the V5-ZC3H11 protein was barely detectable by Western blot after the affinity purification (data not shown).

When using 41°C (Figure 20d), almost all the polyribosomes were disassembled, the reduction observed was similar to cells treated with RNases.

The best condition was heat shock at 39°C (Figure 20c), the effect on translation was clear, the polyribosomes disassembled (fractions 6-9) and the monosomes increased (fractions 4-5). Nevertheless, there were still polyribosomes present to perform the pull down and the V5-ZC3H11 protein was detectable by Western blot. For this reason, heat shock at 39°C was chosen for further heat shock experiments.

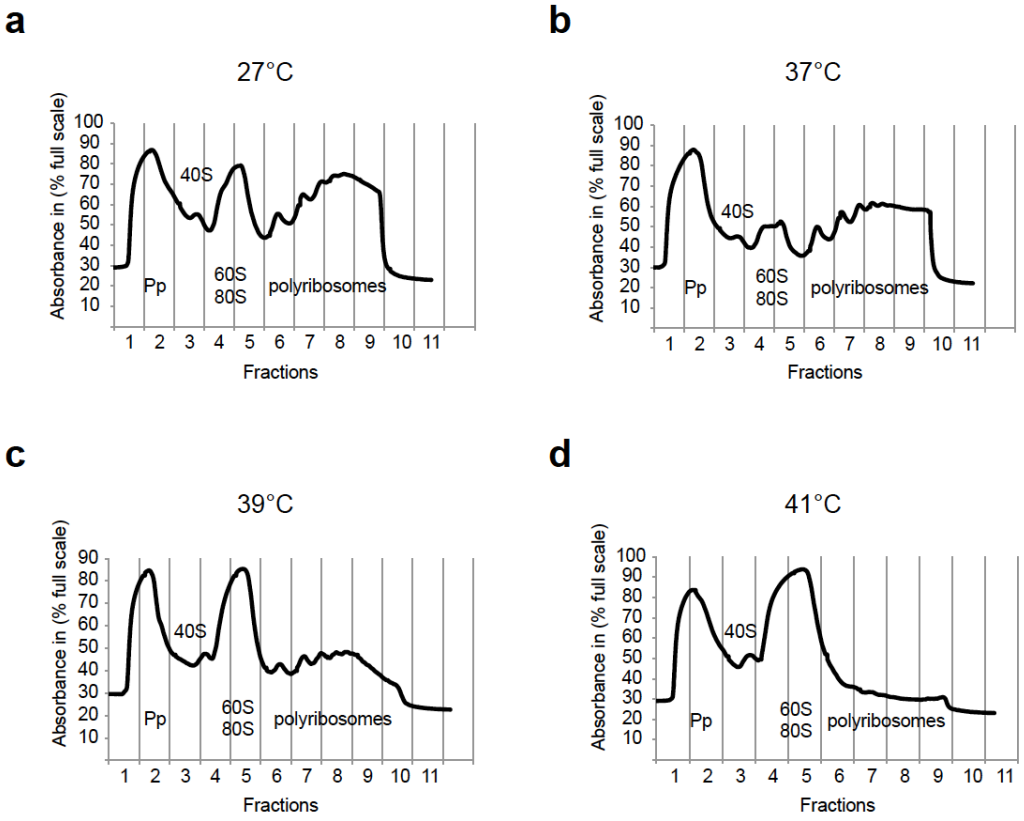


Figure 20. Polyribosome profiles in culture and heat shock conditions
(**a**) Polyribosome profile of untreated cells. Normally procyclic form trypanosomes are cultured at 27°C.
(**b**) Heat shock using 37°C for 1 hour.
(**c**) Heat shock at 39°C for 1 hour.
(**d**) Heat shock at 41°C for 1 hour.
Pp, protein peak; 40S, ribosomal subunit 40S; 60S/80S, ribosomal subunit 60S and monosomes 80S (the peak for these fractions could not always be separated).

Additionally, the presence of the *3SBP-CAT-HSP70* 3'-UTR mRNA was detected by polyribosomal fractionation. Upon 1 hour heat shock at 39°C, the reporter mRNA remained in the polyribosomal fractions and it seems to be evenly distributed among the higher sucrose fractions (8-11, Figure 21).
As loading control, the ribosomal bands of the rRNA are shown (methylene blue staining), indicating that the polyribosomes, corresponding to fractions 8 to 11, were equally loaded.

An additional control was the *HSP70* mRNA that is stable upon heat shock. The signal of the *HSP70* mRNA was more abundant in the higher sucrose fractions (10-11), characteristic of a highly translated mRNA, multiple ribosomes associated to this necessary mRNA upon heat shock. Its pattern was different to the one exhibit by the *CAT* mRNA, despite the fact that both mRNAs contained the same 3'-UTR (Figure 21b), maybe because the *HSP70* mRNA is approximately 2.8Kb and the *CAT* 1.5Kb.
Also it was noticed that the *CAT* mRNA was distributed equally throughout the polyribosomal fractions, maybe because the 5'-UTR of the reporter is different (*EP* 5'-UTR). The advantage of this distribution is that, at least in theory, this

can allow the purification of proteins that are bound initially to the translating mRNPs and of proteins that are recruited later on during translation.

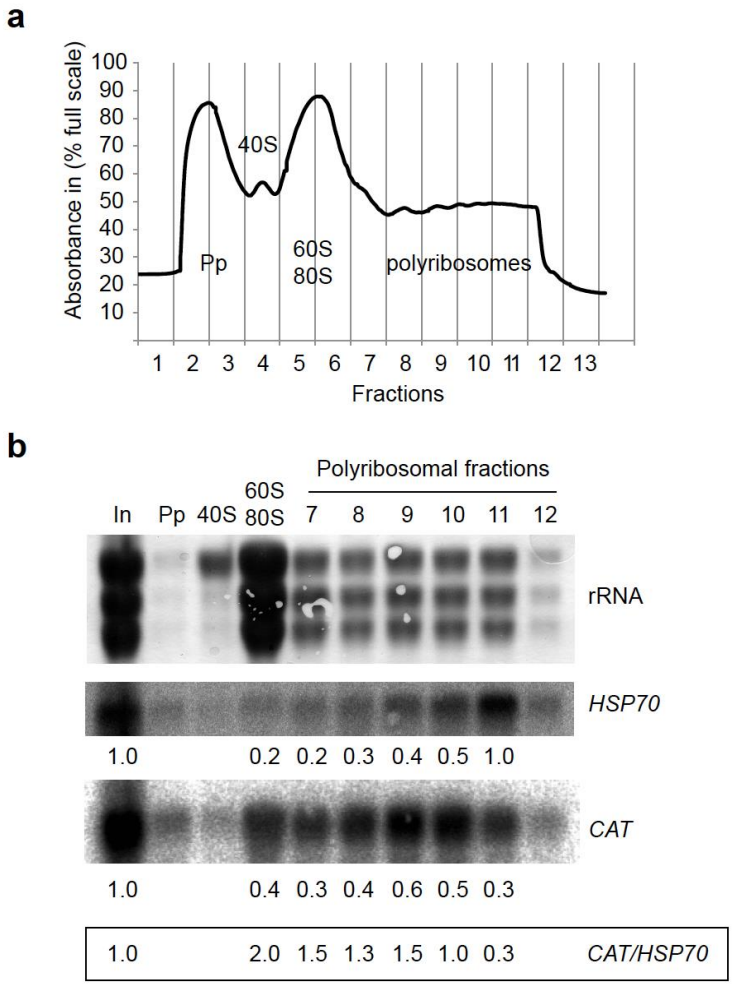


Figure 21. Distribution of the *HSP70* reporter in the polysomal gradient
(**a**) Polyribosome profile of the cell line expressing the *HSP70* reporter. 6x10^8 cells were heat shocked for 1 hour at 39°C
(**b**) Northern blot showing the distribution of the reporter. As heat shock control, the pattern of *HSP70* mRNA is shown. Ratio CAT/HSP70 was shown as a reference of the different translation patterns of the mRNAs throughout the sucrose gradient.
Pp, protein peak (fractions 2-3); 40S, ribosomal subunit 40S (fraction 4); 60S/80S, ribosomal subunit 60S and monosomes 80S (fractions 5-6); rRNA, ribosomal RNA; *CAT*, chloramphenicol acetyltransferase reporter mRNA (contains the 3SBPs and the HSP70 3'-UTR); *HSP70*, heat shock protein 70 mRNA; In, input from lysis (2x10^7 cells used for RNA extraction).

3.9.2 ZC3H11 co-purified with the *HSP70 3'-UTR* reporter

To validate the technique, the detection of proteins bound to the *HSP70* mRNA was necessary. This includes proteins that bind directly to the mRNA, like ZC3H11 [107] and also proteins that do not bind directly to the mRNA, such as MKT1 [108]. For this experiment, 4 cell lines were generated; two expressing the tagged proteins of interest, V5-ZC3H11 and V5-MKT1. Each of them was transfected with the *3SBPs-CAT-HSP70* 3'-UTR reporter. In parallel

cell lines expressing the *3SBPs-CAT-HSP70 AUU-del* reporter were generated.

The cell lines used were:
1. V5-ZC3H11 transfected with *3SBPs-CAT-HSP70* 3'-UTR reporter
2. V5-MKT1 transfected with *3SBPs-CAT-HSP70* 3'-UTR reporter
3. V5-ZC3H11 transfected with *3SBPs-CAT-HSP70 AUU-del* control reporter
4. V5-MKT1 transfected with *3SBPs-CAT-HSP70 AUU-del* control reporter

The conditions established were as follows: Heat shock the cells for 1 hour at 39°C then proceed with the UV cross-linking of RNA-protein complexes in order to detect the protein by Western bot. Addition of cycloheximide and ultracentrifugation of the cleared lysate to obtain the polyribosomes. As a final step the polyribosomes were pooled and affinity purification performed (Figure 11). All procedures were done on ice and as fast as possible to avoid recovery of the cells from heat shock, as the published estimated time of recovery was 30 min [163].

The affinity purifications for each V5-tagged protein was performed in parallel, using cells translating the mRNA containing the complete *HSP70* 3'-UTR and the deleted version. Note that both reporters can bind to the streptavidin beads because both contained the 3SBPs (Figure 22a). Prior to the extraction of RNA samples for Northern blot; Proteinase K treatment was necessary (material and methods 2.5). Northern blots were used in order to confirm that the pull downs worked.
Northern blots using the cell line with the V5-ZC3H11 were repeated more than 3 times obtaining the same results, meaning that both reporters were bound to the beads.
The pull down using a cell line expressing V5-MKT1 was repeated three times but only in one of them the *CAT* signal was detected for both reporters by Northern blot (Figure 22c). The quantitation of this blot was done taking the unbound as 1.00 because the signal for the input seem to be degraded and cannot be consider as 100%, in the purification with the deletion. In the other Northern blots no CAT signal was detected, probably because there was not enough RNA extracted from the sample, since most of it was used for the Western blot (Figure 22b and c).

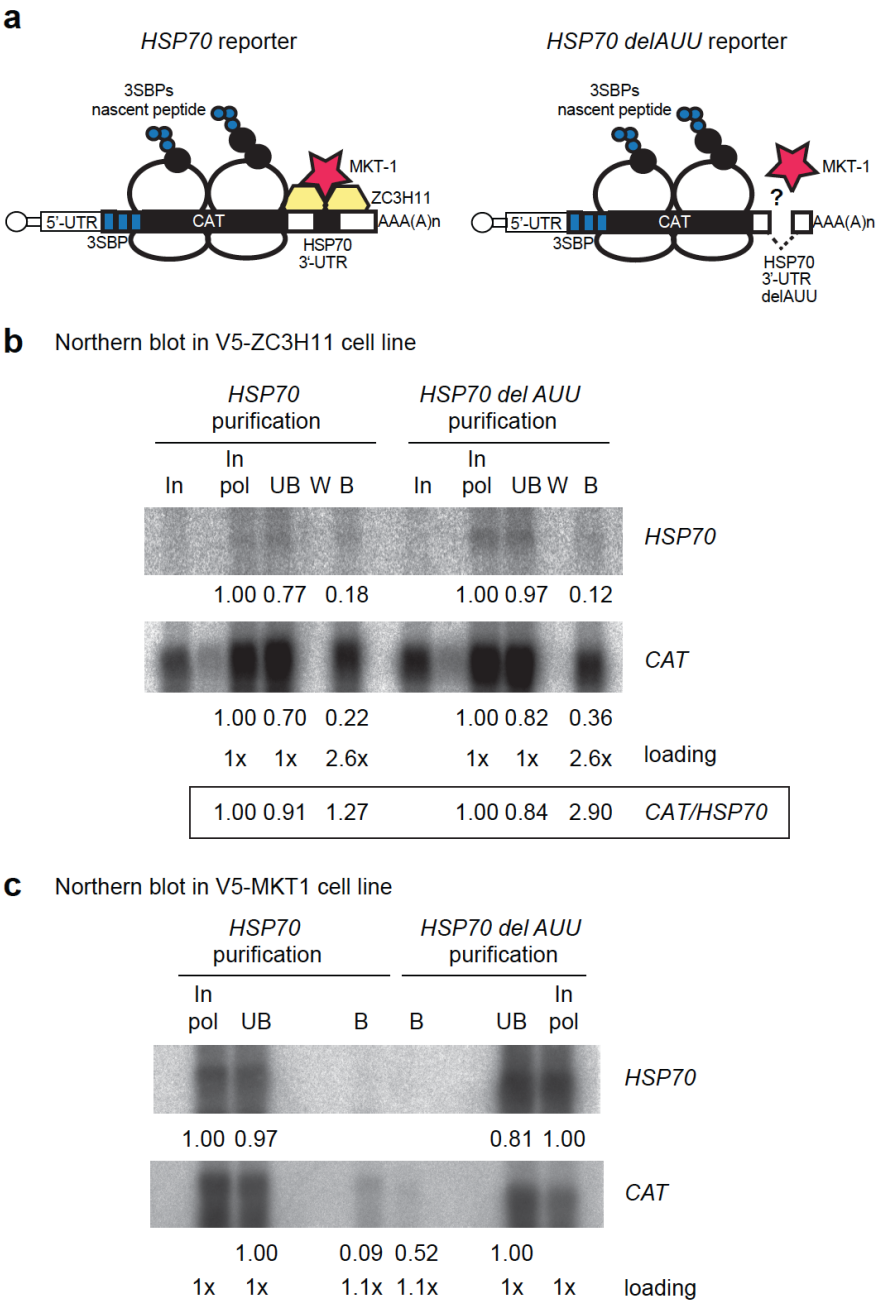


Figure 22. Pulling down the *CAT-HSP70* reporters using the affinity purification method

(**a**) Schematic representation of the reporters used for the purification. The control reporter has a deletion of the AU rich element present in the *HSP70* 3'-UTR. Both reporters were able to bind to the matrix.

(**b**) Northern blot of the affinity purification in V5-ZC3H11 cell line. The signal for *CAT* mRNA was present in both purifications.

(**c**) Northern blot of the affinity purification in V5-MKT1 cell line. The signal for *CAT* mRNA was detected in both purifications. The unbound was taken as 1.00 to measure the *CAT* mRNA signal.

In the Northern blots *HSP70* mRNA was used as loading control.

3SBPs, three streptavidin binding proteins; CAT, chloramphenicol acetyl-transferase; ZC3H11, zinc finger protein 11; MKT-1, maintenance of k2 killer toxin; PABP, poly A binding protein, binding protein; HSP70, heat shock protein 70; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

Among the proteins of interest was V5-ZC3H11, which, as previously published [107], binds directly to the mRNA via the AU-rich element present in the *HSP70* 3'-UTR, when this AU-rich element was not present, then ZC3H11 was not able to bind its target mRNA.

Figure 23b showed that ZC3H11 was enriched 26-fold in the purification using the complete *HSP70* 3'-UTR reporter, whereas, it is impossible to quantify in the purification with the AU rich element deleted. This experiment was repeated at least three times and in all the experiments V5-ZC3H11 was enriched (2 to 5-fold) in the purifications containing the complete *HSP70* 3'-UTR when comparing with the deleted one.

In the case of MKT1, this protein was found to be enriched 4-fold in the purification containing the complete *HSP70* 3'-UTR (Figure 23c). This experiment was repeated four times and in all replicates MKT1 was enriched between 4.7-1.3 fold in the complete *HSP70* 3'-UTR purification compared to the deleted *HSP70* 3'-UTR purification.

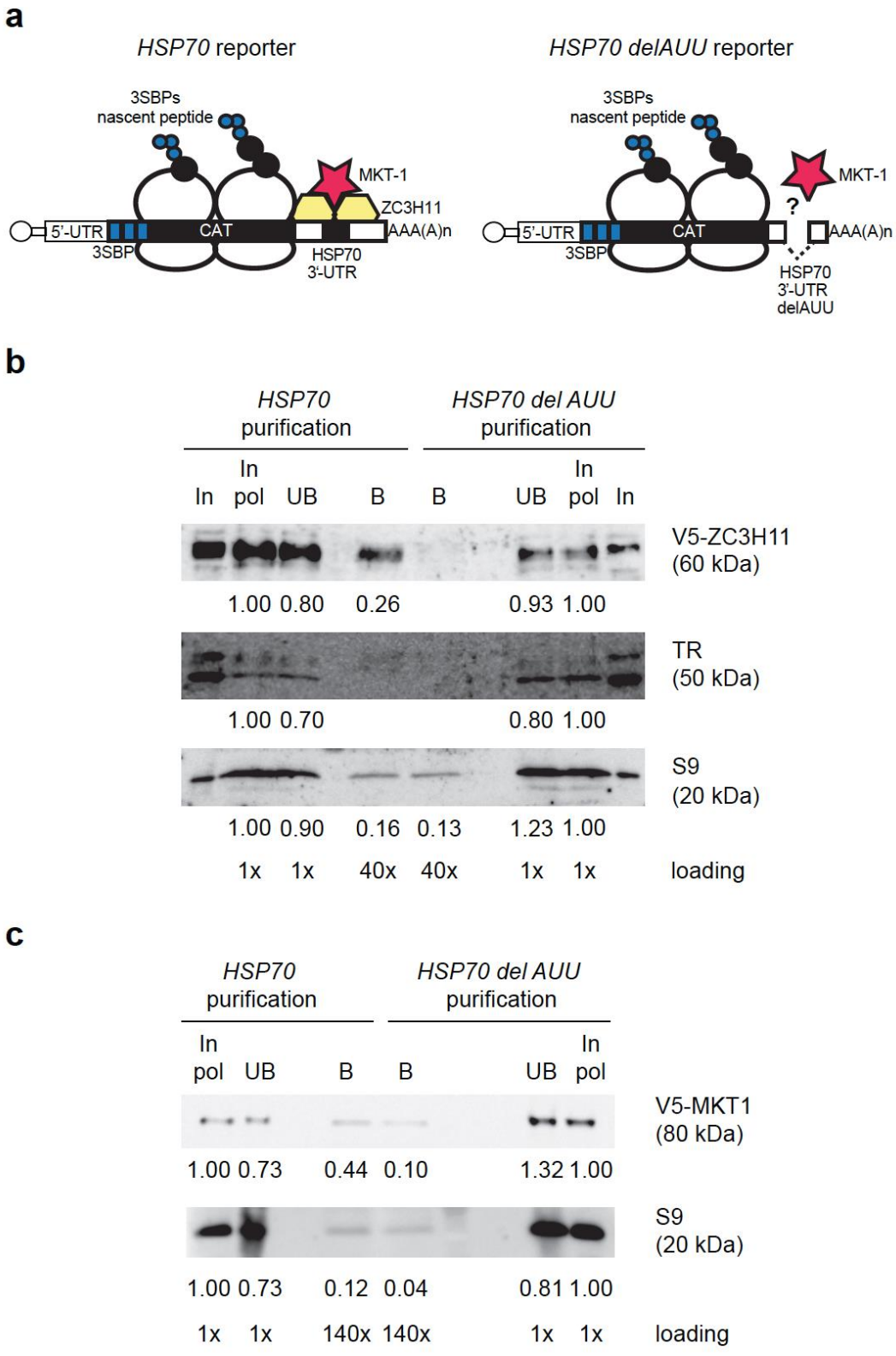


Figure 23. Proteins bound to *HSP70* 3'-UTR detected by the affinity purification method

(**a**) Schematic representation of the reporters used for the *HSP70* 3'-UTR purifications. As in Figure 22a both reporters contained the 3SBPs and were able to bind to the matrix.

(**b**) Western blot comparing both *HSP70* purifications using the cell line expressing V5-ZC3H11. For the beads forty times more sample was loaded in comparison to input polyribosomes and unbound.
(**c**) Western blot in order to detect the co-purification of V5-MKT1 and the *HSP70* 3'-UTR reporters. $5x10^6$ cells were loaded for input from polyribosomes, $3x10^8$ for the unbound, and for the beads the approximate number of $1x10^9$ cells was loaded.
As controls trypanothione reductase (TR) and the ribosomal protein S9 were used, TR is not associated with polyribosomes whereas S9 is part of the ribosome.
3SBPs, three streptavidin binding proteins; CAT, chloramphenicol acetyl-transferase; ZC3H11, zinc finger protein 11 or V5-ZC3H11 (V5-tagged protein); MKT-1, maintenance of k2 killer toxin or V5-MKT1 (V5-tagged protein); HSP, heat shock protein; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

The purification corresponding to the complete *HSP70* 3'-UTR, showed in Figure 23b, was send to MS, the gel was cut into 3 gel pieces, named 4-6. The aim was to detect the proteins bound to this 3'-UTR and specifically peptides from the V5-ZC3H11 protein. Previous attempts to detect this protein by MS and DL-QMS were done in our laboratory, but the protein is highly phosphorylated making its detection a challenge.

Table 2 shows a list of the proteins that co-purify with the *HSP70* 3'-UTR, among them; many RBPs were found as well as proteins of the degradation machinery and translation factors. Interestingly, peptides for MKT1 were not found and only one single modified peptide for V5-ZC3H11 was detected. The reason why no peptides were detected for MKT1 could be the fact that approximately 140 times more of sample was used to detect this protein by Western blot in the purification (Figure 14c) and the peptides from this protein could be masked by peptides from other abundant proteins such as ribosomal and mitochondrial peptides whereas in the Western blot the V5-antibody is very efficient and sensitive. This could also be the case for V5-ZC3H11.

Table 2. Proteins that co-purify with the *HSP70* 3'-UTR
List of selected candidate proteins detected in the purification with the complete *HSP70* 3'-UTR. The sample was cut in 3 pieces (numbers 4 to 6), the number of peptides for each protein is depicted on the table for each gel piece.

Gene number	Proteins found	Category	Number of unique peptides		
			1	2	3
Tb927.10.240	PEX14 peroxin 14	Glycosome biogenesis	0	0	2
Tb927.9.8740	DRBD3 (PTB1)	RNA binding	0	0	2
Tb927.7.2670	ZC3H21	RNA binding	0	0	6
Tb927.10.14950	ZC3H40	RNA binding	0	3	0
Tb927.11.14100	DRBD4 (PTB2)	RNA binding	2	0	0
Tb927.10.4430	PUF1	RNA binding	8	0	0
Tb927.10.11760	PUF6	RNA binding	2	0	0
Tb927.11.510	UBP2	RNA binding	2	0	2
Tb927.11.1980	ZC3H41	RNA binding	7	5	0
Tb927.10.3990	DHH1	RNA degradation	0	0	3
Tb927.10.8720	NOT10	RNA degradation	0	7	0

Tb927.10.6630	ATP-dependent DEAD/H RNA helicase HEL64, putative (cytosolic)	RNA helicase	6	0	0
Tb927.10.4640	eIF-3 subunit L	Translation	0	0	3
Tb927.6.1870	eIF4E4	Translation	0	0	4
Tb927.6.4370	eIF3 subunit 7-like protein	Translation	0	4	0
Tb927.10.2090	EF1-alpha elongation factor 1-alpha, (TEF1)	Translation	2	0	0
Tb927.9.9290	PABP1	Translation	10	0	0
Tb927.10.2100	EF1-alpha elongation factor 1-alpha, (TEF1)	Translation	0	6	19
Tb927.11.1900	T-complex protein 1, beta subunit, putative (TCP-1-beta)	Translation	0	32	3
Tb927.10.14550	DED1-1, ATP-dependent DEAD/H RNA helicase,	Translation	9	5	3
Tb927.9.10770	PABP2	Translation	26	20	22

The co-purification of *HSP70* 3'-UTR with ZC3H11 validate the technique. It showed that the purification of specific mRNPs and detection of proteins bound to these mRNPs was possible, at least by Western blot.

3.10 DRBD3 co-purified with the *AATP11* 3'-UTR reporters

The affinity purification method allows the exchange of almost all the components of the reporters used. Therefore, as another proof of principle, the 3'-UTR of *HSP70* was changed for the amino acid transporter 11 (*AATP11*) 3'-UTR, to proof another known protein-mRNA interaction.

As shown previously, the RNA binding protein DRBD3 (also known as PTB1, *Tb*927.9.8740) binds and stabilizes the *AATP11* mRNA [79, 80]. It has also been proposed that a region between 290 and 618 (328bp) in the *AATP11* 3'-UTR contains regulatory elements that are involved in DRBD3 binding to its target mRNA [79, 174].

The affinity purification technique was used to confirm the interaction between DRBD3 and the *AATP11* 3'-UTR. Two different *CAT* reporters were generated, one containing the complete *AATP11* 3'-UTR and another with a 328 nucleotide deletion (290-618 region deleted). Both reporters contained the 3SBPs at the 5' of the CAT open reading frame (Figure 24a).

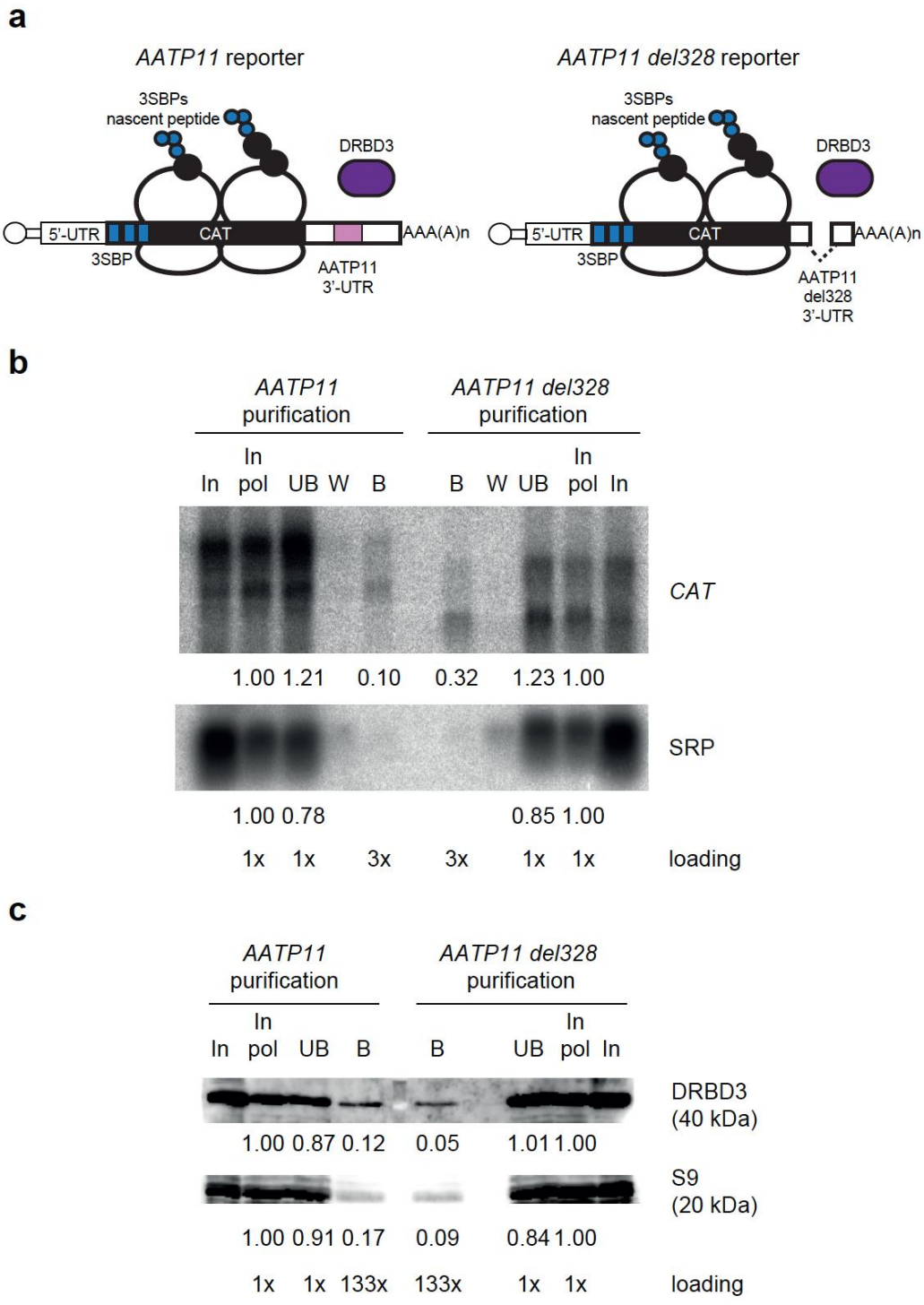


Figure 24. Co-purification of the *AATP11* 3'-UTR reporters and DRBD3
(**a**) Schematic representation of the reporters used in this experiment. Both reporters contained the multi-tag. The control reporter had a 328 nucleotide deletion in the *AATP11* 3'-UTR.
(**b**) Northern blot of the affinity purification, both reporters bind to the beads. The *AATP11* mRNA has two different polyadenylation sites; therefore there were two bands for the *CAT* mRNAs. The *CAT- AATP11* reporters were 1.9 Kb and 1.8 Kb whereas the control reporters were 1.7 Kb and 1.6 Kb. From the lysis input $5x10^6$ cells were used for Northern blot, in case of In pol, UB and W, approximately $1.5x10^7$ cells were loaded and for the beads $4.5x10^7$ cells. SRP was used as loading control; the size of this structural RNA is approximately 0.25 Kb.

(**c**) Western blot showing the co-purification of DRBD3 and the *AATP11* 3'-UTR reporters. The Western blot was performed using and antibody against DRBD3 (kindly provided by Antonio Estevez). Input from lysis contained $5x10^6$ cells, In pol and UB, an approximately of $1.5x10^7$ cells and the beads $2x10^9$ cells.
3SBPs, three streptavidin binding proteins; CAT, chloramphenicol acetyl-transferase; *SRP*, signal recognition particle, an structural RNA; AATP11, amino acid transporter 11; UTR, untranslated region; DRBD3, RNA binding protein; S9, ribosomal protein S9; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

Both reporters could be detected in the beads because both of them contain the 3SBPs. The *AATP11* 3'-UTR presents a second polyadenylation site as previously reported [174]. Interestingly, DRBD3 binds to the reporter containing the complete *AATP11* 3'-UTR as well as to the one carrying the 328mer deletion (Figure 24c). It should be noted that the RNA-protein complexes were UV cross-linked prior to the purification and that DRBD3 is an abundant protein that binds to many mRNAs [79] and it was expected to unspecifically bind to the beads. This experiment was done only once, nevertheless it showed that DRBD3 was 2.4-fold enriched in the complete *AATP11* 3'-UTR purification compared to the deleted one.

3.11 Purification of the *EP* 3'-UTR reporters and detection of the proteins bound

In parallel to the *AATP11* purifications, work on the *EP1* 3'-UTR was carried out. So far, no *trans*-acting factors have been identified to dictate the fate of the *EP1 procyclin* mRNA. The *EP1* mRNA is developmentally regulated, meaning that this mRNA accumulates (is 10-folds higher) in procyclic forms compared to the bloodstream form trypanosomes and its regulatory regions are present mainly on the 3'-UTR [63, 175]. The *EP1* 3'-UTR is composed of three stem-loops containing two positive regulatory elements (LI and LIII) and one negative (LII), the LI regulatory element is present on the first 40 bases; the LIII has a 16mer region that enhances translation while its deletion decreases the mRNA-polyribosome complexes [58, 64, 67, 68]. On the other hand, the 26mer element present in LII is involved in rapid degradation of the mRNA and reduces its steady state levels [65].

To detect the proteins bound to the *EP1* 3'-UTR, affinity purifications using the *3SBPs-CAT* reporter containing the *EP*–UTRs were performed. The affinity purifications were done, as described previously, by pooling the translating *3SBPs-CAT-EP* polyribosomes and incubating them with the streptavidin beads. Samples of the *3SBPs-CAT-EP* and control purifications (reporter without 3SBPs) were sent to mass-spectrometry.

Four independent affinity purifications were done for the reporter containing the *3SBPs-CAT-EP*-UTRs and for the control reporter (Figure 25a). To ensure that the *CAT* reporters were pulled down, samples for Northern blot were taken (Figure 25b) and the rest (corresponding to an equivalent cell number of $3x10^8$ cells) was sent for DL-QMS.

The *EP* purification performed showed that approximately 7% of the *CAT* present in the polyribosomes was purified using this method. The signal for *tubulin* was barely measurable, which indicates that there were few mRNA contaminants in the sample. Also, when measuring the ratio between the two mRNAs, a 7-fold increase of *CAT* versus *tubulin* was observed (Figure 25b).

In the case of the control purification, the signals were barely detectable. A signal for *tubulin* in the wash and beads fraction was observed, but in the case of *CAT*, no signal was detected, even when over-exposure was done for 3 days (Figure 25b). This experiment was repeated more than 3 times with similar results. The samples presented in **Figure 25** were the ones send to DL-QMS.

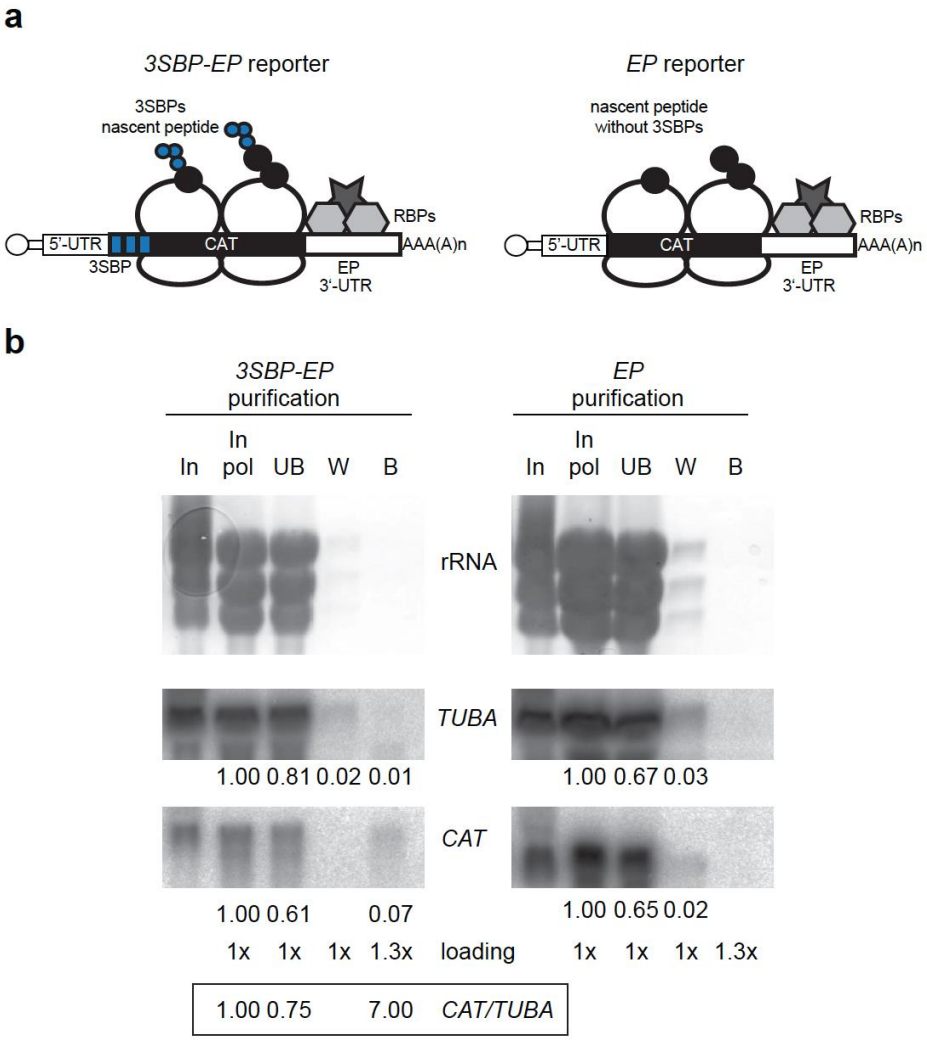


Figure 25: Affinity purification of the *EP* 3'UTR reporters
(**a**) Schematic representation of the reporters used in these purifications. Both reporters contain the same ORF [124], 5' and 3'-UTRs from *EP*. The control reporter does not contain the 3SBPs.
(**b**) Northern blot showing the binding of the *CAT* reporters to the beads. Only the mRNA containing the multi-tag (1.5Kb) was able to bind to the beads, but not the control one (1.3Kb). *Tubulin* (1.8Kb) was used as loading control and to assess the purity of the purification. For input from polyribosomes and unbound the equivalent of

6 x10^7 cells were taken. In the case of the beads, approximately 8x10^7 cells were used for Northern blot.
3SBPs, three streptavidin binding proteins; ORF, open reading frame; CAT, chloramphenicol acetyl-transferase; *TUB*, tubulin mRNA; EP, glutamic acid and proline procyclin protein, the major surface protein on procyclic trypanosomes; rRNA, ribosomal RNA; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

The results obtained from DL-QMS revealed not only translation factors and ribosomal proteins, but also proteins of the degradation machinery such as XRNA and NOT1, and other proteins associated with mRNPs. Interestingly, peptides corresponding to a CCCH zinc-finger protein that has not been detected before in other purifications, ZC3H22 were also detected (Table 3).

Table 3. Proteins co-purifying with the *EP* 3'-UTR reporter
This table shows selected candidate proteins detected by DL-QMS. The enrichment was determined by comparing the peptides found in the multi-tag purification with the ones found in the control purification (same reporter without the 3SBPs). The coverage represents the percentage covered by the detected peptides in terms of the length of the protein.
DHH1, dead box protein 1, an ATP dependent RNA helicase; MKT1, maintenance of K2 killer toxin; NOT1, negative on TATA 1, a protein component of the degradation machinery; XRNA, 5'-3' exoribonuclease; RBP42, RNA binding protein 42; DRBD3, an RNA binding protein; PUF1, pumilio-fem-3-binding factor and RNA binding protein; ZC3H22, Zinc finger protein 22.

Gene number	Proteins found	Category	Enrichment	Coverage (in %)
Tb927.10.3990	DHH1	RNA degradation	26	14
Tb927.6.4770	MKT1	RNA metabolism	5	5
Tb927.10.1510	NOT1	RNA degradation	3	3
Tb927.7.4900	XRNA	RNA degradation	147	4
Tb927.6.4440	RBP42	RNA binding	3	33
Tb927.9.8740	DRBD3	RNA binding	84	7
Tb927.10.4430	PUF1	RNA binding	66	4
Tb927.7.2680	ZC3H22	RNA binding	3	12

Large-scale purifications of the reporters containing the *EP* 3'-UTR were sent once more to DL-QMS. Due to the long time required to process the samples in the MS facility, it was decided to start studying some of the proteins obtained in the first trial (for instance, ZC3H22) and to generate the corresponding cell lines to confirm the results obtained.

The *EP*-big scale purifications were repeated two times more with and without cross-linking but only few proteins were detected by DL-QMS, most of them being abundant proteins that bind unspecifically to the beads. Additionally, it is

important to mention that when using DL-QMS, the sample was diluted because it had to be mixed with the control one compromising the detection of underrepresented proteins, such as peptides belonging to RBPs. Therefore it was decided not to continue processing the samples using DL-QMS.

Further purifications of the *CAT-EP* 3'UTR reporters were performed without cross-linking. Four replicate samples were sent to the Mass Spectrometry Facility University of Dundee. Northern blot results confirmed that the pull down was performed correctly (Figure 25b). The results of MS are listed in table 4. Most of the hits were hypothetical proteins and contaminants, such as tubulin, mitochondrial and nuclear proteins (supplementary table 3).
Only two RBPs were identified, ZC3H13 and ZC3H21. In the case of ZC3H13, a cell line expressing a V5-tagged protein was created and the affinity purification method was used in order to determine if V5-ZC3H13 co-purifies with the *3SBPs-CAT-EP* 3'-UTR reporter. A signal for V5-ZC3H13 was detected in both, the *3SBPs-CAT-EP* 3'-UTR and the control purification (without the tags). Although, V5-ZC3H13 was found to be 6.8-fold enriched in the 3SBP-CAT purification compared to the control. Unfortunately, the protein controls such as the ribosomal protein S9 or the initiation factor eIF4G3 were not detected by Western blot, probably because of blotting problems (data not shown). Furthermore, this experiment was only done once; therefore the repetition of this purification might be of use.

Table 4. Proteins that co-purified with the *EP* 3'UTR *reporters*
Results of four replicates sent to MS. The control reporter did not contain the 3SBPs and therefore was not able to bind to the streptavidin beads.
The ratio of peptides corresponding to a specific gene present in the *3SBP-EP* 3'-UTR purification was compared to the counts in the control sample (no SBPs) for each replicate. The table includes a list of genes that had 2-fold increase for at least two independent replicates. The replicates were arranged accordingly to the highest ratio.

Accession	Description	Replicates (ratio 3SBPs/no SBP)			
		1	2	3	4
Tb927.1.2400	alpha tubulin	34,0	30,0	1,2	1,0
Tb927.1.2390	beta tubulin	29,0	28,0	1,0	1,0
Tb927.3.1010	hypothetical protein, Trypanosoma-specific	9,0	1,0	3,0	1,0
Tb927.3.3300	hypothetical protein	2,0	1,0	8,0	0,3
Tb927.9.8820	hypothetical protein	7,0	0,7	3,0	0,4
Tb927.9.12200	60S ribosomal protein L31	6,0	5,0	1,0	1,7
Tb927.5.2530	hypothetical protein	3,0	1,0	6,0	1,0
Tb927.9.6920	hypothetical protein	3,0	1,0	5,0	1,0
Tb927.11.9080	hypothetical protein	4,0	1,0	5,0	1,0
Tb927.11.510	UBP2	5,0	4,0	0,8	1,3
Tb927.3.2050	hypothetical protein	5,0	1,0	2,0	0,3
Tb927.3.3150	hypothetical protein	5,0	1,0	3,0	0,2
Tb927.7.4500	hypothetical protein	5,0	5,0	2,3	0,1

Tb927.6.2010	hypothetical protein	4,7	1,0	2,5	0,3
Tb927.10.1100	60S ribosomal protein L9	4,0	1,0	1,0	2,0
Tb927.3.3460	hypothetical protein	4,0	1,0	3,0	1,0
Tb927.10.8270	eIF3 subunit 8, putative	4,0	1,0	3,0	1,0
Tb927.10.14700	hypothetical protein	1,0	1,0	4,0	3,0
Tb927.7.5340	hypothetical protein	3,0	1,0	4,0	1,0
Tb927.5.1580	ZC3H13	1,8	4,0	3,0	1,0
Tb927.11.2600	hypothetical protein	3,0	1,0	4,0	0,3
Tb927.7.3550	hypothetical protein	2,0	1,0	4,0	0,3
Tb927.6.4320	hypothetical protein	3,5	1,0	2,3	0,4
Tb927.7.2670	ZC3H21	2,3	1,0	3,3	0,3
Tb927.9.8070	60S ribosomal protein L10	2,2	1,0	3,0	1,8
Tb927.11.9570	hypothetical protein	3,0	1,0	3,0	1,0
Tb927.3.4580	hypothetical protein	3,0	1,0	3,0	0,3
Tb927.11.9780	hypothetical protein	3,0	0,3	2,7	0,3
Tb927.6.5070	hypothetical protein	3,0	1,0	2,2	0,3
Tb927.8.1270	hypothetical protein	2,0	0,3	2,7	0,3

3.12 MKT1 does not interact specifically with the *3SBPs-CAT-EP* reporter

In order to confirm the results from MS (Table 3), the affinity purifications were repeated using cell lines expressing a V5-tagged MKT1 and the *EP* reporters (Figure 26a).

The Northern blot shows that the pull down was successful, by a slight signal for *CAT* present only on the *3SBPs* mRNA (Figure 26b).

The Western blot showed that the V5-MKT1 protein co-purifies with both *CAT* reporters. MKT1 is an abundant protein that binds to PBP, present in all mRNAs with a polyA binding protein [108], therefore it was possible that MKT1 interacted unspecifically with the beads (Figure 26c). This experiment was repeated twice and in both cases the V5-MKT1 signal was found in both purifications (with and without the multi-tags). Nevertheless, for both experiments there was more MKT1 in the purification with the 3SBPs, approximately 2-fold and 3.6-fold increased per replicate, when compared to the control purification.
In Figure26c, the unbound was taken as 1.00 for the quantitation because there was not signal detected in the input of the 3SBP-EP purification.

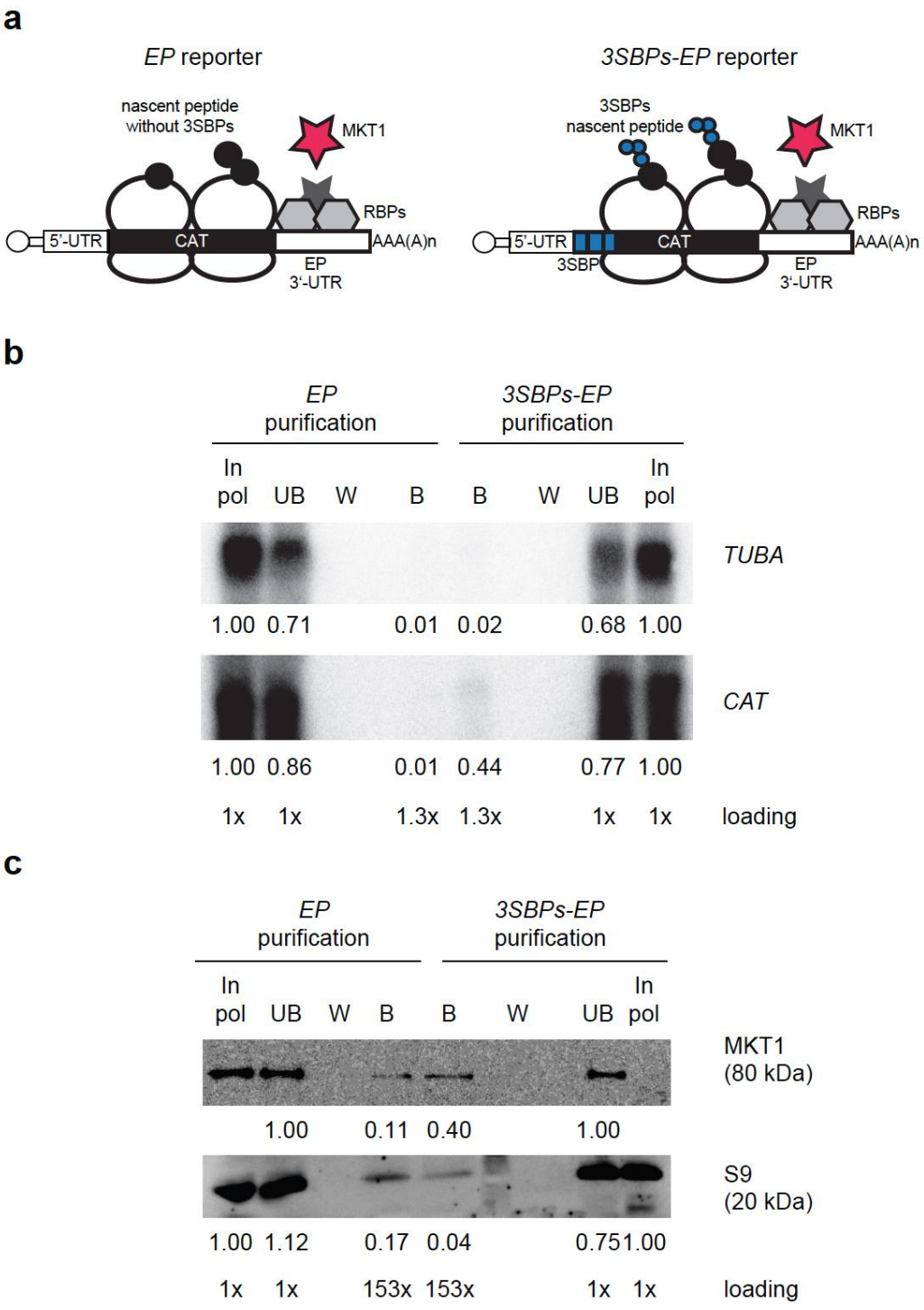


Figure 26. Co-purification of V5-MKT1 with both *EP* reporters
(**a**) Schematic representation of the purified reporters. The purifications were made in stable cell lines expressing the V5-tagged MKT1 protein. Both reporters contain the *EP* 5' and 3'-UTRs, but the control reporter does not contain the *3SBPs*.
(**b**) Detection of the *EP* reporters by Northern blot. The signal for *TUB* (1.8Kb) was used as loading control and control of contamination. For the *CAT* mRNAs the sizes were 1.5Kb (multi-tag) and 1.3Kb (control reporter).
(**c**) Co-purification of V5-MKT1 and the *EP* reporters by Western blot. Equal amount of sample was loaded for In pol and UB; 153 times more sample was loaded for the beads.

3SBPs, three streptavidin binding proteins; CAT, chloramphenicol acetyl-transferase; *TUB*, tubulin mRNA; EP, glutamic acid and proline procyclin, the major surface protein on procyclic trypanosomes; UTR, untranslated region; MKT1, maintenance of K2 killer toxin protein; S9, ribosomal protein S9; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

3.13 ZC3H22 does not interact specifically with the *3SBP-CAT-EP* reporter

Stable cell lines were generated in order to assess the interaction between ZC3H22 and the *EP* 3'-UTR reporter. The purification of the cell line expressing V5-tagged ZC3H22 and the *3SBP-CAT-EP* 3'-UTR was compared with the control purification, a cell line expressing the V5-ZC3H22 protein and the *CAT* reporter without the 3SBPs (Figure 27a).

The Northern blot results showed only a slight signal detected for the *CAT* mRNA in the multi-tag cell line and none for the control purification (Figure 27b). Indicating that the *3SBP* reporter binds to the beads whereas the one without did not.

In the Western blot, the signal for V5-ZC3H22 was found in both purifications, implying that the binding of this protein was not specific for the reporter containing the *3SBPs-CAT-EP*-UTRs. It could be that this protein binds unspecifically to the beads. The ribosomal protein S9 was used as a loading control. Furthermore, there were three bands detected for V5-ZC3H22, meaning that the protein is subjected to post-translational modifications, such as phosphorylation (Figure 27c).

This experiment was repeated two times, but only in one of the replicates the signal for *CAT* mRNA was detected in the Northern blots, in the other one the amount of mRNA was not enough for the detection and the signals for the beads in the Western blot were barely detectable. In the experiment shown in Figure 27c, it seems that V5-ZC3H22 is 2.4-fold more in the multi-tag purification than in the control one.

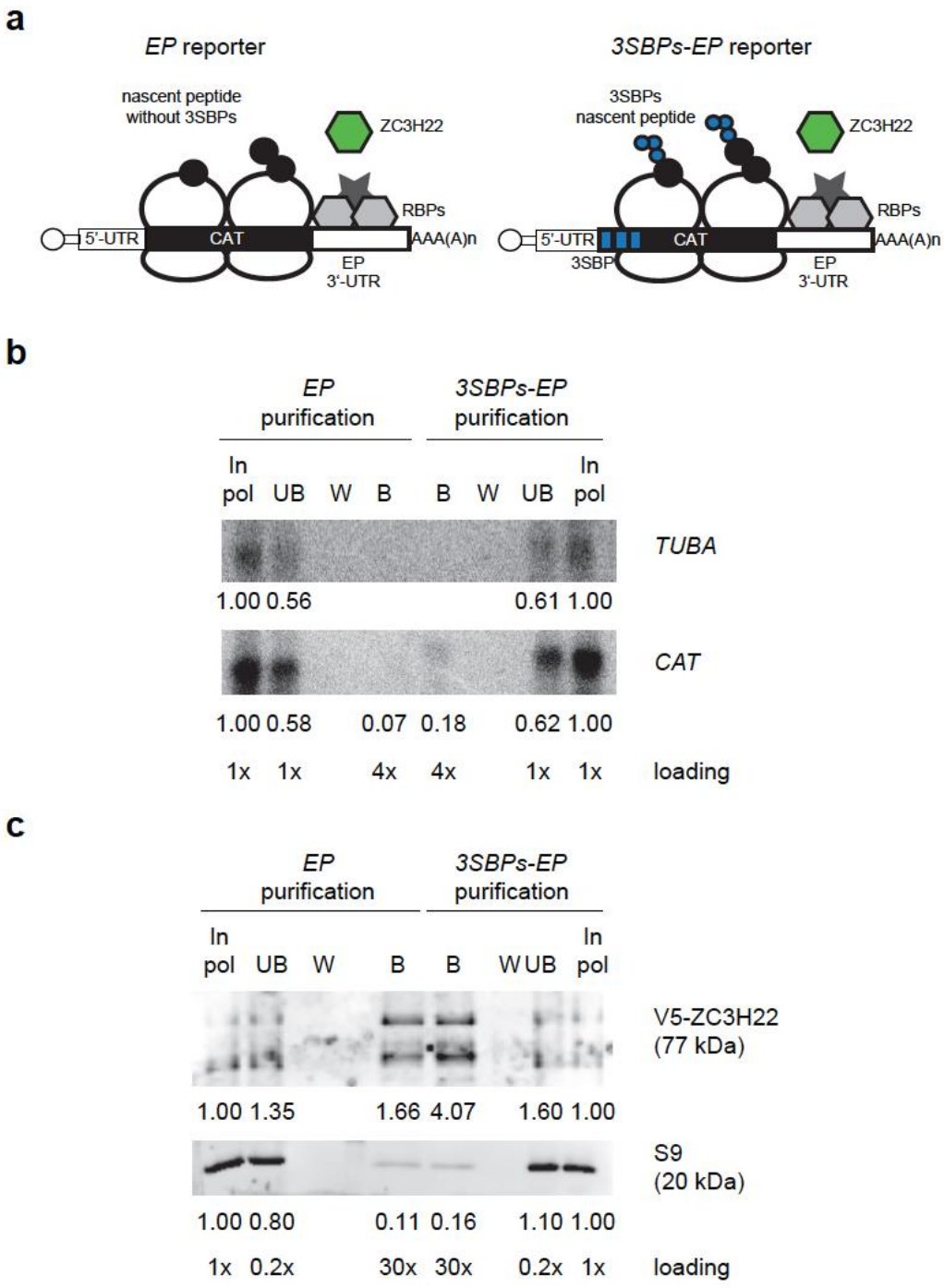


Figure 27. V5-ZC3H22 co-purified with both *EP* reporters

(**a**) Schematic representation of the reporters used in the purification. The purifications were made in stable cell lines expressing the V5-tagged ZC3H22 protein. Both reporters contain the *EP* UTRs, but the control reporter does not contain the multi-tag.

(**b**) Detection of the *EP* reporters by Northern blot. *TUB* (1.8Kb) was used as loading control. The sizes for the *CAT* mRNAs were 1.5Kb (reporter with the 3SBPs) and 1.3Kb (control reporter)

(**c**) Co-purification of V5-ZC3H22 with both *EP* reporters.

3SBPs, three streptavidin binding proteins; *CAT, chloramphenicol acetyl-transferase* mRNA; *TUB*, *tubulin* mRNA; EP, glutamic acid and proline procyclin protein; UTR,

untranslated region; ZC3H22, zinc finger protein 22; S9, ribosomal protein S9; In, input from lysis; In pol, input from polyribosomes; UB, unbound; W, wash; B, beads.

3.14 Zinc Finger Protein 22 (ZC3H22)

The gene of the zinc finger protein 22 (*Tb*427.07.2680) is part of the transcription unit where two other zinc finger proteins are present (*Tb*427.07.2660, ZC3H20 and *Tb*427.07.2670, ZC3H21). Both, ZC3H21 and ZC3H20 presented a growth defect phenotype on procyclic form trypanosomes upon knock-down and overexpression respectively [106].

So far, *Tb*ZC3H22 has not been characterized and it has not been found in any other purifications. According to the transcriptomes obtained by RNA sequencing published in www.tritrypdb.org [176], many reads aligning to a long stretch of the gene were detected, indicating that probably the 3'-UTR is shorter than predicted (Figure 28a). Also, the predicted poly(A) sites seem to be in the wrong position. The 77 KDa protein contains two CCCH zinc finger domains.

The main reason why it was decided to work with this protein was because it was found by quantitative mass spectrometry in one of the affinity purifications using the reporters expressing the *EP* 3'-UTR (Table 3).

The present work on ZC3H22 started while waiting for the mass spectrometry results (*EP* replicates) and was done before obtaining the stable cell lines expressing the *CAT-EP* reporters to confirm the results obtained by DL-QMS (Table 3) and before obtaining the results of Figure 27. Therefore, at that time, it was thought that this protein might be involved in *EP* mRNA regulation.

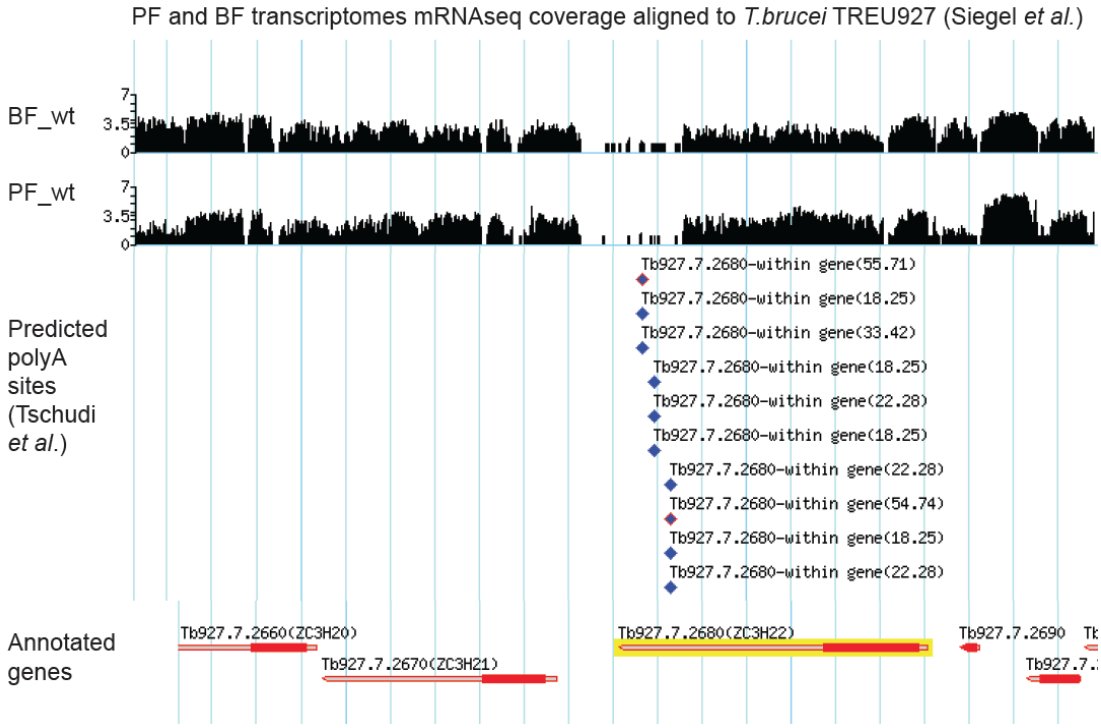


Figure 28. *Tb*ZC3H22 transcription unit
The *Tb*ZC3H22 is located in the same transcription unit as *Tb*ZC3H20 and *Tb*ZC3H21 (source www.tritrypdb.org). The figure shows the predicted polyadenylation sites and the RNAseq coverage of the different reads obtained for the genes of this transcription unit.

3.14.1 The V5-ZC3H22 protein localizes in the cytoplasm

Immunofluorescence was performed in cells expressing the V5-tagged ZC3H22 and compared with wild type cells. DAPI was used as nuclear and kinetoplast staining. The DIC pictures showed the integrity of the trypanosomes. These results showed that V5-ZC3H22 localizes mainly in the cytoplasm (Figure 29). The dots in the V5-ZC3H22 could be an artifact of the V5-antibody, or some precipitation due to the fixation with paraformaldehyde.

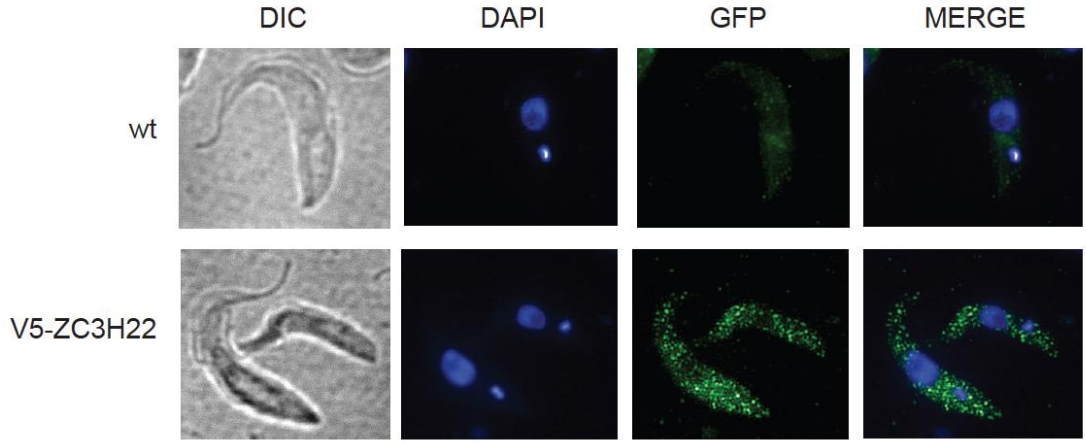


Figure 29. Localization of ZC3H22
Immunofluorescence of cells expressing V5-ZC3H22, compared with wild type cells. ZC3H22, zinc finger protein 22 (V5-tagged); wt, wild type, Lister 927 cells; DIC, differential interference contrast; DAPI, 4',6-diamidino-2-phenylindole, marker for nucleus and kinetoplast; GFP, green fluorescent protein filter, Merge, mixed of DAPI and GFP pictures.

3.14.2 The V5-ZC3H22 was not associated with polyribosomes

The distribution of V5-ZC3H22 protein in the polyribosome profile was also studied (Figure 30a).

$6x10^8$ cells were used in this experiment. The ZC3H22 protein had a similar pattern to the one of the RNA binding protein DRBD3. Only when UV cross-linking of RNA-protein complexes was performed both proteins were detected in the polyribosome gradient. V5-ZC3H22 as well as DRBD3 seemed to leak through the gradient, implying that part of the proteins was associated with polyribosomes, but most of them not. This was easily corroborated by comparing with the pattern exhibited by the ribosomal protein S9, which was included as control for proteins that are part of the polyribosomes. Instead both proteins were detected with higher intensity in the protein peak, meaning

that these proteins were free in the cytoplasm without interacting with the translating mRNAs (Figure 30b). Furthermore, previously it was shown that DRBD3 is not bound to the polyribosomes [79].

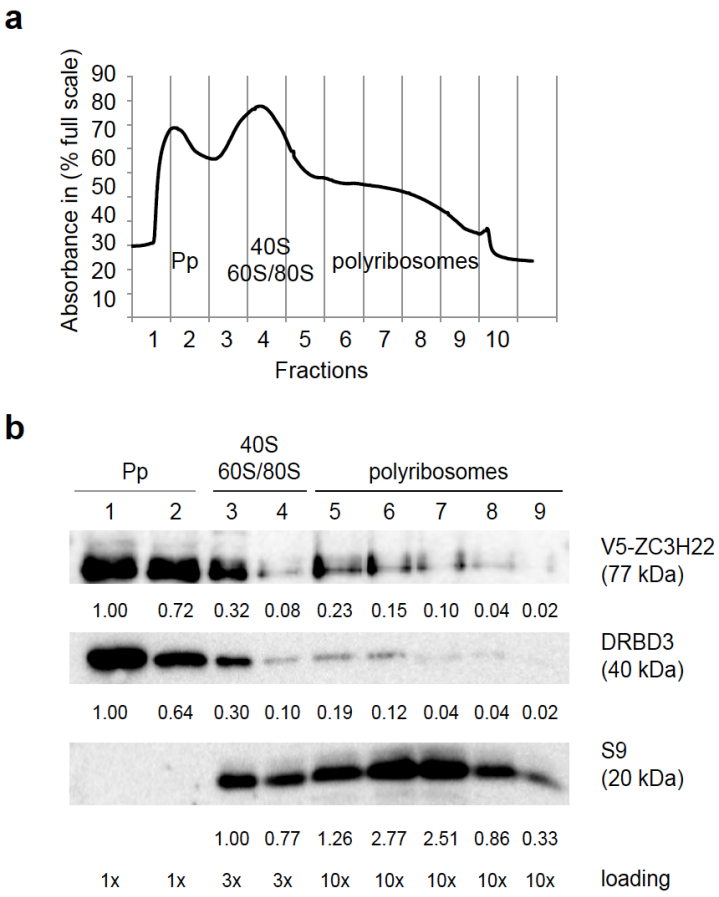


Figure 30. Distribution of the V5-ZC3H22 protein throughout the polyribosomal gradient
(**a**) Polyribosome profile of UV cross-linked complexes in procyclic trypanosomes.
(**b**) Western blot showed a similar distribution for V5-ZC3H22 and DRBD3 throughout the polyribosomal gradient. For the Pp sample (fractions 1-2) only 3µl were loaded. For the 40S, 60S and 80S fractions (3-4) 10 µl were loaded and for the polyribosome fractions (5-9) 30 µl of the sample was used in the Western blot.
Pp, protein peak; 40S, ribosomal subunit 40S; 60S/80S, ribosomal subunit 60S and monosomes 80S; ZC3H22, zinc finger protein 22 (V5-tagged ZC3H22); S9, ribosomal protein S9; DRBD3, RNA binding protein.

3.14.3 ZC3H22 RNAi did not affect growth of procyclic form trypanosomes

A change in growth was not observed when I performed RNAi against *ZC3H22* (Figure 31a), it is possible that the *ZC3H22* mRNA and protein levels could not be reduced sufficiently. After 24 hours, 60% or more of the mRNA was still present in the tested clones (Figure 31b, clone 3).

When testing the presence of ZC3H22 protein in a Western blot, it was observed that the protein was still present even after 48 hours of induction with tetracycline (Figure 31c). Several transfections using different parts of the ORF, as *ZC3H22* mRNA targets for RNAi, and longer growth curves were

done but none of them gave a change in growth or a sufficient reduction of mRNA levels.

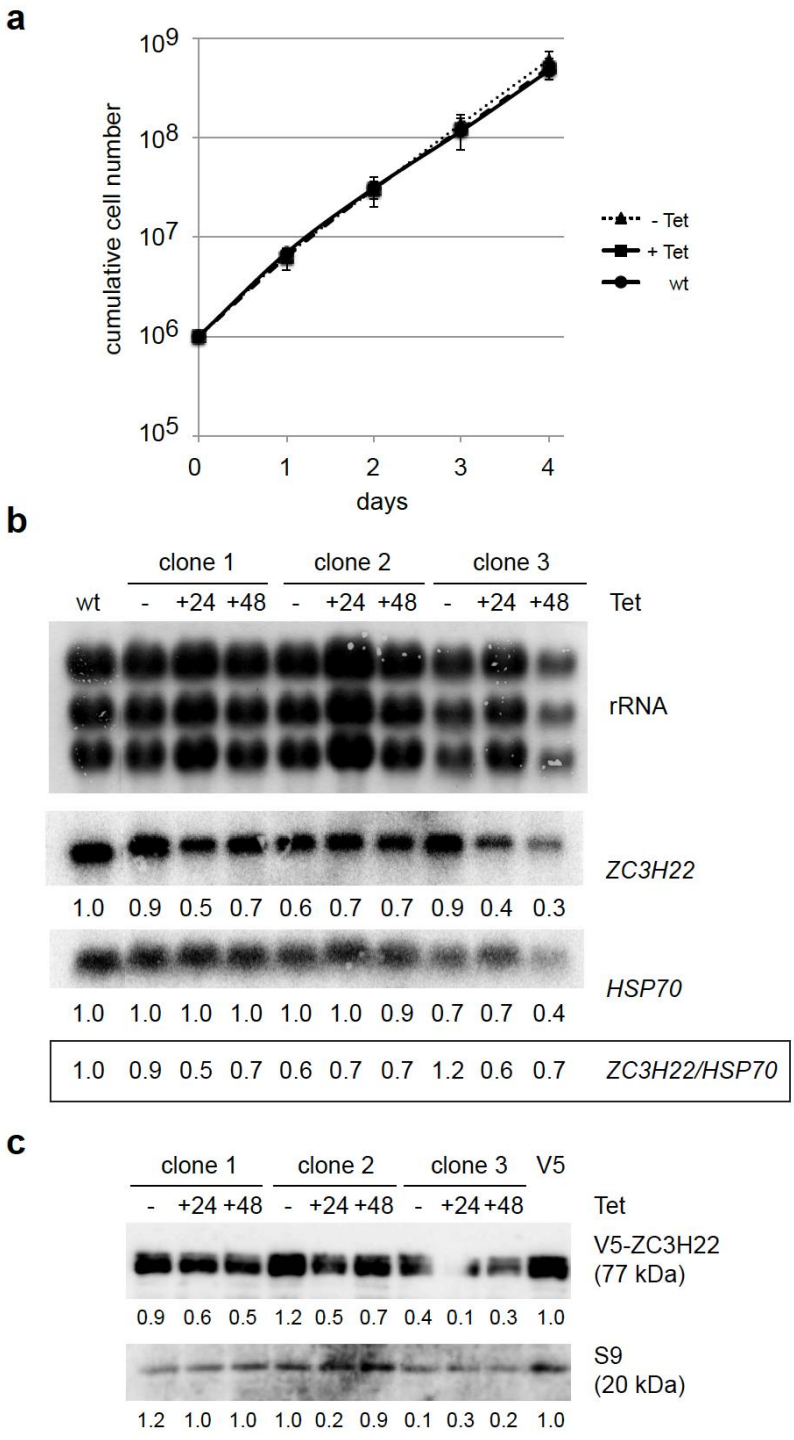


Figure 31. No effect of RNAi on *ZC3H22* mRNA and protein
(**a**) Effect of ZC3H22 depletion on procyclic form trypanosomes. The results of the samples – tet and + tet represent the average of three independent clones.
(**b**) Effect of RNAi against *ZC3H22* in Northern blot. *HSP70* was used as loading control. *ZC3H22* mRNA was approximately 5Kb and *HSP70* 2.7Kb
(**c**) Effect of ZC3H22 depletion on the protein level. Detected by the presence of the protein on Western blot.

wt, wild type, Lister 427 2060; -/+ Tet, with or without tetracycline; rRNA, bands of the ribosomal RNA; *ZC3H22, zinc finger protein 22*, mRNA or V5-tagged protein (V5-ZC3H22); *HSP70, heat shock protein 70* mRNA; S9, ribosomal protein S9.

3.14.4 Tethering of ZC3H22 to a reporter mRNA does not have a significant effect on the reporter

The tethering strategy relies on a λN-peptide that has the capacity to bind to the five boxB elements. Therefore, when fused to the ZC3H22-myc protein the effect of this protein on a reporter mRNA can be tested. These results are usually compared with a cell line that did not contain the boxB elements on the reporter mRNA (Figure 32a). If λN-ZC3H22-myc stabilizes its target mRNAs upon binding then the *CAT* reporter expression will be enhanced and the activity of the CAT protein measured by CAT assay will increase. In the opposite case, the reporter mRNA will be degraded and the signal measured over time will reduce. In case the protein does not have any effect on the mRNA, then the signal will be the same as the one of the control cell lines.

This tethering experiment was done using a cell line which constitutively expressed a *CAT* reporter followed by 5 *boxB* elements and have an actin 3'-UTR (*CAT-boxB*). This cell line was compared with a cell line containing only the *CAT* reporter (without the boxB element). Both cell lines expressed the ZC3H22 fusion protein, with a λN peptide at the N-terminus and a myc-tag at the C-terminus of the protein (Figure 32a).
As additional controls, cell lines expressing the *CAT-boxB* or the *CAT* reporter alone without the presence of the fusion protein were tested.

Upon induction of λN-ZC3H22-myc expression by addition of tetracycline, the relative CAT activity present in the samples was measured by CAT assay. There was no difference between the samples without tetracycline and the ones induced by tetracycline, as shown in Figure 32c, because the expression of the fusion protein was leaky. Therefore, in Figure 32b only samples induced with tetracycline are shown. The effect of the λN-ZC3H22-myc protein on the *CAT-boxB* reporter was very similar to the control cell line (without the λN-ZC3H22-myc protein), suggesting that there might not be an effect of λN-ZC3H22-myc protein when tethered to a reporter mRNA (Figure 32b). Interestingly, the relative CAT activity measured in the cell lines without boxB was 1.5-fold higher than in the cell lines with this element (Figure 32b) and the signal for *CAT* mRNA in the Northern blot seemed to smear slightly in the samples containing the boxB when comparing with the cell line without the element (Figure 32c), this might be due to degradation of the sample.

Interestingly, the CAT activity of the cell lines without the boxB element was higher than the ones with this element.
In order to confirm that the reporter mRNAs and the fusion proteins were being expressed, Northern and Western blots were performed. The expression of the *CAT* and *CAT-boxB* reporters was detected by Northern blot (Figure 32c) and the presence of λN-ZC3H22-myc protein was assessed by Western blot (Figure 32d).

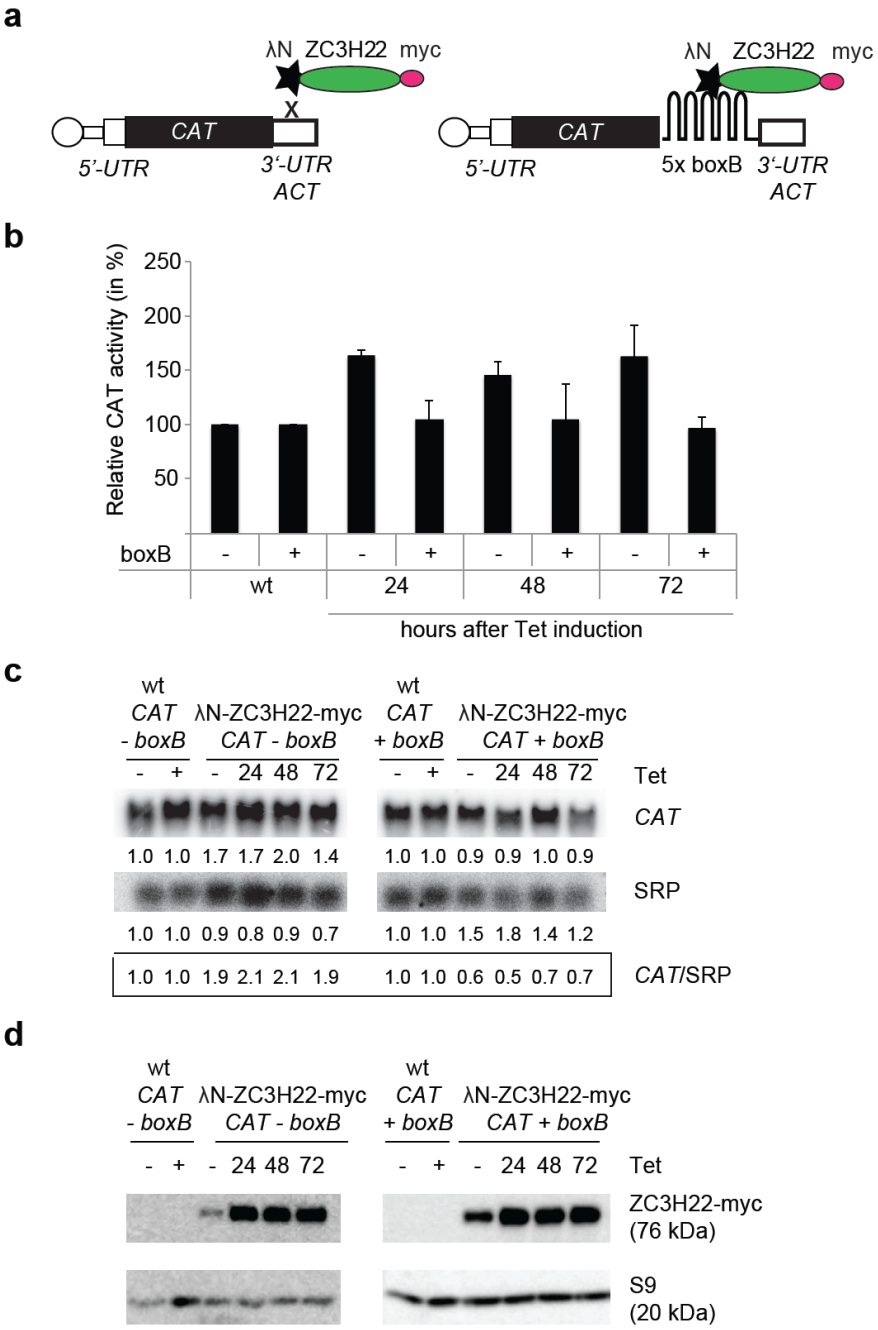


Figure 32. Tethering ZC3H22 to a *CAT* reporter had no effect
(**a**) Schematic representation of ZC3H22 tethering strategy
(**b**) Relative CAT activity measuring the effect of λN-ZC3H22-myc protein on the *CAT* without boxB and on the *CAT-boxB* reporter upon tetracycline [8] induction. The results of 3 independent clones were shown in this experiment over time.
(**c**) *CAT-boxB* mRNA compared to the cell line without *boxB* was detected by Northern blot. These cell lines also express the λN-ZC3H22-myc protein. As controls, wild type (wt) cells without the λN-ZC3H22-myc protein were used. Two controls were used, one that expressed the *CAT-box B* mRNA and an additional control without the *boxB* but only expressing *CAT* mRNA. The signal recognition particle (SRP) was used as loading control.
(**d**) λN-ZC3H22-myc expression as determined by Western blot. The protein was not present in the wild type cell lines. The ribosomal protein S9 was used as loading control.

3.14.5 RNA-Immunoprecipitation of V5-ZC3H22

As mentioned previously, *Tb*ZC3H22 was not characterized previously nor found in other purifications. For this reason, it was interesting to detect which RNAs were bound to it. Initially, it was thought that the *EP1* mRNA was a target of ZC3H22, due to the results of DL-QMS (Table 3).

Therefore, a cell line expressing the *3SBPs-CAT* reporter and a V5-tagged ZC3H22 was generated. The main idea was to pull the V5-ZC3H22 using V5-beads and detect the *3SBPs-CAT* mRNA. Three replicates were done, but only in one of them signals in Northern and Western blot were detected. Figure 33a shows the Northern blot performed where only a slight signal for *3SBPs-CAT* was detected, although the V5-ZC3H22 was efficiently pulled by the V5-beads (Figure 33b). Note that abundant proteins such as TR and S9 were able to bind unespecifically to the beads.

As an alternative, the presence of the endogenous *EP1* mRNA was also assessed by Northern blot. A signal was detected in both, the V5-beads as well as in the control IP (wild type cells), probably because *EP1* is an abundant mRNA in procyclic form trypanosomes (Figure 33a).

The *CAT* mRNA designed in the present study, as well as the *EP1* mRNA are both transcribed by RNAPI and probably expressed in similar levels. Thus, it could be possible that both mRNAs compete for the binding to V5-ZC3H22.

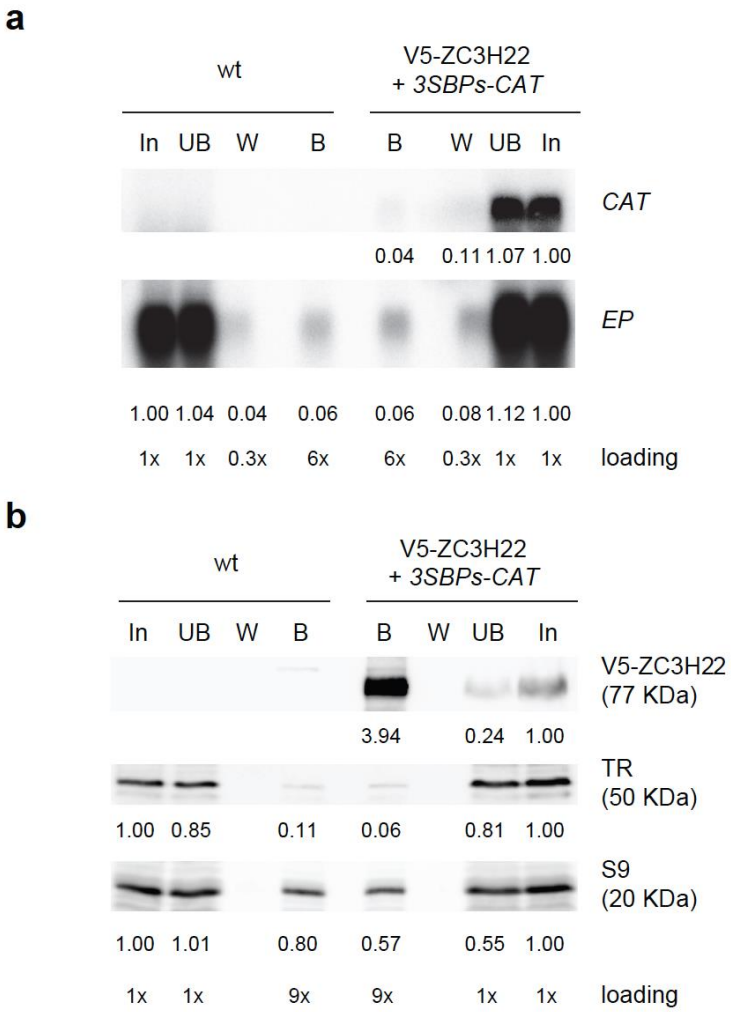


Figure 33. Pull down of V5-ZC3H22 using V5-beads
(**a**) Northern blot showing the pull down of V5-ZC3H22 and the *CAT* mRNA bound to it. The *EP1* mRNA was used to determine if it is a target of ZC3H22 but in the RNA-IP it is shown to also bind to the beads in the wild type sample. *CAT* mRNA was approximately 1.5 Kb and the *EP1* was 0.9 Kb.
(**b**) Western blot showing the pull down of V5-ZC3H22 using V5-beads. $2x10^7$ cells were loaded for input of lysis and unbound, $2x10^6$ for the wash and $1.8x10^8$ for the beads.
In, input of lysis; UB, unbound; W, wash; B, V5-agarose beads; ZC3H22, zinc finger protein 22 (V5-tagged version); TR, trypanothione reductase, a cytosolic protein; S9, ribosomal protein S9; EP, *EP procyclin* mRNA.

3.14.6 Single knockout of *Tb*ZC3H22 had no effect on the growth phenotype of procyclic trypanosomes

Due to the fact that RNAi against *ZC3H22* was not functional (Figure 31) and no growth defect phenotype was observed, it was attempted to knock out both alleles of *Tb*ZC3H22. The strategy consisted in replacing them with puromycin or blasticidin resistance markers (Figure 34a). A puromycin acetyltransferase cassette containing the 5' and 3'-UTR of *Tb*ZC3H22 was transfected in procyclic form trypanosomes and positive clones were analyzed by extraction of genomic DNA followed by PCR (Figure 34b).

Clone 1 was a successful single knock-out, contained the second allele of *Tb*ZC3H22 and was also positive for puromycin resistance. This cell line had no change in the growth phenotype.
In order to have a conditional knockout an over-expression plasmid (with ZC3H22-myc) was transfected in the single knockout cell line (Figure 34c). It was difficult to obtain a cell line over-expressing ZC3H22-myc, most of the ones obtained did not over-express the protein. It seemed that the over-expression of this protein was not well tolerated in procyclic trypanosomes. After five trials of transfection, changing the transfection buffers and the amount of plasmid transfected, one conditional knock out cell line over-expressing ZC3H22-myc was obtained. This cell line had no change in growth phenotype either.

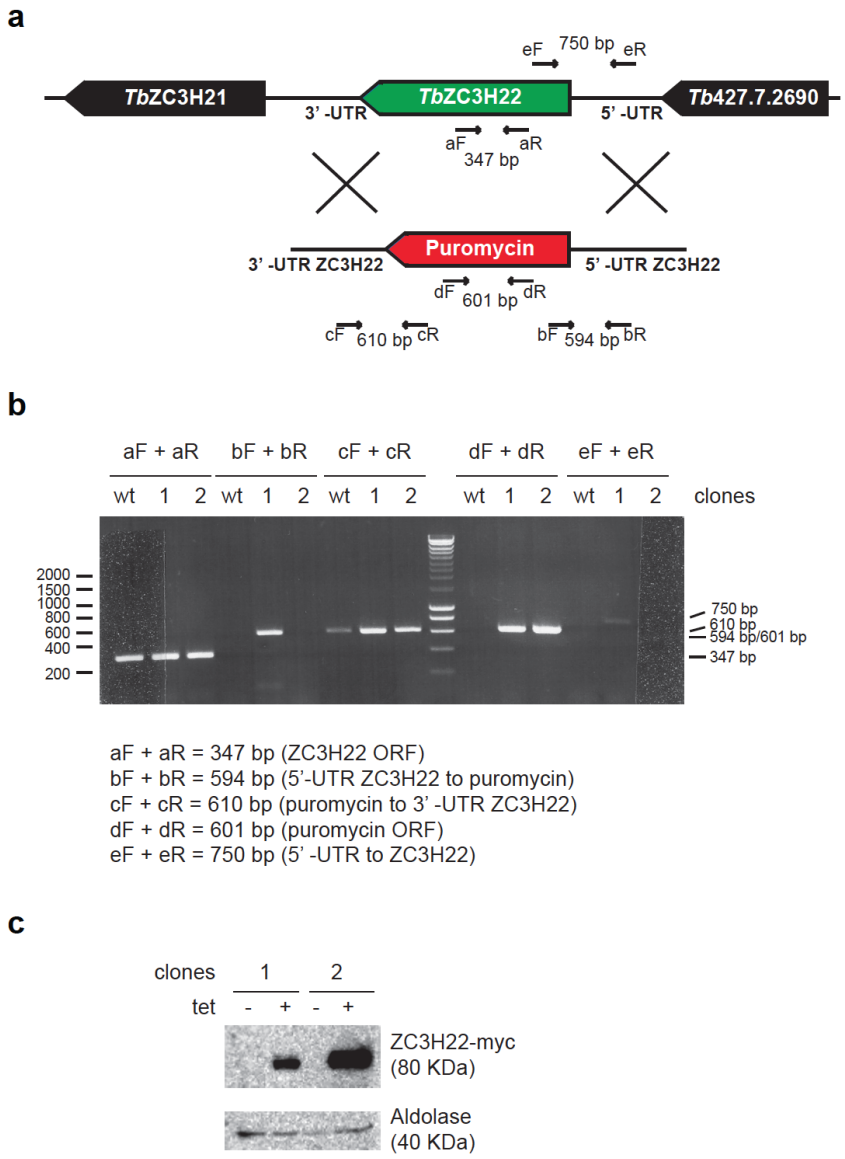


Figure 34. Single knockout of *Tb*ZC3H22
(**a**) Schematic representation of *Tb*ZC3H22 locus. The generation of the single knockout was done by replacing one of the copies of the *Tb*ZC3H22 allele for a

puromycin resistance cassette. Primers used to amplify the *Tb*ZC3H22 alleles are depicted in the picture.
(**b**) PCR products using different sets of primers in order to confirm the knockout clones. Clone 1 contains the puromycin gene and also one of the copies of *Tb*ZC3H22. Primers aF (cz4849) and aR (cz4850) amplified the ORF ZC3H22; bF (cz5078) and bR (cz5155) gave a product that goes from the 5' –UTR of *Tb*ZC3H22 until puromycin ORF; cF (cz5154) and cR (cz5068) amplified a product from the puromycin ORF to the ZC3H22 3'-UTR; dF (cz3294) and dR (cz2460) amplified the puromycin ORF; eF (cz4850) and eR (cz5078) gave a product that comprises the 5'-UTR of ZC3H22 and ZC3H22 ORF.
(**c**) Western blot showing the overexpression of ZC3H22-myc, the single knockout cell line was used as a base to transfect the overexpression plasmid of ZC3H22-myc. This plasmid is under the control of a polymerase I promoter and transcribed 10 times more than with polymerase II. Aldolase was used as loading control.
ZC3H22, zinc finger protein 22 or the tagged version (ZC3H22-myc); UTR, untranslated region; tet, tetracycline.

Unfortunately, it was not possible to generate the double knockout cell line; only the inducible cell line with a single knockout allele for *Tb*ZC3H22 was obtained and exhibited no change in the growth phenotype. Despite the fact that different concentrations of plasmid were transfected, no living clones were observed. After these results and the ones obtained in Figure 27, it was decided to stop the work on ZC3H22 completely.

3.15 The transcription unit of *Tb*ZC3H37 and *Tb*ZC3H38

The RNA binding protein, ZC3H37 has previously been detected in a screen looking for potential post-transcriptional regulators in *T. brucei* made by Dr. Esteban Erben in our laboratory [74]. The results of the screen suggested that this protein might stabilize its target mRNAs [74]. The transcription unit where *Tb*ZC3H37 is located comprises several other RBPs such as *Tb*ZC3H38, *Tb*ZC3H36 and *Tb*ZC3H35, which have not been characterized so far. Interestingly, the main difference between sequences of these two genes is a 117mer region at the 3'-end of the ORF (data obtained using MegaAling). For these reasons, it was decided to study one of them, *Tb*ZC3H38, in blood stream form trypanosomes.

When looking at the transcriptomes of this transcription unit, it was observed that for *Tb*ZC3H38 the reads align throughout the gene without gaps and go on until the adjacent region annotated as *Tb*927.10.12790. According to the trypanosome database www.tritrypdb.org (TritrypDB data base), possible splice sites have not been identified for *Tb*927.10.12790, but instead, the splicing sites are located in the 5'-UTR of the downstream gene *Tb*ZC3H38 (Figure 35a). Furthermore, when looking at the ribosome profiling data, it seems that no ribosomes are on the region annotated as *Tb*927.10.12790. Therefore, it was suspected that *Tb*427.10.12790 was actually the 3'-UTR of *Tb*ZC3H38 and not a real gene. This was confirmed by Northern blot, using a probe specific for *ZC3H38*. The predicted size of the *ZC3H38* mRNA is 3.5kb, but the signal detected in the Northern blot was approximately 5kb, exceeding the predicted size of the annotated *ZC3H38* mRNA, most probably containing

the *Tb*427.10.12790 region. These results were obtained by Jannik Traut and are published in his Bachelor thesis.

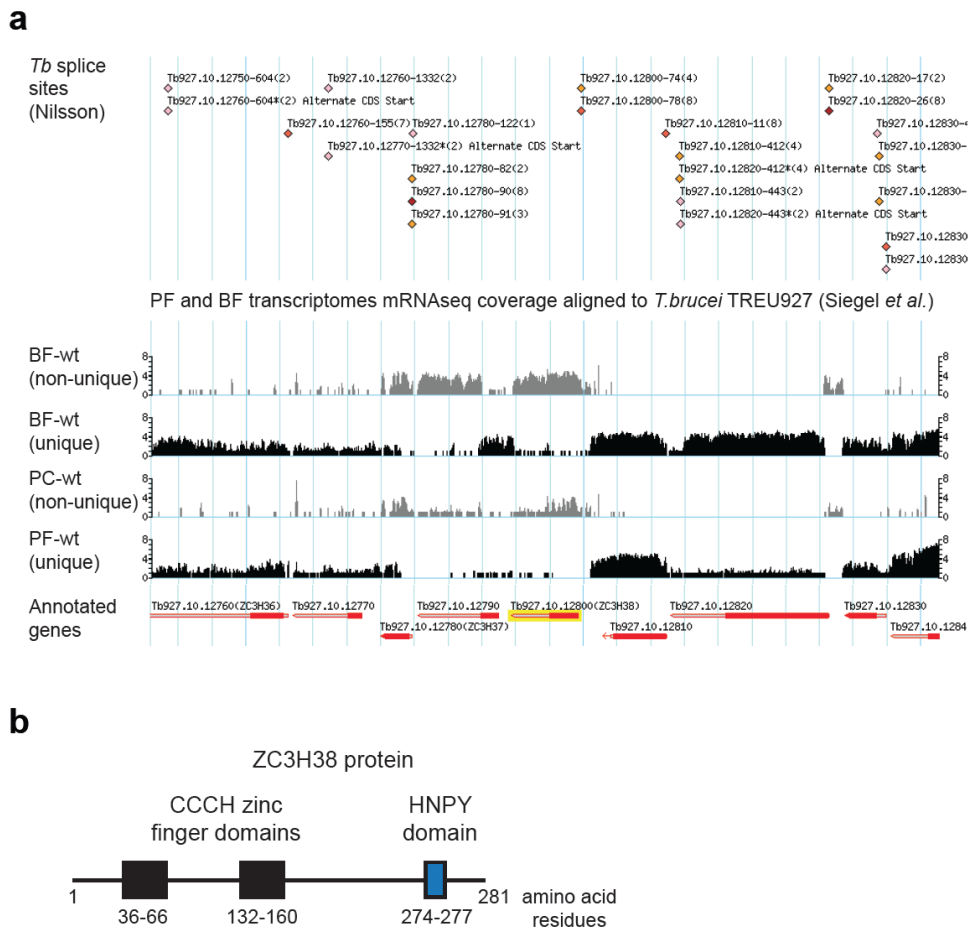


Figure 35. *Tb*ZC3H38 transcription unit
(**a**) Schematic representation of the *Tb*ZC3H38 transcription unit, picture taken from TriTrypDB.
*Tb*ZC3H38 is highlighted in yellow. Reads detected by RNAseq are depicted in black (unique) and grey (non-unique) for both life cycle stages of the parasite. In the upper panel, possible spliced sites are also shown.
(**b**) Schematic representation of the ZC3H38 protein, the two zinc finger domains are depicted in black and the HNPY domain in blue. The numbers of the amino-acid residues for each domain are depicted in the picture too.

ZC3H38 contains two zinc finger domains and a HNPY domain (Figure 35b). The HNPY domain has been reported previously [108], to be necessary for the binding to MKT1, a protein that is involved in the stabilization of its target mRNAs and binds to PBP1, polyA-binding protein binding protein.

3.15.1 ZC3H38 localizes mainly to the cytoplasm of bloodstream form trypanosomes

In order to determine the localization of ZC3H38 a cell fractionation assay was performed using two different lysis methods, glass beads or carbamide. The lysis with both methods does not require the use of detergent therefore the

organelles remained intact. The pellet fraction represents the organellar fraction and the supernatant the cytosolic fraction.

In the case of lysis with glass beads no signal was detected for the V5-tagged ZC3H38, neither for the nuclear protein XRND, only the signal for the cytosolic protein trypanothion reductase (TR) was detected by Western blot, perhaps due to blotting problems or to insufficient lysis. When lysing with carbamide, all proteins could be detected and V5-ZC3H38 presents a similar pattern than TR. When comparing the V5-ZC3H38 signal with the one of XRND, a nuclear protein, it seems that V5-ZC3H38 is 2.8-fold more in the cytosolic fraction than XRND, suggesting that this protein was mainly present in the cytoplasm (Figure 36).

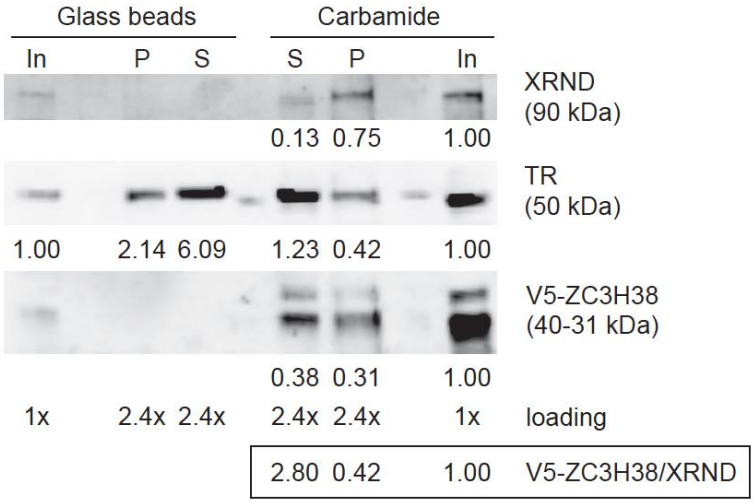


Figure 36. Cellular localization of ZC3H38.
Cell fractionation to determine the localization of ZC3H38. Lysis with carbamide and a pellet pestle. For the input $2.6x10^6$ cells were loaded. In case of pellet and supernatant $6.3x10^6$ cells were loaded.
XRND, exoribonuclease D, nuclear marker; TR, trypanothion reductase used as cytosolic marker; V5-ZC3H38, endogenous V5-tagged ZC3H38, In, input of lysis; P, pellet; S, supernatant.

The predicted molecular weight for ZC3H38 is 31 KDa. The signal detected for V5-ZC3H38 presents several bands that range between 31 to 40 KDa. This pattern implied the presence of post-transcriptional modifications, such as glycosylation or protein phosphorylation. Jannik Traut, a Bachelor student in our laboratory, performed experiments for localization and protein phosphorylation. He corroborated the presence of ZC3H38 in the cytosol and its phosphorylation by immunofluorescence and phosphatase assay respectively. These results were published in his Bachelor thesis.

3.15.1 ZC3H38 was not associated with polyribosomes

In order to determine whether V5-ZC3H38 is associated with polyribosomes, a sucrose gradient centrifugation was performed.
V5-ZC3H38 had the same pattern as trypanothione reductase (TR), a cytosolic protein that is not associated with polyribosomes, but leaks

throughout the gradient (Figure 37a). These experiments were repeated in triplicates and the pattern obtained was similar in all the cases.
When treating the cells with RNases, the polyribosome profile changed. The polyribosomes as well as the monosome peak (80S) and the 40S-60S collapsed, all ribosomes bound to the mRNAs and other proteins disassembled and form only one peak (compare upper panels of Figure 37a and b).
Interestingly, when treating the samples with RNases, V5-ZC3H38 collapsed with the polyribosomes whereas TR was present in almost all the fractions. It was possible that only a fraction of the V5-ZC3H38 protein was associated with polyribosomes (Figure 37b).
As an additional control, the eIF4G3 protein was used; this protein is part of the initiation complex of translation. The signal for this protein seems to leak throughout the gradient, but appeared slightly intense in fraction 4, corresponding to the initiation complex fraction, compared to the polyribosomal fractions (5-7). eIF4G3 protein moves to the first fractions upon RNaseA treatment, showing the characteristic patter of a protein associated to polyribosomes (compare Figure 37a and b).

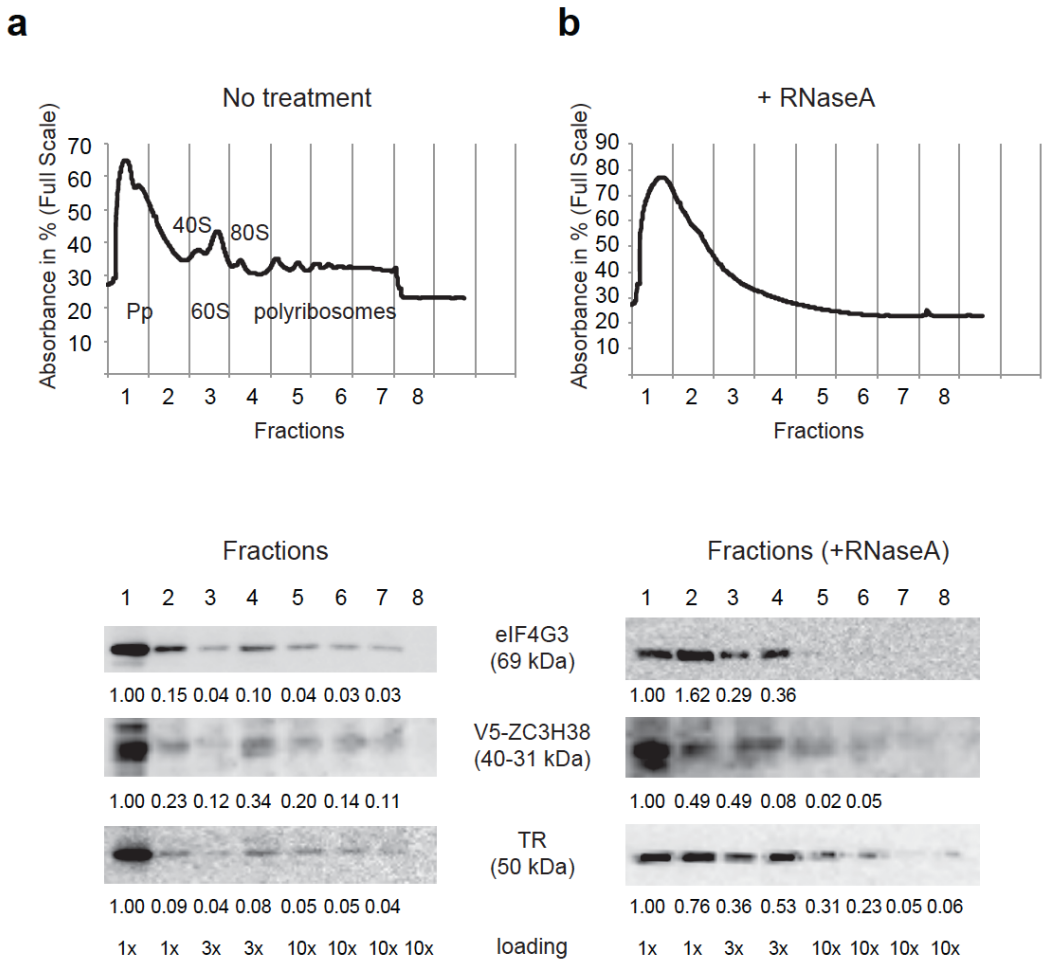


Figure 37. Distribution of ZC3H38 throughout the sucrose gradient.
(**a**) Polyribosome profiles of cells expressing V5-ZC3H38.
(**b**) Like (**a**), but lysis was performed in the presence of 1mg/ml of RNaseA.

Polyribosome profiles shown in the upper panels and Western blots in the lower one. TR was used as cytosolic control, not associated with polyribosomes and the initiation factor eIF4G3 as a control of a protein that is associated with polysomes.
Pp, protein peak; 40S, ribosomal subunit 40S; 60S, ribosomal subunit 60S; 80S, monosomes; eIF4G3, initiation factor of translation; TR, trypanothion reductase an abundant cytosolic protein; V5-ZC3H38 zinc finger protein 38 (endogenous V5-tag).

3.15.2 ZC3H38 and ZC3H37 knock down strategy

In order to elucidate the importance of *Tb*ZC3H38 several RNAi constructs were made and stable cell lines generated (Figure 38a). Since ZC3H38 and ZC3H37 have 77% of protein homology; differing only in 39 amino acids (Figure 38b), RNAi was generated for the specific region of difference, in order to target either *Tb*ZC3H38 or *Tb*ZC3H37 separately (Figure 38). Furthermore, RNAi targeting the 3'-UTR of *Tb*ZC3H38 annotated as *Tb*427.10.12790 was also made (Figure 38a).

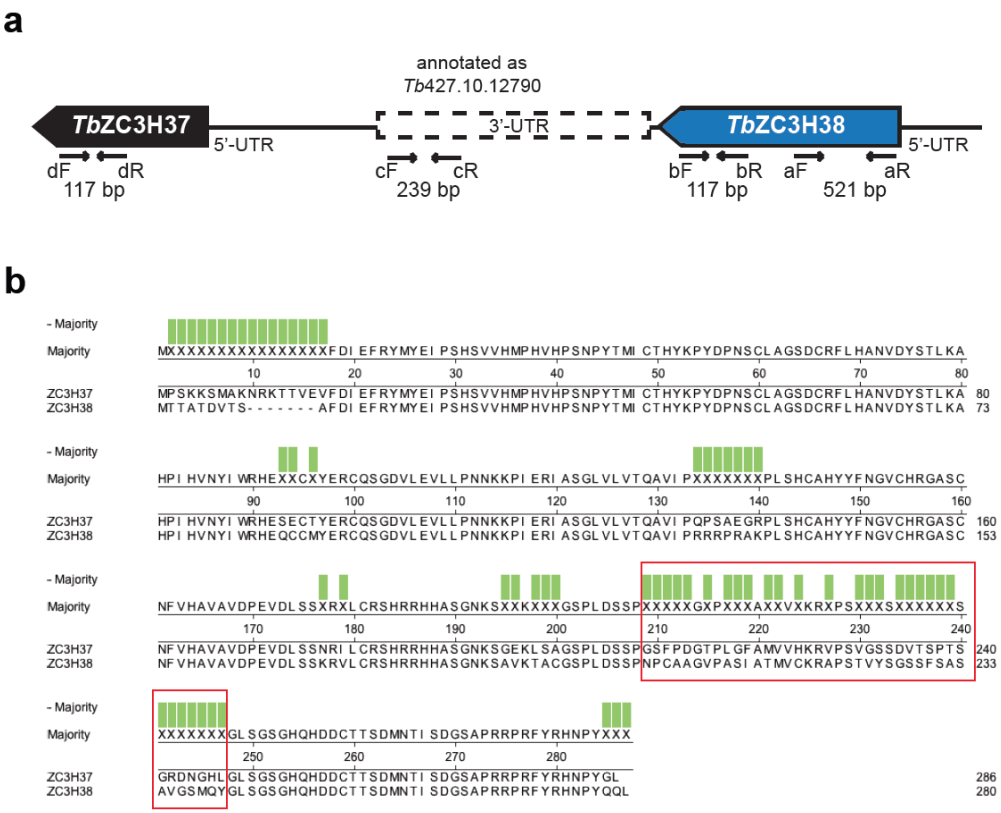


Figure 38. *Tb*ZC3H38 transcription unit and protein homology to *Tb*ZC3H37
(**a**) Schematic representation of the *Tb*ZC3H38 transcription unit and RNAi targets. The primers used to generate the RNAi against a common region for *Tb*ZC3H37 and *Tb*ZC3H38 are aF=CZ4874 and aR=CZ4875. Primers used only for *Tb*ZC3H38 RNAi are bF=CZ5562 and bR=CZ5563. In the case of the *Tb*427.10.12790 gene, the oligos designed to generate the RNAi against the mRNA of this gene are cF=CZ5560 and cR=CZ5561. Finally, the ones used specifically for *Tb*ZC3H37 RNAi are dF=CZ5629 and dR=CZ5630.
(**b**) Sequence alignment of ZC3H38 and ZC3H37 proteins, the regions in green are not homologous. The region in a square is the 39 amino acids that differ between the two proteins; this region was the one chosen to target the specific RNAis against *ZC3H37* and *ZC3H38* mRNAs

Data previously obtained in our laboratory (unpublished) showed that RNAi against a common region of *Tb*ZC3H37 and *Tb*ZC3H38 lead to a slight growth defect. Also, when performing a Northern blot with the same probe to detect *ZC3H38*, another band was also detected, corresponding to the size of the *ZC3H37* mRNA. The intensity of both bands was reduced upon RNAi induction. These results were obtained only once from one of the clones. Unfortunately, it was impossible to reproduce these results, most probably because by freezing and thawing the clones the RNAi effect was lost.

3.15.3 RNAi against ZC3H38 results in a slight growth defect

The RNAi was inducible by tetracycline and was designed using the sequence of the 117mer that differs between *Tb*ZC3H37 and *Tb*ZC3H38, targeting only *ZC3H38* (Figure 39a).

Upon RNAi induction, the cells presented a slight growth defect compared to wild type cells. Due to the fact that the induction by tetracycline was leaky, the cells without tetracycline (-Tet) and the ones were tetracycline was added (+Tet) had the same growth phenotype (Figure 39b). Furthermore the signal for *ZC3H38* mRNA reduces after 24 hours of tetracycline induction, measured by Northern blot (Figure 39c). The V5-ZC3H38 protein signal reduced also after 24 hours of tetracycline induction (Figure 39d), this western blot includes the V5-ZC3H38 protein without the RNAi plasmid and the wild type, without any plasmid, as controls. The Northern and Western blot experiments were repeated at least three times obtaining similar results ZC3H38 decreases after 24 hours of tetracycline induction in the mRNA level and at the protein level, while the signal of the V5-tagged cell line was still present without any variation in the case of the Western blot.

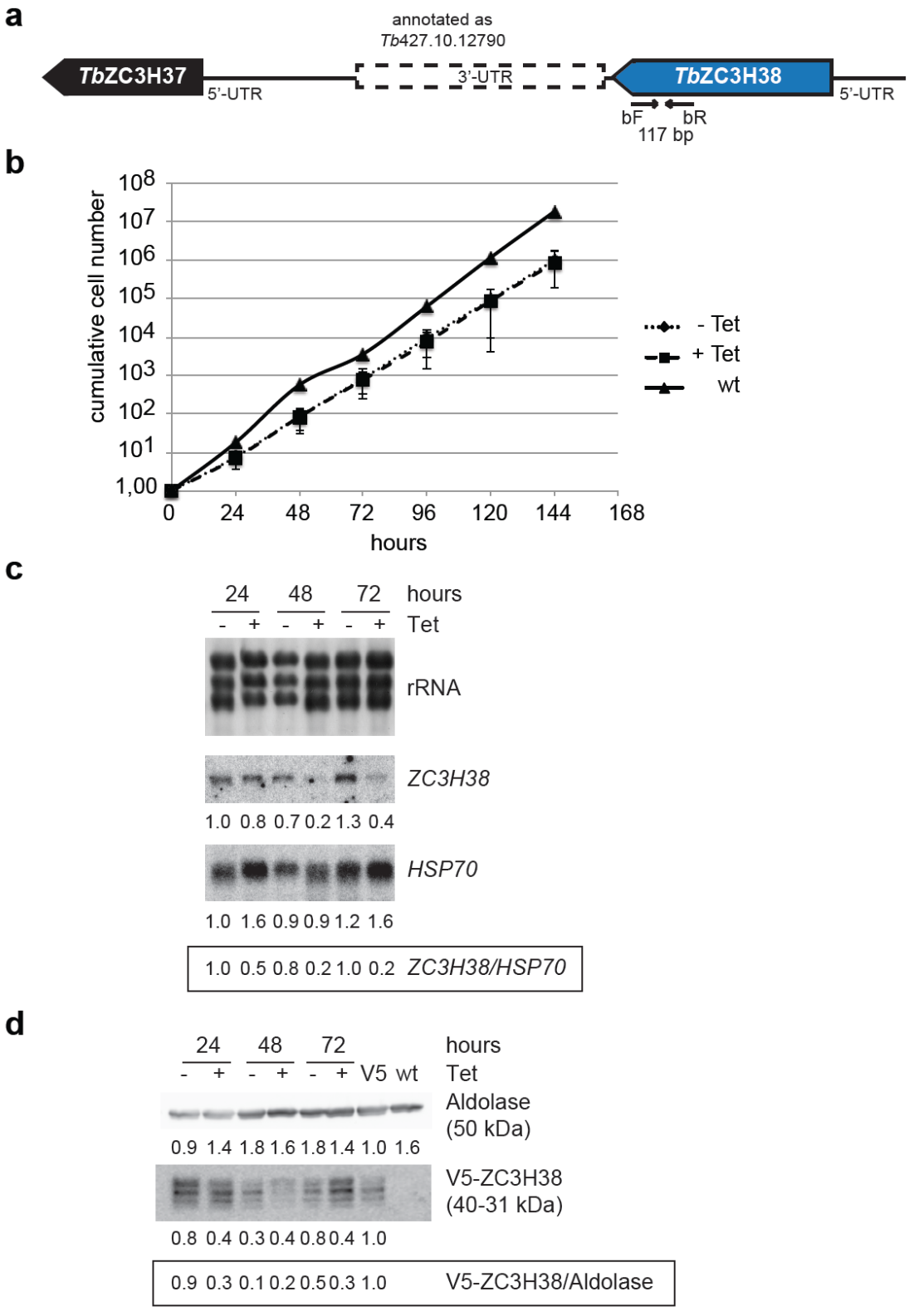


Figure 39. Effect of RNAi against *ZC3H38*

(**a**) Schematic representation of the *Tb*ZC3H38 transcription unit. The primers used to amplify the 117bp probe against *ZC3H38* are depicted as arrows (bF=CZ5562 and bR=CZ5563).

(**b**) Growth curve for cell lines where the RNAi against *ZC3H38* was induced (+Tet) and uninduced (-Tet) compared to the wild type (wt).

(**c**) Northern blot showing the decreased in the *ZC3H38* signal upon RNAi induction. *HSP70*, the *heat shock protein 70* mRNA, was used as loading control.
(**d**) Western blot showing the effect on V5-tagged ZC3H38 protein upon RNAi induction. TR, trypanotion reductase was used as control

Furthermore, a construct that targeted only the 3'-UTR of *Tb*ZC3H38 gene (annotated as *Tb*927.10.12790) was also designed to generate an RNAi specifically for this gene (Figure 40a). A slight growth defect in the phenotype was observed using this RNAi when comparing to wild type cells (Figure 40b). The growth defect obtained by this RNAi was similar to the one caused by the RNAi specifically against *Tb*ZC3H38 ORF. Samples with (+Tet) and without (-Tet) presented the same growth phenotype as in Figure 39b.

In the Northern blot (Figure 40c) and Western blot (Figure 40d) the signal for ZC3H38 also reduced after 24 hours of tetracycline induction. Especially in the Western blot, when comparing the signal for the tagged protein at 24 hours of tetracycline induction with the one of the control cell line containing only the V5-tag and not the RNAi (named V5 in Figure 40d). Approximately, 70% of the V5-ZC3H38 protein has disappeared and it is not being produced anymore.

In order to find the RNAs that can be affected by the reduction of the ZC3H38 protein, it was decided to perform a time course experiment to determine the exact time at which the V5-ZC3H38 is reduced but there is no growth defect. The time course experiment was done using both RNAi cell lines, the one targeting the ORF and the 3'-UTR of *Tb*ZC3H38. Time points were taken from 2 to 24 hours, no reduction in the protein level was observed up to 8 hours, only when inducing the cells for 12 hours there was a reduction of the V5-ZC3H38 protein without presenting a change in the growth phenotype. Further experiments will be conducted by Vikram Kumar to solve this open question.

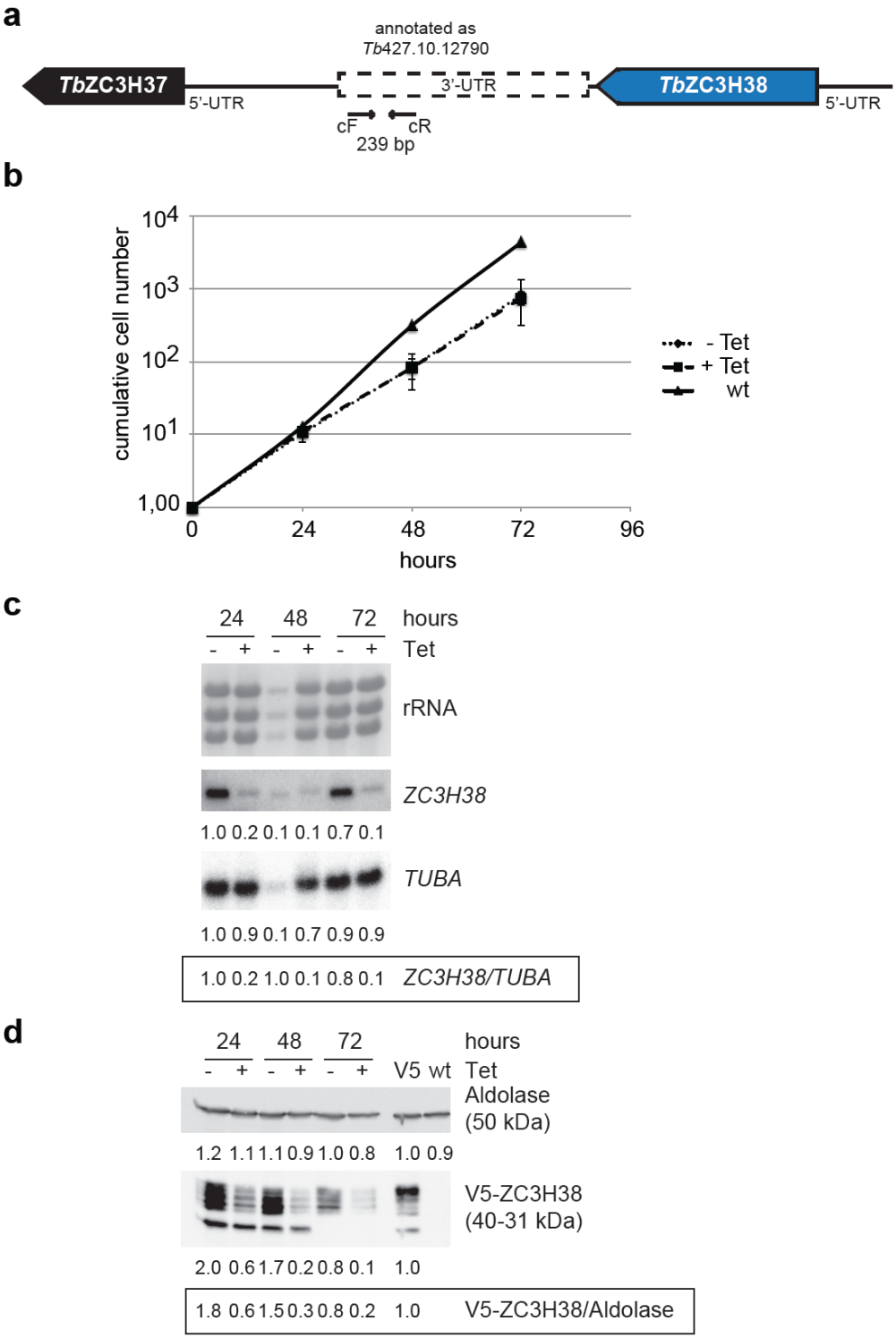


Figure 40. Effect of RNAi against *Tb427.10.12790* mRNA
(**a**) Schematic representation of the *Tb*427.10.12790 transcription unit. The primers used to amplify the 239 bp probe against this mRNA were depicted as arrows (cF=CZ5560 and cR=CZ5561).
(**b**) Growth curve for cell lines where the RNAi against *Tb427.10.12790* was induce (+Tet) and uninduce (-Tet) compared to the wild type (wt).

It was also interesting to determine the effect of RNAi on *ZC3H37*. Therefore, an RNAi construct (that was generated using the primers cz5629 and cz5630)

was used to target specifically *Tb*ZC3H37 (Figure 41a). Preliminary results, showed no changes in the growth phenotype upon the addition of tetracycline compared to the wild type (Figure 41b). The cell line expressing this RNAi behaved in terms of growth like wild type cells. This results was ontained only once with one clone.

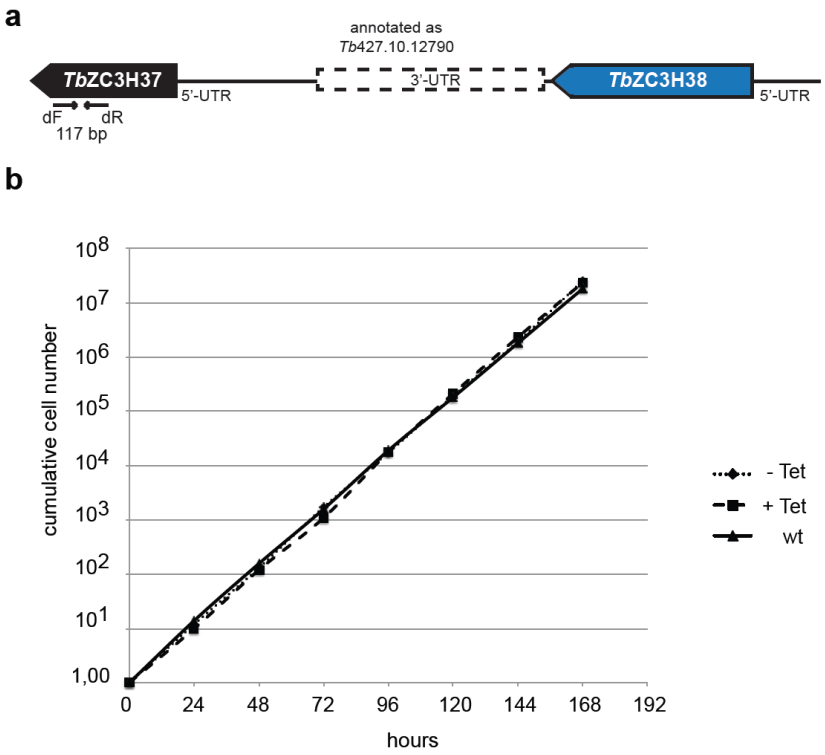


Figure 41. Effect of RNAi against *ZC3H37*
(**a**) Schematic representation of the *Tb*ZC3H37 transcription unit. The primers used to amplify the 117bp probe against this mRNA are depicted as arrows (dF=CZ5629 and dR=CZ5630).
(**b**) Growth curve for cell lines where the RNAi against *ZC3H37* was induce (+Tet) and uninduce (-Tet) compared to the wild type (wt).

Due to the results obtained by the different RNAi against these genes, it was decided that knockout cells lines were required. The plasmids to generate the knockouts for *Tb*ZC3H38 only and for a region containing *Tb*ZC3H38-*Tb*927.10.12790-*Tb*ZC3H37 were designed and cloned. These plasmids were transfected into bloodstream form trypanosomes. So far, only clones for a single knockout against *Tb*ZC3H38 were obtained. Efforts are being made in order to obtain the knockout of the long stretch of the transcription unit corresponding to *Tb*ZC3H38-*Tb*927.10.12790-*Tb*ZC3H37.

3.15.4 Tethering of ZC3H38

In order to determine the effect of ZC3H38 on its target mRNAs, the ZC3H38 protein was tethered to two different reporter mRNAs, one containing a *CAT-5 boxB* and another cell line expressing only the *CAT* reporter without the *5 boxB* elements. The protein generated was a fusion protein (λN-ZC3H38-myc) composed of a λN at the N-terminus and a myc-tag at the C-terminus

(Figure 42a). As additional controls, cell lines that did not express the protein were tested.

Results obtained previously by Dr. Esteban Erben in our laboratory [74] indicated that this protein might be able to stabilize its target mRNAs. Therefore, to determine exactly which region of the ZC3H38 protein was responsible for its effect on the reporter mRNA, different constructs were made (Figure 42b):

1. The complete protein λN-ZC3H38-myc (1104bp)
2. The λN-ZC3H38-myc protein with the two CCCH domains, without the HNPY (deletion of a region compromising the 814-843mer of the ZC3H38 ORF, named CCCHs)
3. The λN-ZC3H38-myc protein with a deletion on the N-terminal part of the protein, this part contains the first CCCH domain (deletion 1-218, named N-term del)
4. The λN-ZC3H38-myc protein and a region containing the HNPY domain, without the two CCCH domains (deletion 1-493, named HNPY)

The tethering assay confirmed the results previously obtained in our laboratory [74]. The complete λN-ZC3H38-myc protein stabilized its target mRNAs, which was indicated by the increase in *CAT* mRNA in the samples containing the *CAT-boxB* reporter, but not in the samples containing the *CAT* reporter alone. The amount of *CAT* reporter RNA containing the *boxB* element was also increased compared to the controls (Figure 42b).

When testing different parts of the protein, it was noticed that the CCCH domains were not required for the stabilization of the tethered target mRNAs. The cell line without the HNPY domain, containing the CCCH domains (deletion 814-843, named CCCHs) exhibits a reduction of CAT activity and *CAT-boxB* mRNA expression compared to the cell line expressing the complete protein and the *CAT-boxB* reporter. Also, the CAT activity and *CAT* mRNA values were similar to the wild type cell lines (Figure 42b, CCCHs).

In the case of the cell lines containing the N-terminal deletion (deletion 1-218), the CAT activity increased almost 1.5-fold when comparing to the wild type and the activity was similar to the complete protein (Figure 42b, N-term del). The *CAT* mRNA results for the N-terminal deletion must be repeated. The Northern blots were quantified using the bands of the rRNA (methylene blue staining) as loading control and some of the Northern blots had problems with blotting. This could generate problems in the quantification, which might be the reason for the apparent 20-fold excess of *CAT* mRNA in the samples for N-terminal deletion with *boxB* and for the cell line expressing the HNPY domain without *boxB* (Figure 42b). In these both cases, the data for the CAT activity rather represent a more reliable result.

The samples containing the HNPY domain (N-term del, deletion 1-218 and HNPY, deletion 1-493) exhibited stabilization of the *CAT-boxB* mRNA, resulting in a 1.5-fold increase of the CAT activity, when comparing to the wild

type. The CAT activity and *CAT* mRNA expression were similar to the ones of the complete fusion protein (Figure 42b, HNPY).

The expression of the λN-ZC3H38-myc protein was tested by Western blot. Figure 42c shows the expression of the complete fusion protein with the characteristic pattern of phosphorylation in cell lines containing *CAT* and *CAT-boxB* reporters. Interestingly, when detecting the λN-ZC3H38-myc protein without the HNPY motif, the phosphorylation reduced and only one single band could be detected (Figure 42d).

These results indicate that ZC3H38 stabilize its target mRNAs and that the regulation of the mRNA abundance depends on the presence of its HNPY domain.
The tethering results were obtained with the help of Jannik Traut.

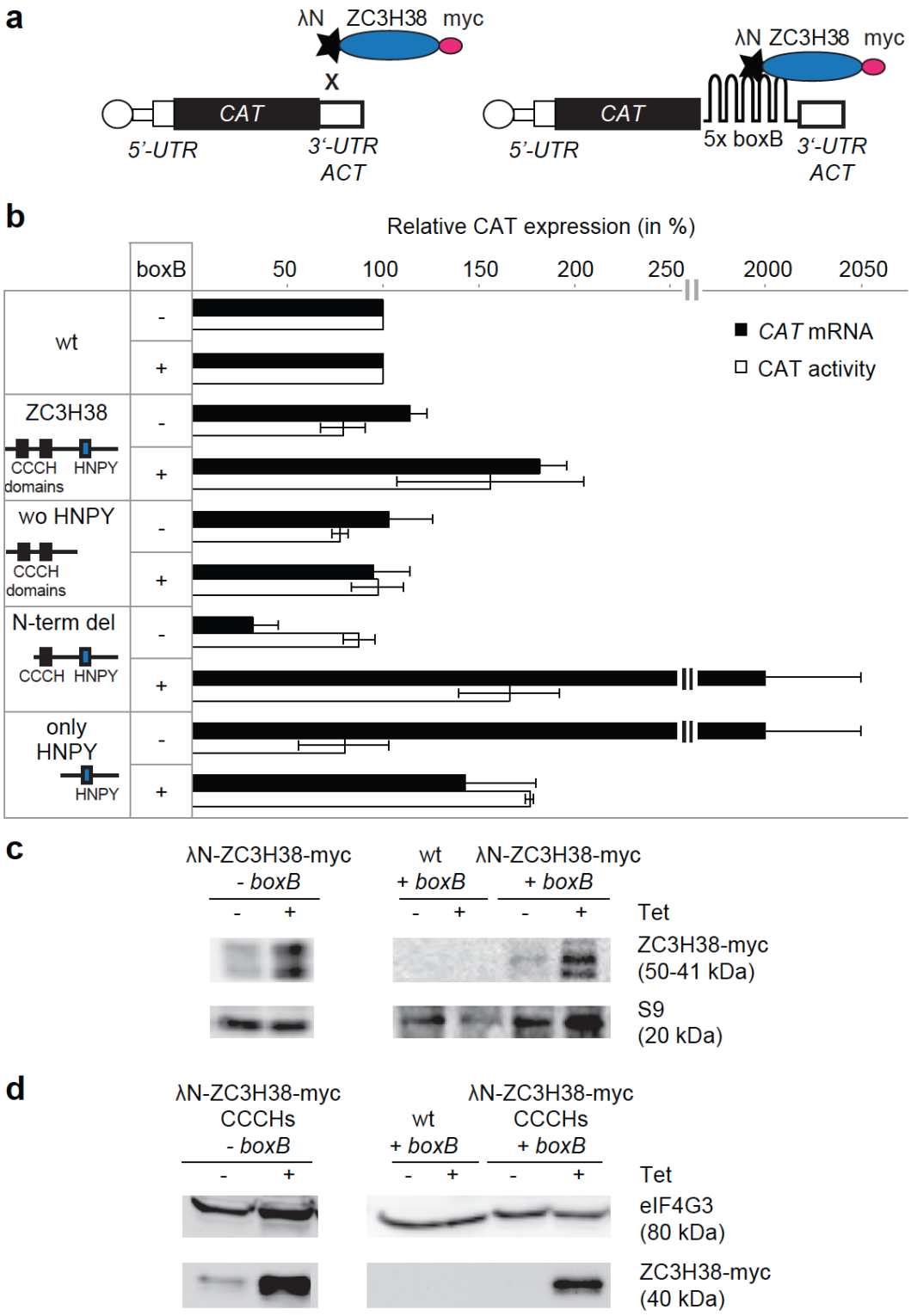


Figure 42. The HNPY domain of ZC3H38 stabilized the *CAT-boxB* reporter
(**a**) Schematic representation of the λN-ZC3H38-myc tethered to a reporter mRNA. (**b**) Relative CAT expression. White bars represent the CAT activity and the black ones represent the *CAT* mRNA. This experiment was done in cell lines that expressed the different parts of the λN-ZC3H38-myc protein on both reporters (with and without boxB). The error bars represent the difference between experiments (standard deviation). Each experiment in this figure was repeated at least three times.

(**c**) Western blot of the cell lines expressing the complete λN-ZC3H38-myc protein. The ribosomal protein S9 was used as loading control.
(**d**) Western blot showing the expression of the λN-ZC3H38-myc protein without the HNPY motif. Note that the phosphorylated bands of ZC3H38 were lost. The eIF4G3 protein was used as loading control.

3.15.5 Yeast two hybrid high-throughput screen of ZC3H38

To further characterize the ZC3H38 protein, it was of interest to know the interaction partners of this protein. Therefore, a genome-wide yeast two hybrid screen was performed.

For this experiments ZC3H38 was used as bait protein. In order to perform the genome-wide screen, our bait protein containing strain was mated with a strain containing random genomic fragments of *T.brucei*. Dr. Esteban Erben made the yeast two-hybrid library in our laboratory.
The mating was performed at 30°C and $1.2x10^5$ diploid cells were obtained with an efficiency of 2%. After the diploids were obtained, a white blue screening was performed and 444 positive blue colonies were taken and grown. The plasmids containing candidate interactors (preys interacting with our ZC3H38-bait plasmid) were isolated and the PCR for the prey plasmids was standardized using different conditions.

The pattern obtained after the amplification with the adaptors is shown in Figure 43. Two different enzymes were tested in order to determine the best conditions for this amplification. Both enzymes gave a similar pattern, so the amplification was done with both, then purified and send for DNA sequencing.

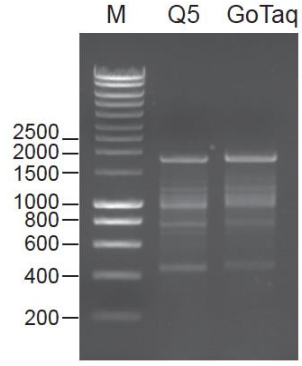


Figure 43. PCR amplification of the positive Y2H clones with ZC3H38 as bait. The primers used for the amplification were CZ5278 and CZ5472. Two different PCRs were done using different polymerases (Q5 and GoTaq) in order to determine the best conditions for the amplification.

The yeast two-hybrid screen was repeated twice and the samples mixed in order to obtain a better coverage of target protein sequences. The results are shown in **Table 5**, the data were selected in order to obtain proteins with the mayor number of reads present in more than one location. The table

compares proteins that were found in other purifications, such as the NOT1 yeast-two hybrid purification and a screen made in our laboratory by Dr. Esteban Erben, which used a plasmid library of random genomic fragments from *T.brucei* to identify proteins that might be involved in the stabilization a reporter mRNA upon the addition of different concentrations of blasticidin (BLA up). Most of the proteins identified were hypothetical proteins and parafagellar rod proteins.

Table 5. Yeast-two hybrid screen using ZC3H38 as bait

The table shows the number of reads obtained for each gene of interest, as well as the number of locations and number of reads per gene. Reads located only in one place in the gene were excluded from the analysis. Strand (+) or (-) correspond to the reads that are in-frame and aligned to the sense strand or anti-sense strand of the *T.brucei* genome.

NOT1 represents a yeast-two hybrid screen of this protein. The table shows common proteins found in the NOT1 and the ZC3H38 genome-wide yeast two-hybrid screens.

BLAup compares a screen made in order to identify proteins involved in the up-regulation of reporter mRNAs (BLAup) with the ZC3H38 yeast two-hybrid screen. The numbers under the BLAup column represent the folds of mRNA up-regulation for each gene. n.d. no data

Accession number	No reads	No locations	ORF length	Strand	Description	NOT1	BLA up
Tb927.11.3300	16 27 17	3	2799	+	hypothetical protein, conserved		6,45
Tb927.3.1580	569 417	2	1641	-	hypothetical protein, conserved		
Tb927.4.3400	11 2268	2	1818	-	hypothetical protein, conserved, ARM repeat	0	
Tb927.7.6920	125 850	2	1875	-	hypothetical protein, conserved		
Tb927.9.4390	185 54	2	1137	+	DNA-damage inducible protein DDI1-like protein		n.d
Tb927.10.11380	13 1532	2	1173	+	hypothetical protein, conserved	25	
Tb927.3.920	3851 20	2	3810	+	hypothetical protein, conserved	69	
Tb927.1.2570	830 15	2	2949	+	coatomer beta subunit	86	
Tb927.9.2700	14 13 74 7548	4	1452	-	hypothetical protein, conserved	113	

Tb927.6.3670	87 79 12	3	9669	-	paraflagellar rod protein PFC8	117	
Tb927.9.7690	27 67 16 17425	4	5574	-	hypothetical protein, conserved	184	15,64
Tb927.3.5310	13 41	2	6387	+	paraflagellar rod protein	318	39,54

3.15.6 RNA-IP of ZC3H38 for RNAseq

In order to determine which RNAs are the targets of ZC3H38, an RNA immunoprecipitation (RNA-IP) was performed using the tethering cell lines (Figure 44a), because the integrity of the mRNA in the preparation could be checked by Northern blot and the success of the pull down by Western blot.

Figure 44b shows that the *CAT* mRNA was not degraded during the pull down and the Western blot confirmed that ZC3H38-myc was efficiently being pulled down using myc-beads (Figure 44c). In the next step, the samples were depleted from the ribosomal RNA using oligos [164], after this step the signal of *CAT* mRNA could not be detected in the Northern blot.

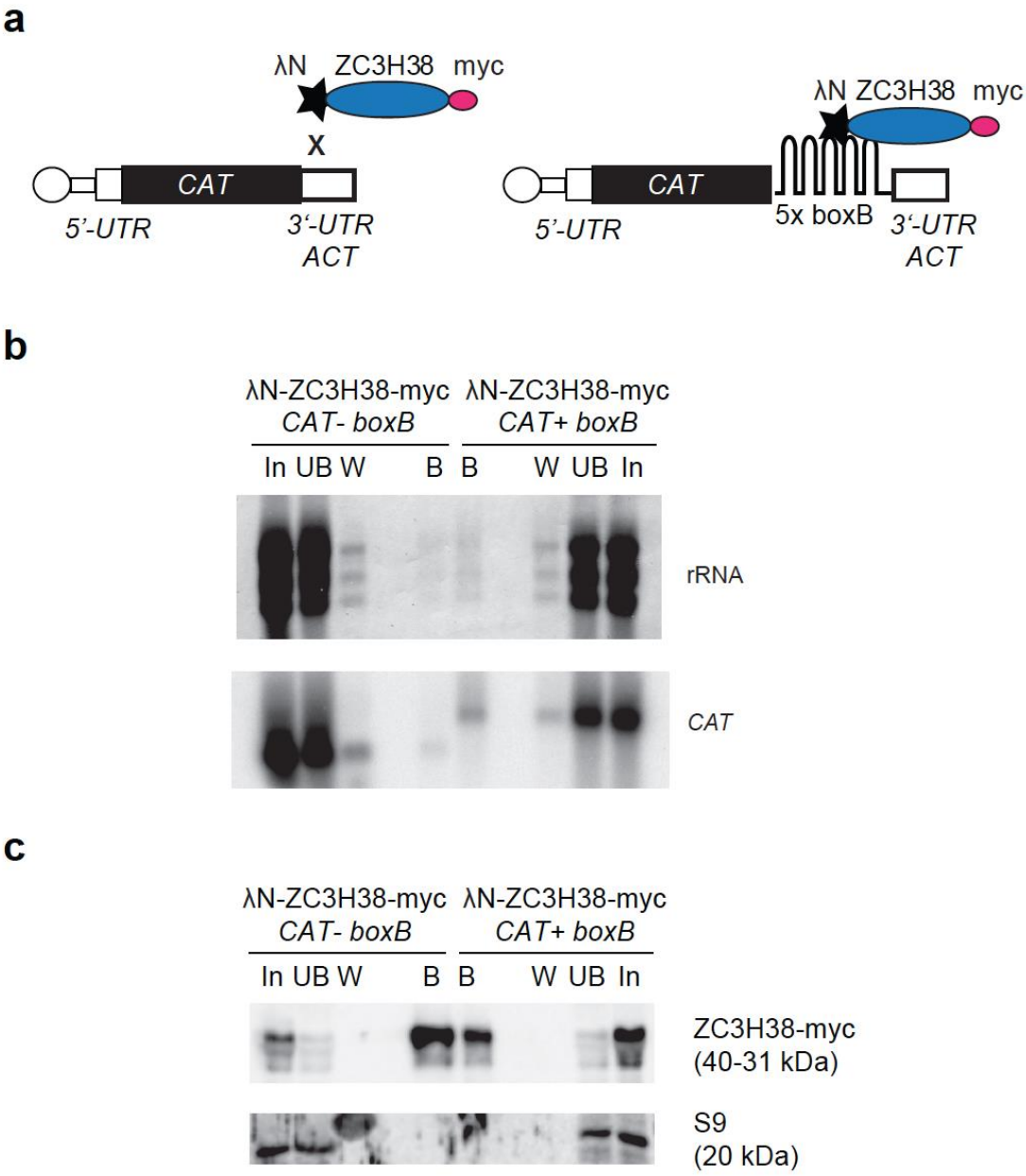


Figure 44. RNA pull down of ZC3H38 for RNAseq
(**a**) Schematic representation of the cell lines used for the pull down.
(**b**) Northern blot shows that *CAT* mRNA can be detected during this pull down and that the mRNA was not degraded at this stage. For In, UB and W, $3x10^7$ cells from each were taken for RNA extraction and loaded onto the gel, in case of the beads, $7.5x10^8$ cells were used.
(**c**) Western blot confirming the pull down. ZC3H38-myc was pulled down using myc-beads. The ribosomal protein S9 was used as a loading control. For In $1x10^7$ cells were leaded, in case of the UB and W $2.5x10^6$ cells were used and for the beads an equivalent to $2x10^7$ cells were taken.
In, Input after lysis; UB, unbound; W, wash; B, beads.

The facility informed us that the samples were degraded. A possible explanation could be that the temperature conditions inflicted by the rRNA depletion procedure caused the degradation of the mRNA. This experiment could be repeated in the future using a modified protocol for rRNA depletion.

3.15.7 Co-IP of ZC3H38 and MKT1

The ZC3H38 protein contains a HNPY motif, which was previously published to be a putative binding motif of MKT1, a protein that binds to the PBP1 and stabilizes its targets mRNAs [108]. A Co-IP was performed in order to determine if these two proteins co-purify.

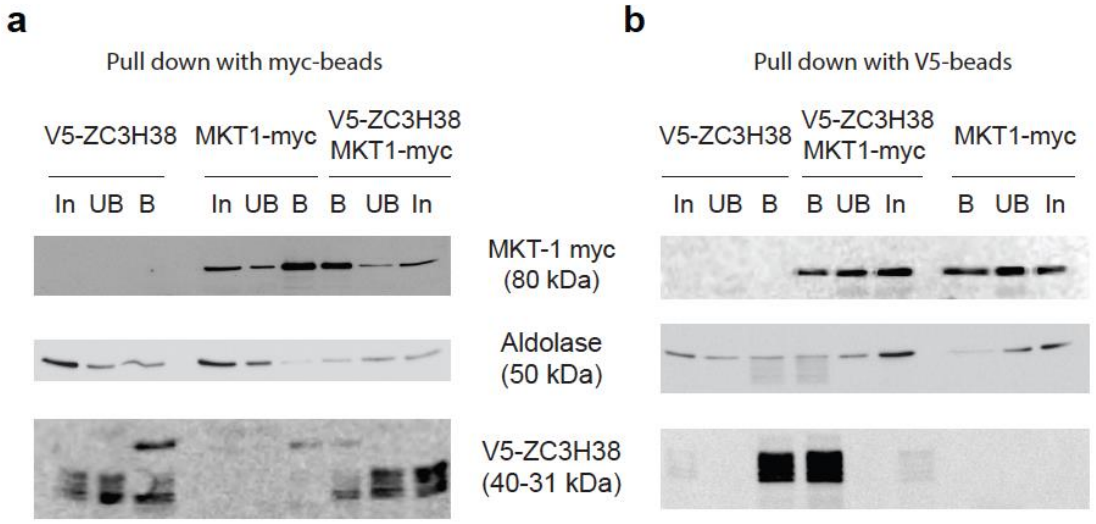


Figure 45. Co-immunoprecipitation of V5-ZC3H38 and MKT1-myc
(**a**) Co-immunoprecipitation of MKT1-myc and V5-ZC3H38 using α-myc beads in bloodstream form cells.
(**b**) Co-immunoprecipitation of MKT1-myc and V5-ZC3H38 using α-V5 beads in bloodstream form cells.
For input and unbound fractions $5x10^6$ cells were taken for the Western blot. In the case of the eluates $1.5x10^8$ cells were loaded in (**a**) and (**b**).
In, input; UB, unbound; B, beads.

Figure 45a shows the results of the co-immunoprecipitation done using α-myc agarose beads. The pull down was done using a cell line expressing the V5-ZC3H38 and MKT1-myc. Control pull downs were done in parallel, using a cell line expressing only V5-ZC3H38 and MKT1-myc, respectively.

When pulling down with α-myc beads, V5-ZC3H38 was detected in the beads of the cell line containing both tagged proteins as well as in the cell line containing only V5-taged ZC3H38. These results imply that V5-ZC3H38 bound unspecifically to the α-myc beads.

In the co-immunoprecipitation using α-V5 beads (Figure 45b), MKT1-myc was found present in the beads of the cell line containing both tagged proteins, but was also found in the control pull down. This indicates that myc-MKT1 binds unspecifically to the V5-beads.

Since there was a signal for aldolase detected in all the beads fractions, it is possible that higher number of washes is necessary.

Taking in consideration the results obtained, it was not possible to reach a conclusion if these proteins co-purify, and therefore different approaches were taken to identify the proteins that can interact with ZC3H38.

3.15.8 Proteins co-purifing with ZC3H38

In order to determine the protein interaction partners of ZC3H38, two approaches were made. TAP purification of ZC3H38 and immunoprecipitation of a V5-ZC3H38 protein using V5-beads.

3.15.8.1 TAP purification of ZC3H38

Small scale TAP-tag purification were performed and it was noticed that the phosphorylation pattern of ZC3H38 was lost, when trying to detect the TAP-ZC3H38 fusion protein by Western blot. Furthermore, it seemed that almost 50% of the TAP fusion protein was not able to bind to the beads; most of it was found to be on the unbound fraction in the Western blot (compare In and UB, Figure 46). In the eluate no signal was detected because the TEV protease released the ZC3H38 with the calmodulin-binding domain of the TAP-tag and the Protein A remains bound to the IgG matrix. Since the antibody used in **Figure 46** recognizes only Protein A, there was no signal in the eluate.
It is important to mention that the TAP-tag is approximately 183 amino acids long and that the TAP-tag was fused to the C-terminal end of the ZC3H38 protein, near the HNPY domain, this might be the cause of the loss of phosphorylation.

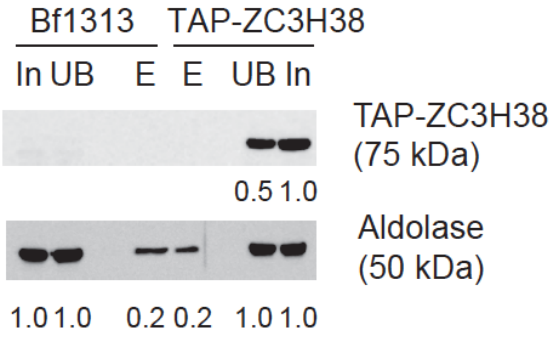


Figure 46. TAP-tag purification
Western blot showing the purification of ZC3H38 using a C-terminal TAP-tag. The purification using a cell line expressing the fusion protein TAP-ZC3H38 was compared to the control purification, using Bf1313 cell line.
For input and unbound an equivalent of $6x10^6$ cells were taken for Western blot. In case of eluate after TEV cleavage $1.5x10^7$ cells were used for Western blot.
In, input; UB, unbound; E, eluate represent the first eluate, after TEV cleavage.

A stable cell line expressing the N-terminal TAP-ZC3H38 fusion protein was obtained. The TAP purification experiment will be conducted in the near future.

3.15.8.2 Immunopurification of V5-ZC3H38

This experiment was performed as an alternative method to identify the proteins that co-purify with ZC3H38. V5-ZC3H38 protein was pulled down using V5-agarose beads and was compared to a control cell line that did not contain the V5-tagged protein (Bf1313 cell line).
The pull down was split in two, a small part of the sample was used for Western blot and the rest was loaded on a gel that was stained with Coomassie blue and sent to DL-QMS.

The pull down was repeated four times and triplicates were tested by Western blot and sent to DL-QMS. Figure 47 shows the results of one of the replicates. The Western blot confirmed that the pull down was successful; the V5-ZC3H38 protein binds specifically to the V5-beads (Figure 47a). The rest of the samples were loaded on a Coomassie blue stained gel as it is shown in Figure 47b. The pattern of proteins between the control pull down and the pull down using V5-ZC3H38 looks similar; therefore it was decided to do DL-QMS.

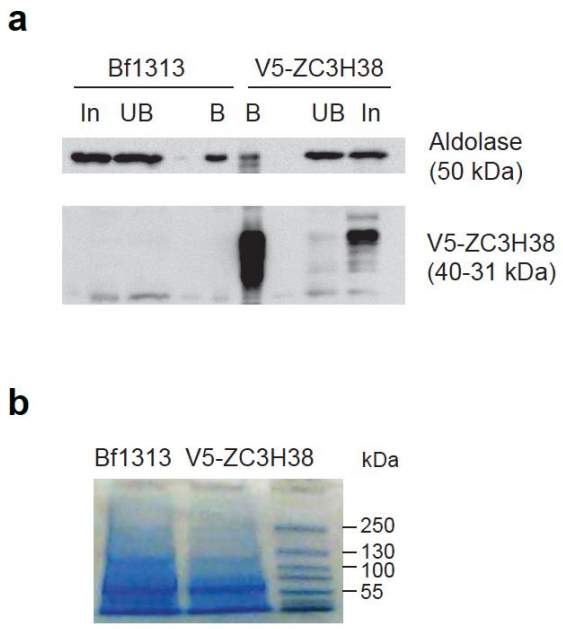


Figure 47. Pull down of V5-ZC3H38 using V5-agarose beads
(**a**) Western blot showing the specificity of the pull down.
In, input; UB, unbound; B, beads eluted by boiling in sample buffer for 10 min.
For input and unbound $5x10^6$ cells were loaded in the Western blot. In case of the beads, $3.7x10^8$ cells were used for Western blot.
(**b**) Coomassie blue staining of the pull down comparing the eluates of the control cell line with the one of the cell line expressing the V5-ZC3H38.
For both samples $7x10^8$ cells were loaded in the Coomassie blue gel.

Due to problems with the trypsinization of the samples, two replicates were lost. Only results from one of the replicates were delivered but the V5-ZC3H38 was not detected among the proteins found.

4. Discussion

4.1 Affinity purification of translating polyribosomes

Several aspects of mRNA metabolism can be controlled by post-transcriptional mechanisms, such as localization, translation, storage and decay, among others. The key components of these mechanisms are regulatory proteins present in the mRNPs. For this reason the study and characterization of the RNP components is fundamental to elucidate these regulators. In the present dissertation, a method to purify translating mRNPs and to detect its protein components is described.

The affinity purification of translating polyribosomes is based on a previously published method from the 70s [143], which purified translating polyribosomes via immunoprecipitation, using an antibody against the ovalbumin nascent peptide in hen oviduct cells. In early studies (methods developed in the 60s-70s), many mRNAs were purified using sucrose gradient centrifugation and immunoprecipitation. The success of these purifications was based on the abundance of the mRNAs purified, as well as the use of very specialized cells, such as the purification of *ovalbumin* mRNA from rabbit reticulocytes [137] or *VSG* from bloodstream form trypanosomes [140-142]. Methods developed later (from 90s on) made use of affinity purification tags [146] to determine the protein components bound to the purified mRNA. More specialized methods used aptamers [156, 159] to purify mRNPs. Most of these approaches used total or concentrated cell lysates for the purification, others have a system that produced abundant mRNAs containing the aptamer-*cis* element of interest and, more important, were *in-vitro*. The problem with the *in-vitro* methods is that such assays may not represent what is happening *in vivo*. Therefore, the method developed here allows the purification of a specific mRNP *in vivo* in order to identify regulatory proteins on the untranslated regions of interest.

Initially, I tried to purify cytosolic polyribosomes from membrane bound ones. Leaving the organelles intact allows the separation of the mature 3SBPs-CAT protein (consider to be a contaminant) from the samples. This strategy was successful (Figure 14), but the main problem encountered was the poor yield obtained. It is possible that part of the polyribosomes with mRNAs containing many ribosomes (present in higher sucrose fractions) were also spun down in the first centrifugation step, along with the organellar fraction (compare polyribosome profiles Figure 16a with Figure 17a). Another possibility is that the mRNAs were degraded during this longer process, which included a double step of ultracentrifugation.

After several optimization steps the purification of 3SBP-CAT-mRNPs from translating polyribosomes consisted on lysis using detergents and only one step of ultracentrifugation (Figure 11). In this affinity purification method, it was expected that many *3SBP-CAT* mRNAs containing several regulatory proteins interact with the multiple streptavidin binding sites in the beads and that these complexes can be easily eluted using biotin. Nevertheless, the

elution with biotin was inefficient (Figure 15). A possible explanation might be that there are many 3SBPs-CAT-mRNPs bound to the beads. Also one must consider that mRNAs can interact with other, sharing regulatory proteins, which can bind to the 3SBP-CAT-mRNPs. These interactions could block the access of eluting agents such as biotin or RNases. Therefore, it was decide to elute the purified mRNPs using SDS, boiling the beads in Laemmli buffer.

4.2 The affinity purification method corroborates known RBP-mRNA interactions

As proof of concept my goal was to detect the well-characterized interaction between ZC3H11 and the AU-rich element present in the *HSP70 3'-UTR* [107] using my purification method. It was noticed that the *3SBP-CAT-HSP70 3'-UTR* reporter is equally distributed in each polyribosomal fraction of the sucrose gradient and this might represent a benefit for the purification (Figure 21). It could allow the purification of proteins that interact at different stages with a specific mRNA, such as proteins that interact at the initiation of translation and proteins of the degradation machinery that could interact when the mRNA is prepared for degradation, most of them present in the polyribosomes.

Western blot results confirm that ZC3H11 co-purified with the *HSP70 3'-UTR* only when the AU-rich element is present (Figure 23). Unfortunately, since the ZC3H11 protein is highly phosphorylated and it cannot be easily detected by mass spectrometry, only one modified peptide for this protein was identified. Other purifications performed in the laboratory also fail to detect this protein by mass-spectrometry. To make possible the detection of this protein, a higher expression might be needed, for instance to expressed the protein using an over-expressed TAP-tag version or maybe in fusion with other protein like the green fluorescent protein (GFP). Maybe these fusion proteins will increase the chance to detect ZC3H11 in the purifications, but will not discard the possibility that the function of the protein is compromised.

Other proteins that co-purified with the *HSP70 3'-UTR* reporter are listed in Table 2. Initiation factors as wells as the poly (A) binding proteins PABP1 and 2 were detected. This was expected because this *HSP70* reporter was purified from translating polyribosomes and is stabilized during heat shock. Interestingly, also protein components of the degradation machinery or factors involved in mRNA degradation, such as NOT10, DHH1 and PUF6 were found to co-purify with this mRNA. For instance, PUF6 in *T. cruzi* has been implicated in the destabilization of mRNAs [177]. This might imply that upon heat shock, when global translation is reduced, proteins of the degradation machinery are in close proximity with translating mRNAs and that the regulatory proteins are in dynamic interaction with the mRNAs. Although more experiments should be done to probe this hypothesis. Some RBPs that are regularly found in purifications were also detected, such as DRBD3, which binds regions of poly-pyrimidines in the mRNAs [178], or abundant proteins such as UBP2 [179].

Purifications in order to detect MKT1, a protein that binds to ZC3H11 but does not directly bind mRNAs were done using the *HSP70* reporters. This protein is found to be 4-fold enriched in the purification of the complete *HSP70 3'-UTR* when comparing with the deleted one (Figure 23). This finding indicates that the method can purify protein components of mRNPs that are indirectly interacting with the target mRNA.

Another well-known mRNA-protein interaction was also tried. The detection of the RNA binding protein DRBD3/PTB1 bound to a reporter containing the *AATP11 3'-UTR.* It is known that this RBP stabilizes *AATP11* mRNA upon binding [79]. Results show that DRBD3/PTB1 co-purified with both reporters, the one containing the complete *AATP11 3'-UTR* as well as the one with the deletion. Nevertheless, quantification of Western blot reveals that DRBD3 is 2.4-fold enriched in the purification of the complete *AATP11 3'UTR* compared to the deleted one. The main problem here is that DRBD3/PTB1 binds to pyrimidine-rich stretches on mRNAs, which might be present in the reporters used.

4.3 Possible regulators of the *EP* mRNA

The purification using the *EP 3'UTR* was one of the most interesting ones, because the *EP 3'-UTR* contains three well characterized regulatory domains, but so far no regulatory proteins have been found to be directly involved in the developmental regulation of this mRNA. I tried to identify regulatory proteins that co-purified with the *EP 3'-UTR*. In a first trial, samples were processed using quantitative mass-spectrometry. Proteins detected to be enriched in the purification of the *EP 3'-UTR* include proteins involved in the stabilization of mRNA and enhance translation, such as MKT1 that binds to PBP1, a protein that binds to poly(A) binding protein. Interestingly, other proteins found were components of the degradation machinery, such as XRNA and NOT1. It is possible that these proteins are in close proximity with the EP-mRNP purified and are inactive or passively waiting for a signal to start the degradation.

One interesting candidate that was not previously found in other purifications was detected, the RNA binding protein ZC3H22. The V5-tagged ZC3H22 seems to be enriched in the *3SBP-CAT-EP 3'-UTR* purification when compared to the control purification (Figure 27). Unfortunately, the RNA-IP using V5-ZC3H22 showed that the *EP* mRNA binds unspecifically to the V5-beads and no signal for the *3SBP-CAT-EP 3'-UTR* mRNA was detected (Figure 33). It came to my interest that although both mRNAs are transcribed by the same polymerase, the expression is not the same, it seems that *EP* is expressed more than the *3SBPs-CAT* reporter (compared inputs in Figure 33). Maybe because the *3SBP-CAT* reporter contains also the *EP* UTRs and the expression of this mRNA is tightly regulated. As it has been reported before, over-expression of the two *EP* isoforms impaired trypanosome growth generating disturbances in the parasite membrane [180]. So it is possible that the *CAT* mRNA is regulated to reduce its expression and also that both mRNAs compete for the binding to V5-ZC3H22. The results obtained for the interaction between *EP* mRNA and ZC3H22 are inconclusive. One of the

problems is that the quantitative mass spectrometry of the *EP* purifications as well as the RNA-IP were repeated only once and peptides from this protein were not found to be enriched in further purifications (Table 4), thus there is a high possibility that ZC3H22 does not really interact with the *EP* mRNA.

There were several problems encountered when performing quantitative mass spectrometry (DL-QMS). One of the main problems was the poor yield, despite the fact that the RBPs could be detected by Western blot and the *CAT* mRNAs by Northern blot, the detection of peptides by DL-QMS in the samples was a big issue. It might be because when performing DL-QMS the control and target sample must be labelled and mixed, this implies the dilution of the sample. There were also technical problems, like failures of the instrument, as well as problems in handling the samples, such as inadequate gel-purification, trypsinization or labelling. For this reason, it was decided not to use DL-QMS and only to count and compare the peptides present in both samples.

Further *EP* purifications were repeated four times. Table 4 shows the ratio of peptides found for a specific protein in the 3SBP purification compared to the control purification for each of the four replicates. Most of the proteins found to co-purify with the *EP 3'-UTR* are conserved hypothetical proteins, with unknown functions. Only two RBPs were found to be enriched at least in two replicates, ZC3H13 and ZC3H21. Both RBPs, ZC3H13 [181] as well as ZC3H21 (data not published only presented on a congress) have an effect impairing growth in procyclic trypanosomes upon over-expression. The affinity purification using V5-ZC3H13 and the reporter mRNAs was done only once but lead to inconclusive results due to the lack of signal for the control proteins in the Western blot. ZC3H13-TAP purification was only performed in bloodstream trypanosomes and the same tag seems to be harmful in procyclic trypanosomes [181]. It would be interesting to see if the same hypothetical proteins found by the affinity purification can be found also in the TAP purification of this proteins (ZC3H13 and ZC3H21) in procyclic forms. Maybe with an N-terminal TAP-tagged and not an over-expressed one, at least in the case of ZC3H13. Knock-out cell lines, neither single nor double, were possible to be obtained for any of these proteins. In the future it might be of use to generate these knockouts and check if the expression of the *EP* mRNA has reduced. More experiments are necessary to corroborate the interaction of these two candidates with the *EP* mRNA.

The results in Table 4 were sorted for proteins with more than 1.5-fold enrichment for all replicates, but most of the proteins found are enriched only in two replicates. This indicates the necessity of more replicates for the selection of candidates. It might also imply that the samples are in the detection limit of mass-spectrometry.

Two hypothetical proteins, *Tb*927.7.4500 and *Tb*927.8.1270 were found to be more than two-fold enriched in three of the replicates. These two proteins do not contain any known functional domains but their mRNAs, judging by the ribosome profiling data available in Trytrip.db, indicates that are really express. It could be possible that some of these hypothetical proteins are

regulators of *EP* mRNA, the fact that no know RBP has been found to be implicated directly in the regulation of this mRNA supports this hypothesis.

4.4 The RNA binding protein ZC3H38 as a possible mRNA regulator

In parallel to my purification method I also started the characterization of an RBP found previously in a screen made in the laboratory [74]. In this screen, the ZC3H38 protein was implied to have a stabilizing effect on a reporter mRNA. The results of my tethering assay corroborate that the full length ZC3H38 protein stabilizes the reporter mRNA and increases the CAT activity (Figure 42). Also, it seems that the HNPY domain, which had previously been found to be a MKT1 interaction motif [108], might be responsible for the effects of this protein on the reporter mRNA. These preliminary results indicate that ZC3H38 can act as a potential mRNA regulator, stabilizing its target mRNAs upon binding. Further studies are being carried out to fully characterize this protein.

4.5 Concluding remarks

The main advantage of this method is that allows the purification of translating mRNPs and the detection of the proteins bound to them *in vivo*. Shown by the co-purification of V5-ZC3H11 and the *HSP70 3'-UTR* reporter, as well as by the detection of MKT1, which does not interact directly with the mRNAs. Another advantage is that all the components of the reporter mRNA can be changed, as it has been demonstrated in the present dissertation by the use of different 3'-UTRs. Furthermore, due to the fact that the 3SBPs-tag is in the N-terminus of the ORF, secondary structures present in the untranslated regions of the mRNA that might be important for regulation are not altered.

However, this technique has its limitations, one of them is the excessive presence of peptides from abundant proteins such as mitochondrial and nuclear proteins and also proteins that bind in a nonspecific manner to the beads. It seems that blocking the beads with tRNA and heparin is not efficient enough to avoid the binding of such proteins. One partial solution could be the addition of DNases to the samples before loading onto the sucrose gradient, at least this way abundant nuclear proteins, which bind unspecifically to the beads should be separated before the affinity purification.

The copy number of *3SBPs-CAT* mRNAs was calculated to be 400 per cell, a number that is comparable to the *tubulin* mRNA, an abundant mRNA in the cell. These results showed that there is enough reporter produced in order to be translated and purified. Nevertheless, the main problem that my method confronted was the detection of the purified samples by mass spectrometry. It seems that the detection of the V5-tagged proteins by western blot gave more reproducible and quantitative results when comparing to mass-spectrometry. This might be possibly due to the high sensitivity and specificity of the used V5-antibody, whereas in mass-spectrometry the peptides for an RBP can be masked by peptides of other abundant proteins. Although, the use of sucrose

gradient centrifugation combined with the affinity purification eliminates most of the contaminants, there are still some present. It seems that the affinity-purified samples are in the limit of detection for mass-spectrometry. In this case the generation of replicates as well as the quantification of the protein signals in Western blot is of vital importance.

As a final remark, I would like to point out that this work shows the development of an *in vivo* method, which allows the purification of translating mRNAs and the detection, at least by Western blot, of the protein components of a specific mRNP.

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6. Supplementary material

Supplementary table 1: Proteins found in the *HSP70 3'-UTR* purification

Three gel pieces were send to mass-spectrometry in order to find the proteins that co-purified with the *3SBP-CAT-HSP70 3'-UTR* reporter.

Accession	Description	Peptide counts		
		gel 1	gel 2	gel 3
Tb927.8.7100	acetyl-CoA carboxylase, putative	104	115	116
Tb927.9.6460	hypothetical protein, conserved	70	74	75
Tb927.2.4230	Nucleoporin NUP-1 protein, putative	63	65	66
Tb927.7.6670	hypothetical protein, conserved	56	63	64
Tb927.8.3950	hypothetical protein, conserved	53	60	61
Tb927.4.2880	Nucleoporin (TbNup225)	49	54	55
Tb927.10.6400	chaperonin HSP60, mitochondrial precursor	53	51	52
Tb927.11.330	Nucleoporin (TbMlp-1)	43	48	49
Tb927.11.980	Nucleoporin (TbNup158)	46	46	47
Tb927.5.3400	calcium-translocating P-type ATPase, calcium pump	45	28	29
Tb927.11.2650	heat shock protein 84, putative, mit HSp90	41	18	19
Tb927.11.2950	Nucleoporin (TbNup89)	36	40	41
Tb927.11.1900	T-complex protein 1, beta subunit, putative (TCP-1-beta)	40	33	34
Tb927.6.3750	heat shock 70 kDa protein, mitochondrial precursor, putative	40	36	37
Tb927.9.10770	PABP2	32	38	39
Tb927.4.4490	multidrug resistance protein E,p-glycoprotein (MRPE)	39	11	12
Tb927.10.2100	EF1-alpha elongation factor 1-alpha, (TEF1)	38	20	21
Tb927.6.890	hypothetical protein, conserved	37	24	25
Tb927.11.11330	heat shock protein 70, major HSP70	37	31	32
Tb927.10.7060	nucleoporin interacting component (NUP93), putative	30	35	36
Tb927.10.9650	hypothetical protein, conserved	32	35	36
Tb927.9.5900	glutamate dehydrogenase (GDH)	35	14	15
Tb927.7.2300	Nucleoporin (TbNup132)	27	34	35
Tb927.10.10900	heat shock protein 83, HSP83	35	13	14
Tb927.9.9290	PABP1	33	26	27
Tb927.11.6630	3-methylcrotonoyl-CoA carboxylase beta subunit, putative	31	32	33
Tb927.10.8170	Nucleoporin (NUP155)	32	16	17
Tb927.9.2470	nucleolar protein (NOP86)	18	31	32
Tb927.10.1510	NOT1	32	10	11
Tb927.9.8820	hypothetical protein, conserved, no yeast or human match	31	27	28
Tb927.9.11150	hypothetical protein, conserved	28	30	31
Tb927.11.7460	BiP	30	22	23
Tb927.11.6280	pyruvate phosphate dikinase (PPDK)	29	1	2
Tb927.11.3600	40S ribosomal protein S4	29	24	25
Tb927.10.4570	EF2 elongation factor 2	29	12	13
Tb927.9.12570	glycerol kinase, glycosomal (glk1)	29	27	28

Tb927.11.15040	chaperonin HSP60, mitochondrial precursor	17	27	28
Tb927.7.4900	XRNA	27	1	2
Tb927.4.1800	Mitochondrial LSU ribosomal protein L3	27	22	23
Tb927.4.4310	Nucleoporin (TbNup64)	22	25	26
Tb927.3.3130	POMP24	25	6	7
Tb927.4.590	hypothetical protein, quinoprotein alchohol dehydrogenase-like domain	25	16	17
Tb927.11.15990	Nucleoporin (TbNup109)	25	23	24
Tb927.3.3180	Nucleoporin (TbNup98)	22	24	25
Tb927.9.6170	arginine kinase (AK) in cytosol and flagellum	25	1	2
Tb927.8.650	cation-transporting ATPase, putative	24	3	4
Tb927.11.7290	pantothenate kinase subunit, putative	24	5	6
Tb927.11.15370	hypothetical protein, conserved (TbKap123)	20	23	24
Tb927.11.11360	guanine nucleotide-binding protein beta subunit- like protein (TRACK)	24	18	19
Tb927.3.5050	60S ribosomal protein L4	24	19	20
Tb927.9.14240	Nucleoporin (Nup82)	20	23	24
Tb927.10.7680	GTPase activating protein, putative	24	17	18
Tb927.2.4550	FtsJ cell division protein, putative (30M24.305)	7	22	23
Tb927.11.11460	POMP9	23	10	11
Tb927.10.1060	T-complex protein 1, delta subunit, putative (TCP-1-delta)	23	7	8
Tb927.3.3270	ATP-dependent phosphofructokinase (TbPFK)	23	13	14
Tb927.11.16760	T-complex protein 1, alpha subunit, putative (TCP-1-alpha)	23	17	18
Tb927.5.440	trans sialidase, putative	17	22	23
Tb927.9.11270	T-complex protein 1, eta subunit, putative, (TCP-1-eta)	23	16	17
Tb927.1.2370	beta tubulin	22	22	23
Tb927.8.2630	Kinesin KIN-C. Trypanosome-specific kinesin family 2	19	21	22
Tb927.2.4400	Mitchondrial SSU ribosomal protein	20	21	22
Tb927.11.4320	hypothetical protein, conserved, PF02622 DUF179	22	1	2
Tb927.9.9660	Archaic translocase of the outer mitochondrial membrane ATOM	22	17	18
Tb927.10.6050	clathrin heavy chain (CHC)	14	21	22
Tb927.2.5610	POMP22	22	5	6
Tb927.8.1590	TOM1, HECT ubiquitin-protein ligase	22	1	2
Tb927.9.6230	arginine kinase (AK)	6	20	21
Tb927.7.990	chaperone protein DNAj, putative	15	20	21
Tb927.11.3240	T-complex protein 1, zeta subunit, putative (TCP-1-zeta)	21	14	15
Tb927.10.2900	importin beta-1 subunit, putative	18	20	21
Tb927.8.4820	eIF4G3	21	14	15
Tb927.3.1380	ATP synthase F1, alpha subunit	21	15	16
Tb927.7.900	hypothetical protein, conserved	21	14	15
Tb927.9.1410	hypothetical protein, conserved	16	20	21
Tb927.10.12500	P-type H -ATPase, putative	9	20	21
Tb927.11.7380	glycerol-3-phosphate dehydrogenase (FAD-dependent), mitochondrial	20	11	12
Tb927.4.3950	cytoskeleton-associated protein CAP5.5, putative,cysteine peptidase	13	19	20
Tb927.11.7780	POMP16	20	14	15

Tb927.10.14710	40S ribosomal protein S2	20	17	18
Tb927.11.13180	POMP10	20	11	12
Tb927.10.8940	hypothetical protein, conserved	20	12	13
Tb927.10.14830	mitochondrial carrier protein, MCP5b	20	17	18
Tb927.10.3940	40S ribosomal protein S3A	20	16	17
Tb927.7.7420	ATP synthase F1, alpha subunit	20	16	17
Tb927.3.4760	dynamin, putative,vacuolar sortin protein 1, putative	17	19	20
Tb927.2.3030	ATP-dependent Clp protease subunit, heat shock protein 78 (HSP78)	20	12	13
Tb927.10.8080	hypothetical protein, conserved	19	3	4
Tb927.5.1060	mitochondrial processing peptidase, beta subunit, MMPbeta	19	14	15
Tb927.11.2340	hypothetical protein, conserved	13	18	19
Tb927.11.2050	60S acidic ribosomal subunit	19	15	16
Tb927.9.8880	actin A	19	16	17
Tb927.10.3260	Long-chain-fatty-acid--CoA ligase 5, Acyl-CoA synthetase 5 (LACS 5)	19	17	18
Tb927.3.2050	hypothetical protein, conserved, no domains, poly(Q) tracts, K-specific	18	5	6
Tb927.8.3150	T-complex protein 1, gamma subunit, putative (TCP-1-gamma)	18	11	12
Tb927.11.1250	kinetoplast poly(A) polymerase complex 1 subunit, MIT ssu-associated	16	17	18
Tb927.4.2850	hypothetical protein, conserved	18	11	12
Tb927.10.13500	60S ribosomal protein L10a	18	15	16
Tb927.9.15150	60S ribosomal protein L5	18	15	16
Tb927.10.7700	ABC transporter, putative	4	17	18
Tb927.7.6850	trans-sialidase (TbTS)	14	16	17
Tb927.8.5200	Mitchondrial SSU ribosomal associated	17	14	15
Tb927.10.560	40S ribosomal protein S11	2	16	17
Tb927.11.9780	hypothetical protein, conserved	10	16	17
Tb11.02.5420	NADPH--cytochrome p450 reductase, putative (CPR)	17	5	6
Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putative	15	16	17
Tb927.7.2680	ZC3H22	17	11	12
Tb927.7.1730	60S ribosomal protein L7	17	15	16
Tb927.11.11080	Nucleoporin (TbNup149)	17	12	13
Tb927.11.2610	hypothetical protein, conserved, no domains, no yeast or human match	17	7	8
Tb927.10.11540	40S ribosomal protein S3	17	15	16
Tb927.11.14020	RNA-binding protein (NRBD2)	16	16	17
Tb927.7.2170	hypothetical protein, conserved	4	16	17
Tb927.3.5370	hypothetical protein, conserved	17	5	6
Tb927.4.4940	hypothetical protein, conserved	16	16	17
Tb927.7.710	heat shock 70 kDa protein, putative (HSP70.4)	16	10	11
Tb927.11.5500	mitochondrial RNA binding protein 1 KRIPP1 (PPR)	12	15	16
Tb927.3.3300	hypothetical protein, conserved	16	14	15
Tb927.11.14730	metalloprotease, putative,cell division protein FtsH homologue, putative	16	9	10
Tb927.10.5770	valosin-containing protein homolog	16	3	4
Tb927.9.8070	60S ribosomal protein L10	16	13	14
Tb927.9.2590	hypothetical protein, conserved	16	1	2

Tb927.8.1270	hypothetical protein, conserved	11	15	16
Tb927.8.1740	Mitochondrial tRNA import	16	14	15
Tb927.10.4430	PUF1	16	7	8
Tb927.11.10760	KIN-D kinesin, associated with sub-pellicular microtubules, essential	11	15	16
Tb927.7.210	proline dehydrogenase	16	12	13
Tb927.8.1330	60S ribosomal protein L7a	13	15	16
Tb927.2.2520	voltage-dependent anion-selective channel	16	15	16
Tb927.10.8030	Mitochondrial ATP synthase subunit, putative	16	5	6
Tb927.10.4310	prohibitin 2, putative (PHB2)	12	15	16
Tb927.6.4280	glyceraldehyde 3-phosphate dehydrogenase, glycosomal (GAPDH)	16	15	16
Tb927.1.1930	TbTOR4 = TOR-like 2	16	1	2
Tb927.11.13520	hypothetical protein, conserved	15	8	9
Tb927.7.6090	hypothetical protein, conserved	15	14	15
Tb927.6.2010	hypothetical protein, conserved	11	14	15
Tb927.10.1100	60S ribosomal protein L9	15	13	14
Tb927.9.14160	rieske iron-sulfur protein, mitochondrial precursor (RISP)	15	4	5
Tb927.7.3550	hypothetical protein, conserved, no yeast or human match	7	14	15
Tb927.11.10150	Mitchondrial SSU ribosomal protein	15	6	7
Tb927.9.4190	fatty acyl CoA syntetase 1 (ACS1)	15	11	12
Tb927.11.3790	hypothetical protein, conserved	15	9	10
Tb927.8.1870	Golgi/lysosome glycoprotein 1 (tGLP1)	15	12	13
Tb927.6.3630	Sphingosine-1-phosphate lyase	11	14	15
Tb927.7.5020	60S ribosomal protein L19	15	14	15
Tb927.11.6210	sterol 14-alpha-demethylase (CYP51)	15	11	12
Tb927.10.11390	60S ribosomal protein L6	15	12	13
Tb927.9.8410	chaperone protein DNAj, putative	11	14	15
Tb927.11.3980	mitochondrial processing peptidase alpha subunit, MMPalpha	15	11	12
Tb927.9.11600	Gim5B protein,glycosomal membrane protein (gim5B)	7	14	15
Tb927.9.3630	hypothetical protein, conserved	15	4	5
Tb927.11.16280	60S ribosomal protein L2 L8	13	14	15
Tb927.10.5340	40S ribosomal protein S18	12	14	15
Tb927.10.5610	40S ribosomal protein S9	12	14	15
Tb927.10.5620	fructose-bisphosphate aldolase, glycosomal (ALD)	15	1	2
Tb927.5.930	NADH-dependent fumarate reductase (FRDg)	15	1	2
Tb927.11.3490	hypothetical protein, conserved, no domains, no good BLASTp matches	14	3	4
Tb927.10.10850	AGO1	14	9	10
Tb927.10.3840	60S ribosomal protein L18	11	13	14
Tb927.5.2930	Mitochondrial ATP synthase subunit, putative	14	10	11
Tb927.3.2600	ATP-dependent DEAD/H RNA helicase, putative	14	1	2
Tb927.11.5560	hypothetical protein, conserved	14	8	9
Tb927.5.520	stomatin-like protein, putative	13	13	14
Tb927.6.1870	eIF4E4	14	8	9
Tb927.10.14180	protein transport protein Sec13, putative	12	13	14
Tb927.9.4680	eIF4A1	14	8	9

Tb927.3.1120	GTP-binding nuclear protein rtb2, putative (rtb2)	14	13	14
Tb927.9.3760	poly(A) export protein, putative (TbGLE2)	9	13	14
Tb927.3.3540	Nucleoporin (TbNup53b)	10	13	14
Tb927.5.1610	60S ribosomal protein L13a	13	13	14
Tb927.10.15760	hypothetical protein, conserved, in flagellar proteomes	14	12	13
Tb927.11.6300	40S ribosomal protein S5	12	13	14
Tb927.7.5170	60S ribosomal protein L23a or L25	14	11	12
Tb927.11.15900	60S ribosomal protein L27	9	13	14
Tb927.3.3320	60S ribosomal protein L13	14	12	13
Tb927.9.11110	PRP8 protein homologue,U5 snRNA-associated splicing factor	14	1	2
Tb927.10.1170	intraflagellar transport protein IFT172	14	3	4
Tb927.9.6310	ABC transporter, putative	5	13	14
Tb927.10.3170	ABC transporter, putative	14	4	5
Tb927.3.1300	hypothetical protein, conserved, no domains, no yeast or human match	14	7	8
Tb927.9.13380	phosphoinositide-binding protein, putative	13	11	12
Tb927.8.2160	multidrug resistance protein A,p-glycoprotein (PGPA)	13	8	9
Tb927.2.5980	ATP-dependent Clp protease subunit, heat shock protein 104 (HSP104)	13	4	5
Tb927.4.1850	hypothetical protein, conserved	13	6	7
Tb927.7.4500	hypothetical protein, conserved	13	7	8
Tb927.7.3940	mitochondrial carrier protein, MCP16	13	5	6
Tb927.11.3120	nucleolar GTP-binding protein 1 (NOG1)	10	12	13
Tb927.3.3460	hypothetical protein, conserved (SDH5 in T cruzi)	13	4	5
Tb927.9.15460	calcium motive p-type ATPase, putative	5	12	13
Tb927.6.4320	hypothetical protein, conserved	13	3	4
Tb927.7.2670	ZC3H21	8	12	13
Tb927.11.4910	hypothetical protein, conserved,predicted ankyrin repeat family protein	13	4	5
Tb927.3.4580	hypothetical protein, conserved	13	6	7
Tb927.8.6580	succinate dehydrogenase flavoprotein, SDH1	13	9	10
Tb927.5.1210	short-chain dehydrogenase, putative	13	8	9
Tb927.5.1300	vacuolar proton translocating ATPase subunit A, putative	8	12	13
Tb927.9.10310	mitochondrial carrier protein, MCP11	13	10	11
Tb927.8.6970	3-methylcrotonyl-CoA carboxylase alpha subunit, putative	10	12	13
Tb927.8.8310	chaperone protein DNAj, putative	13	4	5
Tb927.8.4810	prohibitin 1 (PHB1)	13	12	13
Tb927.8.6250	hypothetical protein, conserved	11	12	13
Tb927.6.4090	chaperonin HSP60, mitochondrial precursor	13	8	9
Tb927.9.3990	40S ribosomal protein S7	13	12	13
Tb927.11.4820	60S ribosomal protein L17	10	12	13
Tb927.10.13280	GCN1 homologue, possibly involved in translation control	13	10	11
Tb927.3.1790	pyruvate dehydrogenase E1 beta subunit, putative	13	1	2
Tb927.10.9440	NADH dehydrogenase (54 NDH2)	13	7	8
Tb927.4.1020	Serine palmitoyltransferase	13	5	6
Tb927.7.4760	hypothetical protein, conserved	11	12	13

Tb927.4.4380	vacuolar-type proton translocating pyrophosphatase 1, putative (PPase1)	9	11	12
Tb927.10.11760	PUF6	12	11	12
Tb927.2.5240	PRP19-like protein, putative (TbPRP19)	12	5	6
Tb927.11.2600	hypothetical protein, conserved	12	8	9
Tb927.3.3940	DRBD11	12	9	10
Tb927.10.3990	DHH1	12	5	6
Tb927.10.13510	zinc metallopeptidase, putative	12	3	4
Tb927.11.16730	dihydrolipoyl dehydrogenase (GCVL-2)	12	1	2
Tb927.11.8060	hypothetical protein, conserved	12	1	2
Tb927.9.8950	CAAX prenyl protease 1, putative,metallo-peptidase, Clan M- Family M48	12	10	11
Tb927.1.4100	cytochrome oxidase subunit IV (COXIV)	12	9	10
Tb927.10.12840	mitochondrial carrier protein, MCP12	12	8	9
Tb927.10.8190	T-complex protein 1, theta subunit, (TCP-1-theta)	12	11	12
Tb927.10.540	ATP-dependent DEAD/H RNA helicase, DDX39-like	12	4	5
Tb927.9.11220	Mitochondrial tRNA import	12	6	7
Tb927.6.3050	aldehyde dehydrogenase family, putative	12	9	10
Tb927.10.14550	DED1-1, ATP-dependent DEAD/H RNA helicase,	12	9	10
Tb927.11.6430	hypothetical protein, conserved	12	3	4
Tb927.10.3660	aspartate aminotransferase	12	1	2
Tb927.3.1840	3-oxo-5-alpha-steroid 4-dehydrogenase, putative	12	9	10
Tb927.8.4330	small GTP-binding protein Rab11 (RAB11)	12	1	2
Tb927.11.6440	hypothetical protein, conserved, Bromodomain and poly(Q)	9	11	12
Tb927.6.2640	importin alpha subunit, putative (TbKap60)	12	10	11
Tb927.11.680	60S ribosomal protein L21e	12	11	12
Tb927.4.2180	60S ribosomal protein L35a	10	11	12
Tb927.11.11820	40S ribosomal protein S17	7	11	12
Tb927.7.1040	40S ribosomal protein S16	7	11	12
Tb927.9.8200	PES1	5	11	12
Tb927.10.13250	hypothetical protein, conserved	12	1	2
Tb927.11.9890	signal recognition particle receptor alpha subunit, putative	12	1	2
Tb927.5.900	oligosaccharyl transferase subunit, putative	12	1	2
Tb927.6.3500	RME8 endosomal trafficking protein, clathrin-associated	12	1	2
Tb927.8.5760	hypothetical protein, conserved	12	1	2
Tb927.8.5770	Elongator-like Protein 3a ELP3a	12	1	2
Tb927.9.10010	Sec63 homologue, chaperone protein DNAj	12	1	2
Tb927.9.1780	sec1 family transport protein, putative (SLY1)	12	1	2
Tb927.2.2970	mitochondrial carrier protein, MCP13	11	4	5
Tb927.9.12200	60S ribosomal protein L31	9	10	11
Tb927.4.4210	ATP-dependent zinc metallopeptidase, putative,metallo-peptidase	11	5	6
Tb927.4.1540	POMP27, NAD-dependent epimerase, aldehyde reductase	11	6	7
Tb927.3.3150	hypothetical protein, conserved	11	4	5
Tb927.4.3300	mitochondrial ATP-dependent zinc metallopeptidase, putative	11	4	5
Tb927.10.520	Mitochondrial ATP synthase subunit, putative	11	8	9
Tb927.4.5200	nucleoporin (NUP54/57, TbNup62)	10	10	11

Tb927.11.4980	ATP-dependent DEAD/H RNA helicase, putative Dbp9	6	10	11
Tb927.10.15310	hypothetical protein, conserved, linked to melarsoprol resistance	11	10	11
Tb927.11.11680	2-oxoglutarate dehydrogenase E2 component, putative	11	6	7
Tb927.9.7590	60S ribosomal protein L11	8	10	11
Tb927.11.10790	40S ribosomal protein SA	11	9	10
Tb927.10.3810	hypothetical protein, in UPF1-TAP	6	10	11
Tb927.11.4540	nucleoporin 48 (TbNup48)	8	10	11
Tb927.8.6150	40S ribosomal protein S8	9	10	11
Tb927.11.2530	mitochondrial RNA binding complex 1 subunit	7	10	11
Tb927.10.6910	Sterol methyltransferase, putative	11	8	9
Tb927.11.6740	PUF10	1	10	11
Tb927.11.9980	2-oxoglutarate dehydrogenase E1 component, putative	10	8	9
Tb927.11.14980	Mitchondrial LSU ribosomal protein	5	9	10
Tb927.10.180	ATP synthase F1 subunit gamma protein, putative	10	3	4
Tb927.8.1420	acyl-CoA dehydrogenase, mitochondrial precursor, putative	10	6	7
Tb927.1.2990	PPR2	10	7	8
Tb927.7.5230	lanosterol synthase	10	5	6
Tb927.3.3580	lipophosphoglycan biosynthetic protein	10	1	2
Tb927.2.2440	proteasome regulatory non-ATPase subunit 6 (RPN6)	10	4	5
Tb927.11.1980	ZC3H41	10	3	4
Tb927.11.1450	2-oxoglutarate dehydrogenase E1 component, putative	10	3	4
Tb927.4.1920	GPI transamidase, putative (TbGPI16)	10	5	6
Tb927.8.890	small GTP-binding protein Rab1, putative	10	6	7
Tb927.10.2320	hypothetical protein, conserved	10	7	8
Tb927.10.12960	ras-related protein rab-5,small GTPase, putative (RAB5A)	10	5	6
Tb927.7.3440	EF hand protein	10	9	10
Tb927.11.10510	ubiquinone biosynthesis methyltransferase, putative	10	7	8
Tb927.10.12700	pyruvate dehydrogenase E1 alpha subunit, putative	10	6	7
Tb927.10.15520	signal recognition particle protein, putative	10	4	5
Tb927.6.4440	RBP42	10	9	10
Tb927.5.1810	lysosomal/endosomal membrane protein p67 (p67)	10	4	5
Tb927.4.1860	40S ribosomal protein S19	10	9	10
Tb927.10.8430	40S ribosomal protein S12	9	9	10
Tb927.3.5520	26S proteasome regulatory non-ATPase subunit (RPN1)	10	4	5
Tb927.10.14620	hypothetical protein, conserved	10	3	4
Tb927.3.5430	hypothetical protein, conserved	10	3	4
Tb927.10.14150	hypothetical protein, conserved, related to yeast BFR1	10	1	2
Tb927.5.3800	glutamine hydrolysingcarbomoyl phosphate synthase, putative	10	1	2
Tb927.9.11380	60S ribosomal protein L23	9	9	10
Tb927.3.3190	serine/threonine-protein kinase, putative,protein kinase, putative	10	7	8
Tb927.8.6640	hypothetical protein, conserved	10	4	5
Tb927.4.2080	CC2D is a FAZ-ER protein, also present on the basal bodies.	6	8	9

Tb927.7.3050	Mitchondrial SSU ribosomal associated	6	8	9
Tb927.5.2530	hypothetical protein, conserved	1	8	9
Tb927.6.1430	hypothetical protein, conserved	9	4	5
Tb927.5.1580	ZC3H13	9	6	7
Tb927.10.6850	MItochondrial SSU S18 or kinetoplast poly(A) polymerase complex 1 sub	9	5	6
Tb927.11.15430	U5 small nuclear ribonucleoprotein U5-116K, putative	9	5	6
Tb927.11.11690	vacuolar ATP synthase subunit b, putative,v-ATPase B sub	4	8	9
Tb927.11.11480	Trichohyalin, putative	1	8	9
Tb927.10.470	choline dehydrogenase, putative	9	3	4
Tb927.11.13090	EF1 gamma, elongation factor 1 gamma, putative	9	1	2
Tb927.11.10690	hypothetical protein, conserved	9	5	6
Tb927.7.2240	hypothetical protein, conserved	9	6	7
Tb927.8.8050	Nucleoporin (TbNup75)	9	8	9
Tb927.10.2240	NTF2-like domain, in Y14 TAP	9	5	6
Tb927.10.6640	COP-coated vesicle membrane protein erv25 precursor,	9	1	2
Tb927.10.600	Mitchondrial LSU ribosomal protein	5	8	9
Tb927.6.4200	Mitchondrial LSU ribosomal protein	3	8	9
Tb927.7.6260	hypothetical protein, conserved	9	1	2
Tb927.1.860	hypothetical protein, conserved	9	1	2
Tb927.6.5040	60S ribosomal protein L15	8	8	9
Tb927.10.7570	dihydrolipoamide acetyltransferase E2 subunit, putative	9	4	5
Tb927.11.9320	hypothetical protein, conserved	9	8	9
Tb927.10.5370	40S ribosomal protein S10	8	8	9
Tb927.11.4300	60S ribosomal protein L18	8	8	9
Tb927.7.2340	40S ribosomal protein S15	8	8	9
Tb927.11.3230	60S ribosomal protein L44	7	8	9
Tb927.11.6200	60S ribosomal protein L28	7	8	9
Tb927.9.14370	60S ribosomal protein L26	6	8	9
Tb927.10.10570	histone H2B	5	8	9
Tb927.11.14170	hypothetical protein, conserved	9	4	5
Tb927.10.4110	60S ribosomal protein L30	3	8	9
Tb927.10.6300	Mitchondrial SSU ribosomal protein S5	9	3	4
Tb927.8.1720	Phosphatidylglycerophosphate synthase	9	1	2
Tb927.9.4500	HSP70, endoplasmatic reticulum, HSP70.a	9	1	2
Tb927.6.1920	hypothetical protein, conserved	9	1	2
Tb927.3.3030	hypothetical protein, conserved, START (lipid-binding) domain	9	1	2
Tb927.5.4040	Mitchondrial SSU ribosomal associated (coiled coil)	8	6	7
Tb927.11.5060	Mitchondrial SSU ribosomal protein	6	7	8
Tb927.9.12730	chaperone protein DNAj, endoplasmatic reticulum	8	4	5
Tb927.11.6000	Mitchondrial LSU ribosomal protein	4	7	8
Tb927.11.10370	glycosyl hydrolase-like protein	4	7	8
Tb927.2.3180	TbPPR1 mitochondrial RNA binding protein 1	6	7	8
Tb927.10.380	PPR5 mitochondrial RNA binding protein 1	4	7	8
Tb927.9.2900	hypothetical protein, conserved	4	7	8
Tb927.7.2410	hypothetical protein, conserved	8	1	2

Tb927.3.5340	Hsc70-interacting protein (Hip), putative	8	1	2
Tb927.7.5820	Monooxygenase, putative	8	6	7
Tb927.2.3370	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase	6	7	8
Tb927.9.11120	Mitchondrial SSU ribosomal associated	8	6	7
Tb927.10.1450	hypothetical protein, conserved, in bilobe	8	4	5
Tb927.3.4920	LETM1 and EF-hand domain-containing protein 1	8	4	5
Tb927.11.5400	signal recognition particle 54 kDa (SRP54)	8	4	5
Tb927.9.9630	hypothetical protein, conserved	8	3	4
Tb927.11.1840	hypothetical protein, conserved	8	3	4
Tb927.10.15410	glycosomal malate dehydrogenase (gMDH)	8	1	2
Tb927.11.14250	T-complex protein 1, epsilon subunit, putative (TCP-1-epsilon)	8	3	4
Tb927.11.13230	TbVAP, flagellar attachment zone	8	3	4
Tb927.9.8740	DRBD3 (PTB1)	8	1	2
Tb927.9.5320	nucleolar RNA binding protein, putative (28G16.220)	4	7	8
Tb927.5.1520	heat shock protein HslU1, in mitochondrion	8	1	2
Tb927.7.2640	hypothetical protein, conserved	8	5	6
Tb927.2.2130	small GTP-binding protein RAB6, putative (25N14.200)	8	1	2
Tb927.8.4500	eIF4G5	6	7	8
Tb927.8.2460	hypothetical protein, conserved	8	7	8
Tb927.10.3640	hypothetical protein, conserved	7	7	8
Tb927.11.2300	ERF1 eukaryotic peptide chain release factor subunit 1, putative	8	3	4
Tb927.3.4380	Tob55, SAM50	8	6	7
Tb927.6.3930	Mitchondrial LSU ribosomal protein	4	7	8
Tb927.6.1770	kinesin	6	7	8
Tb927.11.7140	cell cycle sequence binding phosphoprotein CSB)II	8	1	2
Tb927.9.4310	tricarboxylate carrier, putative	8	6	7
Tb927.7.2190	hypothetical protein, conserved	8	4	5
Tb927.4.2070	antigenic protein, putative	8	4	5
Tb927.8.5640	Homologue of T. cruzi Complex II subunit SDH6	8	1	2
Tb927.5.3980	Mitchondrial LSU ribosomal protein	8	7	8
Tb927.11.5450	malic enzyme	8	1	2
Tb927.7.5940	Protein Associated with Differentiation (TbPAD2)	8	7	8
Tb927.6.720	60S ribosomal protein L14	7	7	8
Tb927.2.5910	40S ribosomal protein S13	8	6	7
Tb927.11.6140	40S ribosomal protein S15A	4	7	8
Tb927.11.6250	Mitochondrial ATP synthase subunit, putative	8	5	6
Tb927.11.14960	PUF7	1	7	8
Tb927.2.3990	hypothetical protein, conserved	8	1	2
Tb927.5.2290	U5 splicing factor U5-200K, putative or BRR2 homologue	8	1	2
Tb927.8.2770	IP3 inositol trisphosphate receptor.	8	1	2
Tb927.9.15290	hypothetical protein, conserved	8	1	2
Tb927.10.1070	cdc2- like protein kinase (CRK1)	8	1	2
Tb927.10.13360	EF-Tu Mitochondrial elongation factor Tu	7	7	8
Tb927.11.15560	Nucleoporin (TbNup53a)	8	7	8
Tb927.11.2410	Flabarin, flagellar membrane protein in Leishmania	7	7	8

Tb927.9.11000	small GTPase, putative,GTP-binding protein, putative (RAB7)	8	6	7
Tb927.10.9910	hypothetical protein, conserved	8	3	4
Tb927.11.3740	proteasome regulatory ATPase subunit 2 (RPT2)	8	3	4
Tb927.10.4080	hypothetical protein, conserved	8	3	4
Tb927.11.13500	paraflagellar rod protein par1	8	1	2
Tb927.10.15850	PEX12 peroxisome assembly protein	8	6	7
Tb927.2.280	retrotransposon hot spot protein 2 (RHS2), putative	6	7	8
Tb927.2.470	retrotransposon hot spot protein 4 (RHS4), putative	8	5	6
Tb927.1.180	retrotransposon hot spot protein 1 (RHS1), putative	8	5	6
Tb927.3.1010	hypothetical protein, conserved, Trypanosoma-specific	7	1	2
Tb927.7.270	ribosome biogenesis protein, putative	1	6	7
Tb927.11.6510	40S ribosomal protein S21	5	6	7
Tb927.7.4290	Nuclear migration protein NudC (animals), 46% identity	7	1	2
Tb927.5.1790	Mitchondrial SSU ribosomal protein	7	3	4
Tb927.10.13730	60S ribosomal protein L7	1	6	7
Tb927.6.1900	U3/U14 snoRNA-associated small subunit rRNA processing protein	7	1	2
Tb927.4.2000	ruvB-like DNA helicase, putative,ATP-dependent DNA helicase, putative	7	4	5
Tb927.6.1470	hypothetical protein, conserved	7	6	7
Tb927.11.540	ABC transporter, putative	7	5	6
Tb927.9.7170	Mitchondrial LSU ribosomal protein	5	6	7
Tb927.10.10360	microtubule-associated protein, putative	2	6	7
Tb927.11.1680	vesicular-fusion protein SEC18, putative	7	1	2
Tb927.9.5040	cAMP-specific phosphodiesterase (PDEB1)	7	1	2
Tb927.4.1300	Amidinotransferase superfamily protein	7	3	4
Tb927.7.3500	glutathione-S-transferase/glutaredoxin, putative	7	3	4
Tb927.10.9900	ABC1 protein, putative	7	4	5
Tb927.10.4850	hypothetical protein, conserved	1	6	7
Tb927.9.2650	POMP2	7	6	7
Tb927.3.3560	hypothetical protein, conserved	1	6	7
Tb927.9.2620	hypothetical protein, conserved	7	6	7
Tb927.6.2790	L-threonine 3-dehydrogenase, putative	7	1	2
Tb927.3.5610	ribosomal protein L3 mitochondrial, putative	4	6	7
Tb927.5.4420	nucleolar RNA helicase 2 human, DDX50 Mouse	6	6	7
Tb927.11.13470	hypothetical protein, conserved	7	1	2
Tb927.9.1380	hypothetical protein, conserved	7	6	7
Tb927.1.3070	hypothetical protein, conserved, poly(Q), poly(H)	7	3	4
Tb927.9.15360	40S ribosomal protein S6	7	5	6
Tb927.6.1500	1-Alkyl-dihydroxyacetonephosphate synthase	7	3	4
Tb927.5.3810	orotidine-5-phosphate decarboxylase/orotate phosphoribosyltransferase	7	3	4
Tb927.10.190	40S ribosomal protein S6	7	6	7
Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD], glycosomal	7	1	2
Tb927.11.6870	14-3-3 protein	7	1	2
Tb927.11.11090	Nucleoporin (TbNup140)	7	5	6
Tb927.8.1570	hypothetical protein, conserved	7	3	4

Tb927.6.4980	40S ribosomal protein S14	7	6	7
Tb927.7.320	RBP8	7	6	7
Tb927.10.7330	40S ribosomal protein S24E	6	6	7
Tb927.8.3060	cytosolic leucyl aminopeptidase, putative,metallo-peptidase, Clan MF, Family M17	7	5	6
Tb927.9.11470	60S ribosomal protein L27a	5	6	7
Tb927.10.14010	RP2 basal body protein, required for axoneme formation	4	6	7
Tb927.10.5460	60S ribosomal protein L24	4	6	7
Tb927.11.8200	40S ribosomal protein S26	4	6	7
Tb927.6.4370	eIF3 subunit 7-like protein	4	6	7
Tb927.8.7340	trans-sialidase, putative,neuraminidase, putative	4	6	7
Tb927.9.15420	60S ribosomal protein L32	4	6	7
Tb927.4.3850	WDR12	3	6	7
Tb927.8.1510	ATP-dependent DEAD/H RNA helicase, putative Dbp3	3	6	7
Tb927.10.8910	hypothetical protein, conserved	7	3	4
Tb927.11.9220	proteasome regulatory non-ATP-ase subunit 2 (RPN2)	7	3	4
Tb927.9.14200	hypothetical protein, possible component of cytochorome oxidase complex	7	3	4
Tb927.9.15090	cytosolic coat protein, putative	7	3	4
Tb927.11.460	hypothetical protein, conserved,predicted WD40 repeat protein	1	6	7
Tb927.8.3520	hypothetical protein, conserved	1	6	7
Tb927.10.15680	hypothetical protein, conserved	7	1	2
Tb927.10.2890	enolase	7	1	2
Tb927.10.5400	hypothetical protein, conserved	7	1	2
Tb927.10.770	hypothetical protein, conserved	7	1	2
Tb927.11.1070	glycosomal transporter (GAT3)	7	1	2
Tb927.11.14700	hypothetical protein, conserved	7	1	2
Tb927.11.4280	hypothetical protein, conserved	7	1	2
Tb927.11.5220	chaperone protein DNAj, putative	7	1	2
Tb927.2.5800	sedoheptulose-1,7-bisphosphatase (SBPase)	7	1	2
Tb927.3.3450	ADP-ribosylation factor-like protein 3A, putative	7	1	2
Tb927.3.750	hypothetical protein, conserved	7	1	2
Tb927.4.4350	hypothetical protein, conserved	7	1	2
Tb927.5.2080	guanosine monophosphate reductase, putative	7	1	2
Tb927.6.2360	adenosine kinase, putative	7	1	2
Tb927.9.3170	cytochrome oxidase subunit V (COXV)	7	1	2
Tb927.10.8660	hypothetical protein, conserved	7	1	2
Tb927.11.5290	mitochondrial carrier protein, MCP9	7	1	2
Tb927.11.11520	PEX11 glycosomal membrane protein	7	5	6
Tb927.8.1500	hypothetical protein, conserved	7	1	2
Tb927.11.13080	hypothetical protein, conserved	4	6	7
Tb927.9.12500	POMP7	7	4	5
Tb927.10.15530	ABC transport system ATP-binding protein, putative	6	6	7
Tb927.9.11840	hypothetical protein, conserved	7	3	4
Tb927.1.120	retrotransposon hot spot protein 4 (RHS4), putative	7	5	6
Tb927.10.4130	NADH-ubiquinone oxidoreductase complex I subunit	7	1	2
Tb927.10.5930	protein kinase, putative	7	1	2

Tb927.10.12240	short-chain dehydrogenase, putative	7	1	2
Tb927.8.1610	MSP-B, putative	7	6	7
Tb927.8.6410	short-chain dehydrogenase, putative	5	6	7
Tb927.11.14380	Kinetoplast polyadenylation/uridylation factor 2	7	3	4
Tb927.1.2100	calpain-like cysteine peptidase, putative,cysteine peptidase	7	1	2
Tb927.2.5160	chaperone protein DNAj, putative	7	6	7
Tb927.10.2090	EF1-alpha elongation factor 1-alpha, (TEF1)	1	6	7
Tb927.4.5350	3-methylcrotonyl-CoA carboxylase, pseudogene	6	4	5
Tb927.2.2950	hypothetical protein, conserved	3	5	6
Tb927.4.4670	hypothetical protein, conserved	6	3	4
Tb927.1.2570	coatomer beta subunit (beta-coP)	6	1	2
Tb927.3.1910	hypothetical protein, conserved, histone RNA binding domain 259-326	3	5	6
Tb927.10.9810	hypothetical protein, conserved	6	1	2
Tb927.8.1890	cytochrome c1	6	1	2
Tb927.7.2400	MCP1 Arc1p homologue, assists aa tRNA synthetases	6	3	4
Tb927.7.4310	hypothetical protein, conserved	6	3	4
Tb927.9.6510	Mitchondrial SSU ribosomal associated	6	5	6
Tb927.1.2410	beta tubulin, pseudogene	1	5	6
Tb927.10.6320	NOC3, nuclear export of ribosomes, assoc with NRG1	1	5	6
Tb927.10.14510	hypothetical protein, conserved	6	1	2
Tb927.7.3980	immunodominant antigen, putative,tc40 antigen-like	6	1	2
Tb927.2.4090	hypothetical protein, conserved	6	1	2
Tb927.8.5460	flagellar calcium-binding protein,44 kDa calflagin, (Tb-44)	5	5	6
Tb927.7.5700	pATOM36	6	4	5
Tb927.4.1270	ruvB-like DNA helicase, putative	6	1	2
Tb927.9.5890	solanesyl-diphosphate synthase, putative (28G16.440)	6	5	6
Tb927.6.5070	hypothetical protein, conserved	6	1	2
Tb927.10.510	LOK1=POMP19 Mitochondrial membrane formation	6	1	2
Tb927.3.5240	Mitchondrial SSU ribosomal associated KRIPP8 (PPR)	5	5	6
Tb927.5.890	oligosaccharyl transferase subunit, putative	1	5	6
Tb927.11.14090	hypothetical protein, conserved, 2 very weak RRMs	4	5	6
Tb927.6.1090	proteasome regulatory ATPase subunit 3 (RPT3)	6	1	2
Tb927.6.4740	importin-alpha re-exporter protein, putative	6	4	5
Tb927.11.6230	pretranslocation protein, alpha subunit, SEC61	6	5	6
Tb927.11.14460	ADP-ribosylation factor GTPase activating protein 1, putative	6	1	2
Tb927.8.750	nucleolar RNA-binding protein, putative	6	5	6
Tb927.10.2290	chaperone protein DNAj, endoplasmatic reticulum	6	4	5
Tb927.8.6660	paraflagellar rod protein PFC1	6	1	2
Tb927.11.10780	Voltage-dependent anion channel (VDAC), putative	6	4	5
Tb927.8.3750	nucleolar protein (Nop56 homologue)	1	5	6
Tb927.10.14090	transporter, putative	6	5	6
Tb927.3.680	cytochrome P450, putative	6	5	6
Tb927.6.2210	hypothetical protein, conserved	6	5	6
Tb927.10.7620	mitochondrial ATP-dependent zinc metallopeptidase	5	5	6

Tb927.5.1780	hypothetical protein, conserved	5	5	6
Tb927.8.6030	60S ribosomal protein L12	5	5	6
Tb927.9.15210	60S ribosomal protein L36	5	5	6
Tb927.5.3360	Mitchondrial LSU ribosomal protein L2, putative	4	5	6
Tb927.9.11880	Mitchondrial SSU ribosomal protein	6	4	5
Tb927.7.2820	histone H2A	3	5	6
Tb927.9.1850	60S ribosomal protein L35	3	5	6
Tb927.9.2220	SUMO1/Ulp2, putative	3	5	6
Tb927.10.1550	proteasome regulatory non-ATP-ase subunit 5,19S proteasome regulatory subunit	6	3	4
Tb927.1.1370	rRNA biogenesis protein, putative	1	5	6
Tb927.1.2430	histone H3	1	5	6
Tb927.1.450	retrotransposon hot spot protein (RHS, pseudogene), putative	1	5	6
Tb927.10.15660	hypothetical protein, conserved	1	5	6
Tb927.11.6790	BOP1/Erb1p	1	5	6
Tb927.4.1080	V-type ATPase, A subunit, putative	1	5	6
Tb927.4.3890	ATP-dependent DEAD/H RNA helicase, weak match to HsDDX39	1	5	6
Tb927.5.1560	ATP-dependent DEAD/H RNA helicase, putative, no clear yeast homologue	1	5	6
Tb927.5.4230	histone H4	1	5	6
Tb927.6.5090	hypothetical protein, conserved	1	5	6
Tb927.9.13350	hypothetical protein, conserved	1	5	6
Tb927.10.13620	NADH-ubiquinone oxidoreductase complex I subunit, NDUFA9 subunit, putative	6	1	2
Tb927.10.16150	ATP-dependent zinc metallopeptidase, putative,metallo-peptidase	6	1	2
Tb927.10.1860	hypothetical protein, conserved, tryp specific	6	1	2
Tb927.10.3520	protease regulatory ATPase subunit 4, putative (RPT4)	6	1	2
Tb927.10.4040	3-Ketosphinganine reductase	6	1	2
Tb927.11.14910	protein phosphatase 2C, putative	6	1	2
Tb927.11.3270	squalene monooxygenase, putative	6	1	2
Tb927.2.4210	glycosomal phosphoenolpyruvate carboxykinase (PEPCK)	6	1	2
Tb927.3.1080	POMP23	6	1	2
Tb927.4.2890	hypothetical protein, conserved	6	1	2
Tb927.4.730	hypothetical protein, conserved	6	1	2
Tb927.7.2570	guide RNA associated protein, GAP2	6	1	2
Tb927.7.7130	TWY1 homologue, tRNA modification	6	1	2
Tb927.7.7470	GRESAG 4, putative receptor-type adenylate cyclase	6	1	2
Tb927.8.4890	endoplasmic reticulum oxidoreductin, Ero1, oxidises PDI	6	1	2
Tb927.9.10520	hypothetical protein, possible component of cytochorome oxidase complex	6	1	2
Tb927.9.6090	PTP1-interacting protein, 39 kDa PIP39	6	1	2
Tb927.7.3040	hypothetical protein, conserved	6	1	2
Tb927.10.8490	glucose transporter, putative	5	5	6
Tb927.9.9550	hypothetical protein, conserved, no yeast or human match	6	1	2
Tb927.8.4450	RBP11	4	5	6
Tb927.11.6390	hypothetical protein, conserved, START domain	6	1	2

Tb927.10.11270	RBP23	6	4	5
Tb927.11.10570	Mitchondrial LSU ribosomal protein	5	5	6
Tb927.9.2320	POMP1 (possible S-Adenosyl methionine methyl transferase	5	5	6
Tb927.10.730	ATP synthase, putative	3	5	6
Tb927.11.9730	60S ribosomal protein L34	5	5	6
Tb927.11.4200	ERGIC53 paralogue, lectin-like, ER quality control	6	1	2
Tb927.6.5000	hypothetical protein, conserved	6	1	2
Tb927.8.3690	isocitrate dehydrogenase [NADP], mitochondrial precursor, putative (IDH)	6	1	2
Tb927.10.6610	chaperone protein DNAj, putative	6	1	2
Tb927.10.240	PEX14 peroxin 14	6	4	5
Tb927.2.4130	enoyl-CoA hydratase/Enoyl-CoA isomerase/3-hydroxyacyl-CoA dehydrogenase, putative	3	4	5
Tb927.9.9310	hypothetical protein, conserved	4	4	5
Tb927.10.7090	alternative oxidase (AOX)	5	3	4
Tb927.7.1790	Adenine phosphoribosyltransferase, putative	5	1	2
Tb927.11.510	UBP2	5	4	5
Tb927.8.3110	Mitchondrial SSU ribosomal protein S9	5	4	5
Tb927.3.970	Mitchondrial SSU ribosomal protein	4	4	5
Tb927.8.3170	Mitchondrial LSU ribosomal protein	4	4	5
Tb927.7.7450	GTP-binding protein, putative	4	4	5
Tb927.11.8870	mitochondrial DEAD box protein,KREH1 (KREH1)	5	4	5
Tb927.10.8270	eIF3 subunit 8, putative	5	3	4
Tb927.10.14700	hypothetical protein, conserved, no human or yeast homologue	5	3	4
Tb927.3.4600	hypothetical protein, conserved	1	4	5
Tb927.7.4220	hypothetical protein, conserved	1	4	5
Tb927.2.100	retrotransposon hot spot protein 1 (RHS1), putative	3	4	5
Tb927.11.6170	protein transport protein Sec31, putative,cytosolic coat protein, putative	5	3	4
Tb927.11.9940	hypothetical protein, conserved	5	3	4
Tb927.8.560	GEM1, putative	5	3	4
Tb927.7.570	prefoldin, putative	5	1	2
Tb927.7.6460	FG-GAP repeat protein, putative,intergrin alpha chain protein, putative	5	1	2
Tb927.9.7110	hypothetical protein, conserved	5	1	2
Tb927.10.1490	Possible splicing factor	5	1	2
Tb927.5.1460	hypothetical protein, conserved	5	1	2
Tb927.5.3010	MRB complex protein	4	4	5
Tb927.11.10140	hypothetical protein, possible component of cytochorome oxidase complex	5	1	2
Tb927.10.11220	procyclic form surface glycoprotein (PSSA-2)	5	4	5
Tb927.10.13800	hypothetical protein, conserved, no yeast or human match	5	3	4
Tb927.8.7530	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor, putative	5	4	5
Tb927.8.580	hypothetical protein, conserved	1	4	5
Tb927.11.17000	AIR9	5	3	4
Tb927.9.12510	DED1-2, ATP-dependent DEAD/H RNA helicase, DED1-2	5	1	2
Tb927.10.13780	glycogen synthase kinase 3 (GSK3)	3	4	5
Tb927.7.4180	Elongase 3	5	3	4

Tb927.10.12710	heat shock protein, HSP110	5	1	2
Tb927.10.4060	hypothetical protein, conserved	5	1	2
Tb927.3.640	hypothetical protein, conserved	5	1	2
Tb927.8.6080	POMP42	5	1	2
Tb927.1.4830	Phospholipase A1	5	1	2
Tb927.11.2370	MEX67	5	4	5
Tb927.11.9720	40S ribosomal protein S27	5	4	5
Tb927.3.5130	hypothetical protein, conserved	5	4	5
Tb927.7.240	40S ribosomal protein S33	5	4	5
Tb927.7.540	chaperone protein DNAj, putative	5	4	5
Tb927.10.1080	40S ribosomal protein S23	4	4	5
Tb927.3.2440	serine/threonine-protein kinase, putative,protein kinase, putative	4	4	5
Tb927.3.4190	endosomal integral membrane protein, putative	4	4	5
Tb927.5.450	NADH-ubiquinone oxidoreductase, mitochondrial, putative	4	4	5
Tb927.7.5970	protein associated with differentiation 5, PAD5	4	4	5
Tb927.10.12290	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase, putative	3	4	5
Tb927.10.2610	hypothetical protein, conserved, DUF1935 domain	3	4	5
Tb927.10.2840	40S ribosomal protein S25	3	4	5
Tb927.10.6370	60S ribosomal protein L37a	3	4	5
Tb927.10.8040	adaptin complex 1 subunit, putative,beta-adaptin, fragment (BAD1)	5	3	4
Tb927.11.10260	hypothetical protein, conserved, no known domains	5	3	4
Tb927.11.3380	Ran-binding protein 1, putative	3	4	5
Tb927.4.3060	hypothetical protein, conserved, PLP dependent transferases like domain	3	4	5
Tb927.7.4550	Mitchondrial LSU ribosomal protein	3	4	5
Tb927.8.1200	vacuolar-type Ca2 -ATPase 2 (TbA2)	3	4	5
Tb927.8.2760	Mitchondrial LSU ribosomal protein	3	4	5
Tb927.8.6980	hypothetical protein, conserved	3	4	5
Tb927.9.7690	hypothetical protein, conserved	5	4	5
Tb927.10.2810	hypothetical protein, conserved	3	4	5
Tb927.11.4650	Mitchondrial LSU ribosomal protein	5	3	4
Tb927.11.4130	ubiquitin-like protein, putative	4	4	5
Tb927.10.12430	hypothetical protein, conserved	1	4	5
Tb927.10.3280	60S ribosomal protein L38	1	4	5
Tb927.11.2120	hypothetical protein, conserved	1	4	5
Tb927.2.4890	ribosomal protein L11, putative	1	4	5
Tb927.8.5490	hypothetical protein, conserved	1	4	5
Tb927.1.5030	leucine-rich repeat protein (LRRP)	5	1	2
Tb927.10.14200	syntaxin 5	5	1	2
Tb927.10.14530	proteasome regulatory non-ATPase subunit 8,26S proteasome regulatory subunit (Rpn8)	5	1	2
Tb927.10.14860	hypothetical protein, conserved	5	1	2
Tb927.10.15430	hypothetical protein, conserved	5	1	2
Tb927.10.15720	19S proteasome regulatory subunit (RPN9)	5	1	2
Tb927.10.4050	serine palmitoyltransferase, putative	5	1	2
Tb927.10.4640	eIF-3 subunit L	5	1	2
Tb927.10.4760	hypothetical protein, conserved	5	1	2

Tb927.10.5300	eIF-6	5	1	2
Tb927.10.7100	delta-4 fatty acid desaturase	5	1	2
Tb927.11.15150	1-acyl-sn-glycerol-3-phosphate acyltransferase, putative	5	1	2
Tb927.11.16670	hypothetical protein, conserved	5	1	2
Tb927.11.2690	succinyl-coA:3-ketoacid-coenzyme A transferase, mitochondrial precursor, putative	5	1	2
Tb927.11.2750	POMP12	5	1	2
Tb927.11.3570	aminopeptidase, putative,metallo-peptidase, Clan MA(E) Family M1	5	1	2
Tb927.11.3860	hypothetical protein, conserved	5	1	2
Tb927.11.4160	hypothetical protein, conserved,predicted C2 domain protein	5	1	2
Tb927.11.5110	hypothetical protein, conserved	5	1	2
Tb927.2.5760	hypothetical protein, conserved, small unknown domain, SET domain	5	1	2
Tb927.3.3850	hypothetical protein, conserved, Sec23-binding domain of Sec16	5	1	2
Tb927.3.4820	acyltransferase, putative	5	1	2
Tb927.6.1040	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	5	1	2
Tb927.7.1220	hypothetical protein, conserved	5	1	2
Tb927.7.2980	hypothetical protein, conserved	5	1	2
Tb927.8.3810	hypothetical protein, conserved	5	1	2
Tb927.8.4940	hypothetical protein, conserved	5	1	2
Tb927.8.6050	hypothetical protein, conserved	5	1	2
Tb927.8.7040	hypothetical protein, conserved	5	1	2
Tb927.8.8120	hypothetical protein, conserved	5	1	2
Tb927.9.3400	endo-beta-N-acetylglucosaminidase, putative	5	1	2
Tb927.9.7800	hypothetical protein, conserved	5	1	2
Tb927.6.1000	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	3	4	5
Tb927.10.15180	nucleosome assembly protein, putative	5	1	2
Tb927.11.3030	phosphoribosylpyrophosphate synthetase, putative (PRS)	1	4	5
Tb927.11.8880	hypothetical protein, conserved	5	4	5
Tb927.3.1920	NOT5	3	4	5
Tb927.11.5140	ubiquitin carboxyl-terminal hydrolase, Clan CA cysteine peptidase, family C12, putative	5	1	2
Tb927.10.9740	19S proteasome regulatory subunit (RPT6)	4	4	5
Tb927.7.2550	proteasome regulatory ATPase subunit 5 (RPT5)	4	4	5
Tb927.11.9920	polyubiquitin, putative	5	4	5
Tb927.6.3940	hypothetical protein, conserved	5	3	4
Tb927.4.330	hypothetical protein, conserved	5	1	2
Tb927.11.2130	proteasome regulatory non-ATP-ase subunit 3 (RPN3)	5	1	2
Tb927.9.12550	glycerol kinase, glycosomal (glk1)	5	1	2
Tb927.6.850	NOT2	5	4	5
Tb927.11.2110	hypothetical protein, conserved	5	1	2
Tb927.2.1330	retrotransposon hot spot protein (RHS6, pseudogene), putative	5	4	5
Tb927.10.2350	pyruvate dehydrogenase complex E3 binding protein, putative	5	1	2
Tb927.11.14220	hypothetical protein, conserved, Phyre2 gives 97% probability of RNA-binding domain	5	3	4

Tb927.5.4330	dihydrolipoamide branched chain transacylase, putative	1	3	4
Tb927.11.9080	hypothetical protein, conserved	4	3	4
Tb927.11.15850	kinetoplast poly(A) polymerase complex 1 subunit	4	3	4
Tb927.4.760	AP-1 adapter complex gamma subunit, putative	4	3	4
Tb927.11.10560	eIF4G4	3	3	4
Tb927.10.5760	adenylate kinase, putative	4	1	2
Tb927.10.5880	Proteophosphoglycan, putative	1	3	4
Tb927.3.1940	hypothetical protein, possible component of cytochorome oxidase complex	4	1	2
Tb927.9.13990	DRBD2	3	3	4
Tb927.10.12660	PUF2	4	1	2
Tb927.11.15760	GPI transamidase subunit Tta1 (TTA1)	4	1	2
Tb927.5.780	hypothetical protein, conserved	4	1	2
Tb927.5.1510	Mitchondrial SSU ribosomal protein	4	3	4
Tb927.9.5840	tryparedoxin peroxidase (TRYP1)	4	3	4
Tb927.6.4560	Mitchondrial SSU ribosomal protein	3	3	4
Tb927.8.7650	amino acid transporter, putative	3	3	4
Tb927.9.15380	NADH-ubiquinone oxidoreductase complex I subunit, putative,NDUFA9 subunit, putative	3	3	4
Tb927.11.5970	phosphoinositide-specific phospholipase C, putative	1	3	4
Tb927.7.4970	glutamine synthetase, putative	1	3	4
Tb927.10.4610	dolicholphosphate-mannose synthase, putative	4	1	2
Tb927.11.14190	hypothetical protein, conserved	4	1	2
Tb927.11.3130	glycosomal transporter (GAT2)	4	1	2
Tb927.2.2210	hypothetical protein, conserved	4	1	2
Tb927.7.1290	hypothetical protein, conserved. DUF2012, peptidase superfamily	4	1	2
Tb927.9.2450	electron transport protein SCO1/SCO2, putative	4	1	2
Tb927.11.530	RBP3	4	3	4
Tb927.11.9570	hypothetical protein, conserved	4	1	2
Tb927.8.1150	KKT9 Kinetochore protein	4	1	2
Tb927.8.1190	hypothetical protein, conserved	3	3	4
Tb927.10.4880	hypothetical protein, possible component of cytochorome oxidase complex	3	3	4
Tb927.11.11590	eIF3E eukaryotic translation initiation factor, putative	4	3	4
Tb927.3.2230	succinyl-CoA synthetase alpha subunit, putative	4	1	2
Tb927.10.2770	eIF5	3	3	4
Tb927.10.15390	kinesin, clathrin-associated, Trypanosome-specific kinesin family 2	1	3	4
Tb927.11.4210	hypothetical protein, conserved	4	3	4
Tb927.11.6600	hypothetical protein, conserved	4	3	4
Tb927.9.5730	nucleosome assembly protein-like protein	4	1	2
Tb927.8.1960	NOT11	4	3	4
Tb927.11.720	hypothetical protein, conserved	4	1	2
Tb927.11.7520	hypothetical protein, conserved	4	1	2
Tb927.5.4380	kinetoplastid-specific phospho-protein phosphatase, putative	4	1	2
Tb927.9.13970	hypothetical protein, conserved	3	3	4
Tb927.10.3930	40S ribosomal protein S3A	2	3	4
Tb927.10.2980	19S proteasome regulatory subunit, (RPN11)	4	1	2

Tb927.6.1440	Mitchondrial LSU ribosomal protein	4	1	2
Tb927.10.16170	potassium voltage-gated channel, putative	4	1	2
Tb927.10.10610	protein tyrosine phosphatase, putative	4	3	4
Tb927.11.1630	Mitchondrial LSU ribosomal protein	4	3	4
Tb927.5.4120	Mitchondrial LSU ribosomal protein	4	3	4
Tb927.5.960	MSP1, Mitochondrial protein involved in sorting of proteins in the mitochondria, putative	4	3	4
Tb927.6.1520	aquaporin 3, putative (AQP1)	4	3	4
Tb927.6.1880	aspartyl-tRNA synthetase, putative	4	3	4
Tb927.7.2330	hypothetical protein, conserved	4	3	4
Tb927.7.3430	Mitchondrial LSU ribosome-associated cyclophilin type peptidyl-prolyl cis-trans isomerase	4	3	4
Tb927.9.4230	fatty acyl CoA synthetase 4 (ACS4)	4	3	4
Tb927.9.6620	hypothetical protein, conserved	4	3	4
Tb927.1.420	retrotransposon hot spot protein 5 (RHS5), putative	3	3	4
Tb927.11.11780	acyl-CoA dehydrogenase, putative	3	3	4
Tb927.11.1850	hypothetical protein, conserved	3	3	4
Tb927.11.6720	hypothetical protein, conserved	3	3	4
Tb927.11.9750	hypothetical protein, conserved	3	3	4
Tb927.3.3220	RNA polymerase-associated protein CTR9, putative	3	3	4
Tb927.3.3520	POMP25	3	3	4
Tb927.4.2040	ALBA3	3	3	4
Tb927.5.2140	UPF1	3	3	4
Tb927.6.4500	hypothetical protein, conserved	3	3	4
Tb927.7.4470	hypothetical protein, conserved	3	3	4
Tb927.7.5960	protein associated with differentiation 4, PAD4	3	3	4
Tb927.8.6650	DRBD13	3	3	4
Tb927.8.6820	hypothetical protein, conserved	3	3	4
Tb927.1.190	retrotransposon hot spot protein (RHS, pseudogene)	4	2	3
Tb927.11.6040	hypothetical protein, conserved	2	3	4
Tb927.4.4910	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor, putative	4	3	4
Tb927.1.720	phosphoglycerate kinase (PGKA)	1	3	4
Tb927.10.15170	NRG1, nucleolar regulator of GPEET expression	1	3	4
Tb927.10.1560	hypothetical protein, conserved	1	3	4
Tb927.10.3790	hypothetical protein, conserved	1	3	4
Tb927.10.6090	tRNA pseudouridine synthase A, putative	1	3	4
Tb927.10.7630	hypothetical protein, conserved,transportin2- like protein	1	3	4
Tb927.10.9330	hypothetical protein, conserved, partial PSP1 superfamily (signal peptidase-like)	1	3	4
Tb927.11.16530	hypothetical protein, conserved	1	3	4
Tb927.11.6360	60S ribosomal protein L24	1	3	4
Tb927.11.8040	Mitochondrial LSU-associated	1	3	4
Tb927.11.8050	hypothetical protein, conserved	1	3	4
Tb927.2.5020	acyl-CoA oxidase, putative	1	3	4
Tb927.2.510	retrotransposon hot spot protein 4 (RHS4), putative	1	3	4
Tb927.2.6240	adenosine transporter 2 (TbNT5)	1	3	4
Tb927.3.4080	hypothetical protein, conserved	1	3	4
Tb927.4.1610	VDAC-like	1	3	4

Tb927.4.2790	hypothetical protein, conserved	1	3	4
Tb927.4.3070	hypothetical protein, conserved	1	3	4
Tb927.4.3840	nucleolar protein, putative	1	3	4
Tb927.4.4600	Mitchondrial LSU ribosomal protein	1	3	4
Tb927.4.4720	KRIPP7/TbPPR7 Kinetoplast ribosomal PPR-repeat containing protein	1	3	4
Tb927.5.4020	hypothetical protein no clear yeast or human matches	1	3	4
Tb927.7.3060	hypothetical protein, conserved	1	3	4
Tb927.7.3380	hypothetical protein, conserved	1	3	4
Tb927.7.650	hypothetical protein, conserved	1	3	4
Tb927.8.2600	hypothetical protein, conserved	1	3	4
Tb927.8.3300	Mitchondrial LSU ribosomal protein	1	3	4
Tb927.9.10560	POMP6	1	3	4
Tb927.9.14410	RNA 3'-terminal phosphate cyclase-like protein	1	3	4
Tb927.9.4200	fatty acyl CoA synthetase 2 (ACS2)	1	3	4
Tb927.9.8290	Mitchondrial LSU ribosomal protein	1	3	4
Tb927.1.90	retrotransposon hot spot protein (RHS, pseudogene), putative	4	1	2
Tb927.10.13860	GPI-anchor transamidase subunit 8 (GPI8)	4	1	2
Tb927.10.13990	hypothetical protein, conserved	4	1	2
Tb927.10.15330	hypothetical protein, conserved	4	1	2
Tb927.10.15710	mitochondrial carrier protein, MCP7	4	1	2
Tb927.10.3210	delta-1-pyrroline-5-carboxylate dehydrogenase, putative	4	1	2
Tb927.10.4280	Mitochondrial cytochrome bc1 complex component	4	1	2
Tb927.10.5220	hypothetical protein, possible component of cytochorome oxidase complex	4	1	2
Tb927.10.5640	TbGemin2	4	1	2
Tb927.10.660	2-oxoisovalerate dehydrogenase alpha subunit, putative	4	1	2
Tb927.10.8720	NOT10	4	1	2
Tb927.10.8730	hypothetical protein, conserved	4	1	2
Tb927.11.1030	KKT7 Kinetochore protein	4	1	2
Tb927.11.12890	POMP17	4	1	2
Tb927.11.14270	protein kinase, putative	4	1	2
Tb927.11.15230	cytosolic coat protein, putative	4	1	2
Tb927.11.15870	hypothetical protein, conserved, no human or yeast homologue	4	1	2
Tb927.11.16110	FG-GAP repeat protein, putative,intergrin alpha chain protein, putative	4	1	2
Tb927.11.16810	dynein light intermediate chain D1bLIC, putative	4	1	2
Tb927.11.4490	long-chain-fatty-acid-CoA ligase, putative,acyl-CoA synthetase	4	1	2
Tb927.11.5520	triosephosphate isomerase (TIM)	4	1	2
Tb927.11.9820	hypothetical protein, conserved	4	1	2
Tb927.11.9900	phytoene synthase, putative, complex I	4	1	2
Tb927.2.4830	TFIIF-stimulated CTD phosphatase, putative	4	1	2
Tb927.2.5930	hypothetical protein, conserved	4	1	2
Tb927.3.3950	hypothetical protein, conserved	4	1	2
Tb927.4.2760	hypothetical protein, conserved	4	1	2
Tb927.4.4570	hypothetical protein, conserved, no domains, no yeast or human match	4	1	2

Tb927.5.1130	hypothetical protein, conserved	4	1	2
Tb927.5.1930	hypothetical protein, conserved	4	1	2
Tb927.5.3190	hypothetical protein, conserved	4	1	2
Tb927.5.770	hypothetical protein, conserved	4	1	2
Tb927.6.1140	dolichyl-P-Man:GDP-Man5GlcNAc2-PP-dolichyl alpha-1,2-mannosyltransferase, putative	4	1	2
Tb927.6.1990	hypothetical protein, conserved, no yeast or human match	4	1	2
Tb927.6.2560	hypothetical protein, conserved	4	1	2
Tb927.6.3510	tRNA modification enzyme, putative	4	1	2
Tb927.6.3720	hypothetical protein, conserved	4	1	2
Tb927.6.4070	hypothetical protein, conserved	4	1	2
Tb927.6.4080	Mitchondrial LSU ribosomal protein	4	1	2
Tb927.7.2500	proteasome regulatory ATPase subunit 1	4	1	2
Tb927.7.3180	Mu-adaptin 1, putative,adaptor complex AP-1 medium subunit, putative	4	1	2
Tb927.7.3370	intraflagellar transport protein IFT74	4	1	2
Tb927.7.3880	protein kinase, putative	4	1	2
Tb927.7.4980	ZC3H23 POMP35	4	1	2
Tb927.7.5080	ATP-NAD kinase-like protein	4	1	2
Tb927.7.6930	ATPase, putative	4	1	2
Tb927.8.1290	hypothetical protein, conserved, no domains	4	1	2
Tb927.8.3580	mitochondrial chaperone BCS1, putative	4	1	2
Tb927.8.3770	mitogen-activated protein kinase, putative	4	1	2
Tb927.8.4050	flagellar membrane protein that interacts with FLA1, similar to FLA3	4	1	2
Tb927.8.4610	small GTP-binding protein Rab1 (Trab1)	4	1	2
Tb927.8.5560	NADH-ubiquinone oxidoreductase complex I subunit, putative	4	1	2
Tb927.8.6360	hypothetical protein, conserved	4	1	2
Tb927.9.10450	hypothetical protein, conserved	4	1	2
Tb927.9.11540	hypothetical protein, conserved	4	1	2
Tb927.9.13580	hypothetical protein, conserved	4	1	2
Tb927.9.13650	ADP-ribosylation factor, putative	4	1	2
Tb927.9.14080	vesicle-associated membrane protein, putative	4	1	2
Tb927.9.15000	hypothetical protein, conserved	4	1	2
Tb927.9.7180	adenosine monophosphate deaminase, putative,AMP deaminase, putative	4	1	2
Tb927.9.9150	GTP-binding protein, putative	4	1	2
Tb927.10.7710	40S ribosomal protein S8	1	3	4
Tb927.10.6810	guanylate kinase, putative	4	1	2
Tb927.6.4770	MKT1	4	1	2
Tb927.10.14930	ZC3H39	4	1	2
Tb927.4.3590	EF1 beta, translation elongation factor 1-beta, putative	4	1	2
Tb927.8.6110	hypothetical protein, conserved	4	3	4
Tb927.10.2560	mitochondrial malate dehydrogenase (mMDH)	4	1	2
Tb927.6.4690	60S ribosomal protein L9	4	1	2
Tb927.11.14750	hypothetical protein, conserved, PSP1 C-terminal region domain	3	3	4
Tb927.6.5080	hypothetical protein, conserved	1	3	4
Tb927.8.7160	UDP-Gal or UDP-GlcNAc-dependent	4	1	2

	glycosyltransferase pseudogene			
Tb927.3.2150	protein phosphatase 2C, putative	4	3	4
Tb927.3.1680	hypothetical protein, conserved	3	3	4
Tb927.7.6800	Mitchondrial LSU ribosomal protein	1	3	4
Tb927.8.4540	PBP1	1	3	4
Tb927.8.980	Phosphoacetylglucosamine mutase PAGM, acts as phosphoglucomutase	1	3	4
Tb927.10.10130	MRB complex component mitochondrial RNA binding complex 1 subunit	4	1	2
Tb927.11.10020	short-chain dehydrogenase, putative	4	1	2
Tb927.3.2900	EF-2 alpha, elongation initiation factor 2 alpha subunit,	4	1	2
Tb927.7.2160	hypothetical protein, conserved, no domains, no yeast or human match	4	1	2
Tb927.7.2710	NADH-cytochrome b5 reductase, putative	4	1	2
Tb927.8.3380	electron transfer protein, SDH2N,succinate dehydrogenase complex	4	1	2
Tb927.2.1170	retrotransposon hot spot protein 5 (RHS5), putative	3	3	4
Tb927.5.1100	PEX5 peroxisome targeting signal 1 receptor	3	3	4
Tb927.8.6390	lysophospholipase, putative,alpha/beta hydrolase, putative (TbLysoPLA)	3	1	2
Tb927.6.1250	Mitchondrial SSU ribosomal protein S29	3	1	2
Tb927.10.15650	tRNA pseudouridine synthase A-like protein	3	1	2
Tb927.7.5340	hypothetical protein, conserved	3	1	2
Tb927.6.4210	aldehyde dehydrogenase, putative (ALDH)	3	1	2
Tb927.11.370	repressor activator protein 1 (RAP1)	3	1	2
Tb927.3.4680	RAB GDP dissociation inhibitor alpha, putative	3	1	2
Tb927.4.5110	KKT8 Kinetochore protein	3	1	2
Tb927.8.3970	oxidoreductase, putative	3	1	2
Tb927.11.760	protein phosphatase 2C, putative	3	1	2
Tb927.10.2730	hypothetical protein, conserved	3	1	2
Tb927.6.4000	small glutamine-rich tetratricopeptide repeat protein, putative	3	1	2
Tb927.11.870	Mitchondrial LSU ribosomal protein	3	1	2
Tb927.4.1930	EIF3D	3	1	2
Tb927.10.12330	ZC3H34	3	1	2
Tb927.5.3640	Mitchondrial SSU ribosomal protein	3	1	2
Tb927.7.1110	asparagine synthetase a, putative	3	1	2
Tb927.10.10160	hypothetical protein, conserved	3	1	2
Tb927.1.1130	glycerol-3-phosphate dehydrogenase (FAD-dependent), putative	3	1	2
Tb927.1.1530	protein kinase, putative	3	1	2
Tb927.1.2120	calpain, putative,cysteine peptidase, Clan CA, family C2, putative	3	1	2
Tb927.1.3450	hypothetical protein, conserved	3	1	2
Tb927.1.710	phosphoglycerate kinase (PGKB)	3	1	2
Tb927.10.10170	hypothetical protein, conserved,predicted WD40 repeat protein	3	1	2
Tb927.10.10280	microtubule-associated protein, putative	3	1	2
Tb927.10.11310	intraflagellar transport protein IFT55/IFT57	3	1	2
Tb927.10.11340	hypothetical protein, conserved	3	1	2
Tb927.10.12740	ZC3H35	3	1	2
Tb927.10.12930	hypothetical protein, conservedn no domains or good BLASTp hits	3	1	2

Tb927.10.13040	GRESAG 4, putative receptor-type adenylate cyclase	3	1	2
Tb927.10.13290	ethanolamine phosphotransferase (EPT)	3	1	2
Tb927.10.14730	chaperone protein DNAj, putative	3	1	2
Tb927.10.1500	methionyl-tRNA synthetase, putative (MetRS)	3	1	2
Tb927.10.1660	hypothetical protein, conserved	3	1	2
Tb927.10.2020	hexokinase (HK2)	3	1	2
Tb927.10.3080	methionine biosynthetic protein, putative	3	1	2
Tb927.10.3330	hypothetical protein, conserved	3	1	2
Tb927.10.430	hypothetical protein, conserved	3	1	2
Tb927.10.4900	TPR-repeat-containing chaperone protein DNAJ, putative,TPR repeat protein	3	1	2
Tb927.10.5030	40S ribosomal protein S27+ ubiquitin	3	1	2
Tb927.10.7230	hypothetical protein, conserved, 7 nucleoside diphostate kinase domains	3	1	2
Tb927.10.760	hypothetical protein, conserved	3	1	2
Tb927.10.8920	TbGRP ras-like small GTPase	3	1	2
Tb927.10.9200	hypothetical protein, conserved	3	1	2
Tb927.10.9800	60S ribosomal protein L22	3	1	2
Tb927.11.1010	chaperone protein DNAj, putative	3	1	2
Tb927.11.10870	32 kDa ER-associated protein (ERAP32)	3	1	2
Tb927.11.11470	Mitchondrial SSU ribosomal associated KRIPP14 (PPR)	3	1	2
Tb927.11.11630	Mitchondrial LSU ribosomal protein	3	1	2
Tb927.11.11740	membrane-bound acid phosphatase, putative	3	1	2
Tb927.11.13250	eIF-2-gamma	3	1	2
Tb927.11.13270	hypothetical protein, conserved	3	1	2
Tb927.11.13360	AAA ATPase, putative	3	1	2
Tb927.11.13750	small GTPase, putative,ras-related rab-4 (RAB4)	3	1	2
Tb927.11.13890	RNA ligase asociated with SSU	3	1	2
Tb927.11.14330	hypothetical protein, conserved	3	1	2
Tb927.11.15530	C-14 sterol reductase, putative	3	1	2
Tb927.11.15750	AMP deaminase, putative	3	1	2
Tb927.11.2670	Nucleoporin (TbNup59)	3	1	2
Tb927.11.3320	ras-like small GTPase, putative (TbGTR)	3	1	2
Tb927.11.3750	NADH-cytochrome b5 reductase, putative (B5R)	3	1	2
Tb927.11.3940	hypothetical protein, conserved	3	1	2
Tb927.11.4180	hypothetical protein, conserved	3	1	2
Tb927.11.4330	hypothetical protein, conserved, no clear yeast or human matches	3	1	2
Tb927.11.4680	hypothetical protein, conserved	3	1	2
Tb927.11.4850	hypothetical protein, conserved	3	1	2
Tb927.11.5420	hypothetical protein, conserved	3	1	2
Tb927.11.5510	dynein light chain p28, axonemal, putative	3	1	2
Tb927.11.8320	hypothetical protein, conserved	3	1	2
Tb927.2.2230	hypothetical protein, conserved, no domains, Kineto-specific	3	1	2
Tb927.2.2940	hypothetical protein, conserved	3	1	2
Tb927.2.3800	mRNA processing protein, putative GAP1 MRB complex protein	3	1	2
Tb927.2.450	retrotransposon hot spot protein 4 (RHS4), putative	1	2	3

Tb927.2.5270	IAD-3 inner arm dynein heavy chain	3	1	2
Tb927.2.5280	trans-sialidase, putative (30J2.90)	1	2	3
Tb927.2.5810	hypothetical protein, conserved	3	1	2
Tb927.3.2080	hypothetical protein, conserved	3	1	2
Tb927.3.3260	hypothetical protein, conserved, kinetoplastid-specific, poly(E) repeats	3	1	2
Tb927.3.3820	hypothetical protein, conserved	3	1	2
Tb927.3.4040	hypothetical protein, conserved	3	1	2
Tb927.3.4120	hypothetical protein, conserved	3	1	2
Tb927.3.4390	dihydrolipoamide dehydrogenase, putative (GCVL-1)	3	1	2
Tb927.3.4640	POMP26	3	1	2
Tb927.3.5490	flagellar transport protein, putative (PIFTB2)	3	1	2
Tb927.4.1600	hypothetical protein, conserved	3	1	2
Tb927.4.1660	mitochondrial carrier protein, MCP6	3	1	2
Tb927.4.1970	hypothetical protein, conserved	3	1	2
Tb927.4.2280	hypothetical protein, conserved	3	1	2
Tb927.4.2600	hypothetical protein, conserved	3	1	2
Tb927.4.5300	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase, putative	3	1	2
Tb927.5.1020	disulfide isomerase, putative	3	1	2
Tb927.5.1710	ribonucleoprotein p18, mitochondrial precursor, putative	3	1	2
Tb927.5.2060	cell division control protein CDC5f, putative part of PRP19 complex	3	1	2
Tb927.5.2150	hypothetical protein, conserved	3	1	2
Tb927.5.3020	hypothetical protein, conserved	3	1	2
Tb927.5.3220	signal peptidase type I, putative	3	1	2
Tb927.6.2170	co-chaperone GrpE, putative	3	1	2
Tb927.6.2490	Succinate dehydrogenase complex component (SDH7 in T cruzi)	3	1	2
Tb927.6.2930	hypothetical protein, conserved	3	1	2
Tb927.6.3540	zinc-finger protein, conserved	3	1	2
Tb927.6.3650	ADP-ribosylation factor, putative	3	1	2
Tb927.6.4990	ATP synthase, epsilon chain, putative	3	1	2
Tb927.6.580	hypothetical protein, conserved	3	1	2
Tb927.7.2070	heat shock protein DNAJ, putative	3	1	2
Tb927.7.4160	Elongase 1	3	1	2
Tb927.7.4950	NAD(p)-dependent steroid dehydrogenase-like protein	3	1	2
Tb927.7.5130	hypothetical protein, conserved	3	1	2
Tb927.7.5250	hypothetical protein, conserved	3	1	2
Tb927.7.5320	hypothetical protein, conserved	3	1	2
Tb927.7.5710	hypothetical protein, conserved	3	1	2
Tb927.7.6200	chaperone protein DNAj, putative	3	1	2
Tb927.7.6440	v-SNARE like protein	3	1	2
Tb927.7.6660	chaperone protein DNAj, putative	3	1	2
Tb927.7.680	chaperone protein DNAj, putative	3	1	2
Tb927.7.740	chaperone protein DNAj, putative	3	1	2
Tb927.7.830	telomerase-associated protein, putative	3	1	2
Tb927.8.1940	endosomal integral membrane protein, putative	3	1	2

Tb927.8.2240	tryptophanyl-tRNA synthetase, putative	3	1	2
Tb927.8.2540	Acetyl-CoA acetyltransferase	3	1	2
Tb927.8.2910	mannosyl-oligosaccharide 1,2-alpha-mannosidase IB, ER quality control	3	1	2
Tb927.8.4150	hypothetical protein, conserved	3	1	2
Tb927.8.4770	small GTP-binding protein Rab18 (TbRAB18)	3	1	2
Tb927.8.5280	Mitchondrial SSU ribosomal associated TbMRPS34	3	1	2
Tb927.8.5370	hypothetical protein, conserved, no yeast or human match	3	1	2
Tb927.8.570	proteasome regulatory non-ATP-ase subunit 10	3	1	2
Tb927.8.6200	tubulin folding cofactor D, putative	3	1	2
Tb927.8.7120	Squalene synthase	3	1	2
Tb927.8.760	nucleolar RNA-binding protein (Nopp44/46)	3	1	2
Tb927.9.12710	hypothetical protein, conserved	3	1	2
Tb927.9.14070	short-chain dehydrogenase, putative	3	1	2
Tb927.9.15010	NADH-ubiquinone oxidoreductase complex I subunit, putative	3	1	2
Tb927.9.2560	hypothetical protein, conserved	3	1	2
Tb927.9.2670	POMP3	3	1	2
Tb927.9.3620	hypothetical protein, conserved	3	1	2
Tb927.9.3640	Mitchondrial LSU ribosomal protein	3	1	2
Tb927.9.3820	syntaxin, putative	3	1	2
Tb927.9.5280	Mitchondrial SSU ribosomal protein	3	1	2
Tb927.9.7830	mitochondrial tRNA import complex, putative	3	1	2
Tb927.9.9710	hypothetical protein, conserved	3	1	2
Tb927.7.3030	hypothetical protein, conserved	3	1	2
Tb927.7.4710	Mitchondrial LSU ribosomal protein	3	1	2
Tb927.9.11580	Gim5A protein,glycosomal membrane protein (gim5A)	3	1	2
Tb11.02.5400	cystathionine beta-synthase, putative	3	1	2
Tb927.2.3780	IF2 translation initiation factor IF-2, putative	3	1	2
Tb927.2.5060	GTP binding protein, putative	3	1	2
Tb927.1.3180	40S ribosomal protein S11	3	1	2
Tb927.10.13710	hypothetical protein, conserved	3	1	2
Tb927.4.2240	hypothetical protein, conserved, D-aminoacid aminotransferase domain	3	1	2
Tb927.7.6890	hypothetical protein, conserved	3	1	2
Tb927.9.10580	3-demethylubiquinone-9 3-methyltransferase, putative	3	1	2
Tb927.5.3970	adenylate kinase, putative (ADKE)	3	1	2
Tb927.9.4210	fatty acyl CoA synthetase 3 (ACS3)	3	2	3
Tb927.10.5810	hypothetical protein, conserved	3	1	2
Tb927.1.2400	alpha tubulin	1	1	2
Tb927.1.2390	beta tubulin	1	1	2
Tb927.9.12610	glycerol kinase, glycosomal (glk1)	1	1	2
Tb927.9.4420	hypothetical protein, conserved	1	1	2
Tb927.4.3680	protein phosphatase 2C, putative	1	1	2
Tb927.11.7710	Gp63-1 surface protease homolog, putative	1	1	2
Tb927.1.1620	ATP-dependent DEAD/H RNA helicase, putative, no clear yeast homologue	1	1	2
Tb927.10.12510	P-type H -ATPase, putative	1	1	2

Tb927.2.5530	POMP22	1	1	2
Tb927.1.2670	flagellar protein PF16	1	1	2
Tb927.9.6920	hypothetical protein, conserved	1	1	2
Tb927.1.1700	hypothetical protein, conserved	1	1	2
Tb927.10.14330	hypothetical protein, conserved	1	1	2
Tb927.6.1020	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (Rhodesain)	1	1	2
Tb927.7.3970	hypothetical protein, conserved	1	1	2
Tb927.7.5110	hypothetical protein, conserved	1	1	2
Tb927.9.12070	hypothetical protein, conserved	1	1	2
Tb927.9.15330	hypothetical protein, conserved	1	1	2
Tb927.9.7540	calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative	1	1	2
Tb927.7.5930	Protein Associated with Differentiation (TbPAD1)	1	1	2
Tb927.10.70	retrotransposon hot spot (RHS), putative, (fragment)	1	1	2
Tb927.10.14320	hypothetical protein, conserved	1	1	2
Tb927.10.1540	ZC3H30	1	1	2
Tb927.10.2550	malate dehydrogenase-related	1	1	2
Tb927.10.9660	CRN/SYF3 part of splicing PRP19 complex, putative	1	1	2
Tb927.11.12880	hypothetical protein, conserved	1	1	2
Tb927.11.14970	hypothetical protein, conserved	1	1	2
Tb927.11.4700	prostaglandin f synthase	1	1	2
Tb927.11.9530	14-3-3-I protein	1	1	2
Tb927.4.1790	60S ribosomal protein L3	1	1	2
Tb927.6.2550	TRRM2	1	1	2
Tb927.7.2390	hypothetical protein, conserved	1	1	2
Tb927.8.1980	hypothetical protein, conserved	1	1	2
Tb927.9.11850	structural maintenance of chromosome 1 (SMC1)	1	1	2
Tb927.11.9710	60S ribosomal protein L10a	1	1	2
Tb927.10.10920	heat shock protein 83, HSP83	1	1	2
Tb927.2.1080	retrotransposon hot spot protein 5 (RHS5), putative	1	1	2
Tb927.4.410	CAF40	1	1	2
Tb927.10.6630	ATP-dependent DEAD/H RNA helicase HEL64, putative (cytosolic)	1	1	2
Tb927.11.8990	cation transporter, putative	1	1	2
Tb927.9.8160	chaperone protein DNAj, putative	2	1	2
Tb10.v4.0052	microtubule-associated protein 2s	1	1	2
Tb927.1.2730	hypothetical protein, conserved	1	1	2
Tb927.10.12860	hypothetical protein, conserved	1	1	2
Tb927.10.14630	fibrillarin, putative	1	1	2
Tb927.10.3010	hypothetical protein, conserved, in bilobe	1	1	2
Tb927.10.7500	fibrillarin (NOP1)	1	1	2
Tb927.10.7810	Homologue of yeast ESF1,nucleolar protein involved in pre-rRNA processing	1	1	2
Tb927.10.8870	hypothetical protein, conserved	1	1	2
Tb927.10.9780	ATP-dependent DEAD/H RNA helicase, putative Mak5	1	1	2
Tb927.10.9890	hypothetical protein, conserved, no domains	1	1	2
Tb927.11.12830	acyl-CoA binding protein, putative	1	1	2
Tb927.11.3550	NPA3/XAB1 homologue, interacts with XPA and with RNA pol II	1	1	2

Tb927.11.9490	hypothetical protein, conserved	1	1	2
Tb927.2.5210	3-oxoacyl-ACP reductase, putative	1	1	2
Tb927.3.2490	hypothetical protein, conserved	1	1	2
Tb927.3.5020	hypothetical protein, conserved	2	1	2
Tb927.3.5250	ZC3H8	1	1	2
Tb927.4.3670	hypothetical protein, conserved	1	1	2
Tb927.4.920	hypothetical protein, conserved	1	1	2
Tb927.5.2500	hypothetical protein, conserved	1	1	2
Tb927.5.980	hypothetical protein, conserved	1	1	2
Tb927.6.2140	Mitochondrial RNA binding complex I component, putative	1	1	2
Tb927.7.1780	Adenine phosphoribosyltransferase, putative	1	1	2
Tb927.7.4440	hypothetical protein, conserved	1	1	2
Tb927.7.6620	hypothetical protein, conserved	1	1	2
Tb927.7.700	hypothetical protein, conserved	1	1	2
Tb927.8.3310	acetyltransferase, putative	1	1	2
Tb927.8.5040	hypothetical protein, conserved	1	1	2
Tb927.8.7170	inositol polyphosphate 1-phosphatase, putative	1	1	2
Tb927.9.8680	cytochrome c oxidase assembly factor, putative	1	1	2
Tb927.9.4910	protein kinase, putative	1	1	2
Tb927.10.4560	EF2 elongation factor 2	1	1	2
Tb927.6.2300	adenosine kinase, putative	1	1	2
Tb927.11.12230	heat shock protein HslU2, in mitochondrion	1	1	2
Tb927.9.13520	hypothetical protein, conserved	1	1	2
Tb927.11.3300	hypothetical protein, conserved, HA tagged is in mitochondrion, poly(Q), rich in E, K.	1	1	2
Tb927.1.1560	vesicular-fusion protein nsf, putative,N-ethylmaleimide sensitive factor (NsF)	1	1	2
Tb927.3.4720	dynamin, putative,vacuolar sortin protein 1, putative	1	1	2
Tb927.6.3890	replication factor C, subunit 2, putative	1	1	2
Tb927.3.1710	Mitchondrial LSU ribosomal protein	1	1	2
Tb927.10.14160	aquaporin 3, putative	1	1	2
Tb927.6.1550	POMP30 leucine-rich repeat protein (LRRP)	1	1	2
Tb927.8.7480	hypothetical protein, conserved, no clear yeast or human matches	1	1	2
Tb927.7.7430	ATP synthase F1, alpha subunit	1	1	2
Tb927.10.14780	protein kinase, putative,mitogen-activated protein kinase kinase kinase, putative	1	1	2
Tb427tmp.160.5200	0	1	1	2
Tb927.9.3280	acidocalcisomal exopolyphosphatase, putative (3C4.175)	1	1	2
Tb927.7.2140	ZC3H18	1	1	2
Tb927.10.4980	ubiquitin-like protein DSK2, putative (DSK2)	1	1	2
Tb927.3.3310	60S ribosomal protein L13	1	1	2
Tb927.11.4770	retrotransposon hot spot protein (RHS, pseudogene), putative	1	1	2
Tb927.6.140	retrotransposon hot spot protein 5 (RHS5), putative	1	1	2
Tb927.6.2080	Mitchondrial SSU ribosomal associated KRIPP22 (PPR)	1	1	2
Tb927.11.10910	40S ribosomal protein SA	1	1	2
Tb927.10.6880	glyceraldehyde 3-phosphate dehydrogenase, cytosolic (GAP)	1	1	2

Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative	1	1	2
Tb927.9.12630	glycerol kinase, glycosomal (glk1)	1	1	2
Tb927.8.6160	40S ribosomal protein S8	1	1	2
Tb927.6.2780	U3 small nuclear ribonucleoprotein (snRNP), putative	1	1	2
Tb927.10.10700	splicing factor Prp31	1	1	2
Tb927.8.5810	mitochondrial carrier protein, MCP24	1	1	2
Tb927.10.2010	hexokinase (HK1)	1	1	2
Tb927.10.15830	hypothetical protein, conserved	1	1	2
Tb927.5.3410	Mitchondrial LSU ribosomal protein	1	1	2
Tb927.7.2110	KKT11 Kinetochore protein	1	1	2
Tb927.3.2100	hypothetical protein, conserved	1	1	2
Tb11.1790	retrotransposon hot spot protein (RHS, pseudogene), putative	1	1	2
Tb10.v4.0053	hypothetical protein	1	1	2
Tb927.10.14820	mitochondrial carrier protein, MCP5a	1	1	2
Tb927.8.1340	60S ribosomal protein L7a	1	1	2
Tb927.11.8100	hypothetical protein, conserved	1	1	2
Tb927.10.8440	glucose transporter 1B (THT1-)	1	1	2
Tb427.11.01.v4	hypothetycal prot conserved	1	1	2
Tb927.7.1750	60S ribosomal protein L7	1	1	2
Tb927.6.4800	hypothetical protein, conserved	1	1	2
Tb10.v4.0247	s-adenosyl-L-methionine-c-24-delta-sterol-methy lt ransferase a, putatives	1	1	2
Tb927.10.2110	EF1-alpha elongation factor 1-alpha, (TEF1)	1	1	2
Tb927.11.14000	RNA-binding protein (NRBD1)	1	1	2
Tb927.1.2190	RAPTOR-like (TOR complexes)	2	1	2
Tb927.1.4310	hypothetical protein, conserved	2	1	2
Tb927.1.4420	ABC transporter, putative	2	1	2
Tb927.10.1970	hypothetical protein, conserved	2	1	2
Tb927.10.2880	calcium channel protein, putative	2	1	2
Tb927.10.3100	Glycerol-3-phosphate acyltransferase	2	1	2
Tb927.10.5350	IAD-4 inner arm dynein heavy chain	2	1	2
Tb927.10.6330	KKT1 kinetochore protein	2	1	2
Tb927.10.740	structural maintenance of chromosome 4 (SMC4)	2	1	2
Tb927.10.8450	glucose transporter,glucose transporter 1E (THT1E)	2	1	2
Tb927.10.8780	P-loop nucleoside hydrolase domain, like a human adenylate kinase domain protein	2	1	2
Tb927.11.10520	KKT2 kinetochore protein, tyrosine kinase, putative	2	1	2
Tb927.11.15450	hypothetical protein	2	1	2
Tb927.11.3100	POMP13, Trypanosome-specific, putative TM domain at Cterminus	2	1	2
Tb927.11.5820	hypothetical protein, conserved, probably in mitochondrion	2	1	2
Tb927.11.6350	AAA ATPase, putative	2	1	2
Tb927.2.1260	ESAG4 pseudogene	2	1	2
Tb927.2.2370	hypothetical protein, conserved	2	1	2
Tb927.2.3080	fatty acid desaturase, putative,oleate desaturase, putative	2	1	2
Tb927.2.5870	hypothetical protein, conserved	2	1	2
Tb927.3.4030	hypothetical protein, conserved	2	1	2

Tb927.4.1500	RNA editing associated helicase 2 (REH2) MRB 1 subunit	2	1	2
Tb927.4.2530	hypothetical protein, conserved	2	1	2
Tb927.4.5020	RNA polymerase IIA largest subunit (RPB1)	2	1	2
Tb927.5.1120	hypothetical protein, conserved	2	1	2
Tb927.5.3510	structural maintenance of chromosome 3 (SMC3)	2	1	2
Tb927.6.870	myotubularin, putative	2	1	2
Tb927.6.920	helicase, putative	2	1	2
Tb927.7.220	CDP-diacylglycerol synthetase, putative	2	1	2
Tb927.7.4170	Elongase 2	2	1	2
Tb927.7.5280	hypothetical protein, conserved	2	1	2
Tb927.7.7400	hypothetical protein, conserved	2	1	2
Tb927.8.2200	terbinafine resistance locus protein (yip1), putative	2	1	2
Tb927.8.2820	hypothetical protein, conserved	2	1	2
Tb927.8.3630	folate transporter, ESAG10, putative	2	1	2
Tb927.8.4950	kinesin	2	1	2
Tb927.8.6620	hypothetical protein, conserved	2	1	2
Tb927.9.12700	phospholipase A1, putative	2	1	2
Tb927.9.1770	hypothetical protein, conserved	2	1	2
Tb927.9.3680	hypothetical protein, conserved	2	1	2
Tb927.9.5700	hypothetical protein, conserved	2	1	2
Tb927.9.9450	ZC3H28	2	1	2
Tb927.9.9740	AMP deaminase, putative	2	1	2
Tb927.9.9810	hypothetical protein, conserved	2	1	2
Tb927.6.960	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	1	1	2
Tb927.11.5300	kinesin, Kif13-3, predicted centromere-associated	1	1	2
Tb927.11.6540	hypothetical protein, conserved, kinetoplastid-specific	1	1	2
Tb927.2.370	retrotransposon hot spot protein 1 (RHS1), putative	1	1	2
Tb927.3.1590	MRB complex protein mitochondrial RNA binding complex 1 subunit	1	1	2
Tb927.3.4500	fumarate hydratase, class I (FHc)	1	1	2
Tb927.4.630	hypothetical protein, conserved	1	1	2
Tb927.5.1090	threonyl-tRNA synthetase, putative	1	1	2
Tb927.5.1470	NADH-cytochrome b5 reductase, putative (B5R)	1	1	2
Tb927.5.3920	PEX6 peroxisome assembly protein, AAA ATPase	1	1	2
Tb927.5.590	protein phosphatase 1, regulatory subunit, putative	1	1	2
Tb927.5.920	hypothetical protein, conserved	1	1	2
Tb927.7.1300	protein disulfide isomerase,ERp72-like, cofactor wth calnexin, putative	1	1	2
Tb927.7.1360	hypothetical protein, conserved	1	1	2
Tb927.7.1920	paraflagellar rod protein PFC5	1	1	2
Tb927.7.2660	ZC3H20	1	1	2
Tb927.7.2700	NADH-cytochrome b5 reductase, putative (B5R)	1	1	2
Tb927.7.3080	hypothetical protein, conserved, kinetoplastid-specific	1	1	2
Tb927.7.4390	threonine synthase, putative	1	1	2
Tb927.7.7030	hypothetical protein, conserved	1	1	2
Tb927.7.7460	hypothetical protein, conserved	1	1	2
Tb927.8.2030	hypothetical protein, conserved	1	1	2

Tb927.9.11050	4E-IP, 4E-interacting protein, putative	1	1	2
Tb927.9.11910	hypothetical protein, conserved	1	1	2
Tb927.9.6450	PEX16, putative	1	1	2
Tb927.9.9870	MCAK-like kinesin, putative	1	1	2
Tb927.9.6100	TFIIF-stimulated CTD phosphatase, in UPF1-TAP	1	1	2
Tb927.9.7470	purine nucleoside transporter NT10	2	1	2
Tb927.11.14900	coatomer epsilon subunit, epsilon-COP	1	1	2
Tb927.10.2640	intraflagellar transport protein IFT81	1	1	2
Tb927.11.16160	ATP binding protein-like protein	1	1	2
Tb927.11.3770	hypothetical protein, conserved	1	1	2
Tb927.2.4980	hypothetical protein, conserved	1	1	2
Tb927.7.1500	Thiomodification of cytiosolic tRNAs (NBP 2)	1	1	2
Tb927.9.8850	actin A	1	1	2
Tb927.11.4900	guanine nucleotide-binding beta subunit-like protein	1	1	2
Tb927.9.6060	2Fe-2S iron-sulfur cluster binding domain containing protein, POMP4	1	1	2
Tb927.1.3110	soluble N-ethylmaleimide sensitive factor (NSF) attachment protein, putative	1	1	2
Tb927.4.1170	ankyrin, putative	1	1	2
Tb927.7.1970	retrotransposon hot spot protein 7 (RHS7), putative	1	1	2
Tb927.11.670	epsinR	1	1	2
Tb927.10.9430	phosphoribosylpyrophosphate synthetase, putative (PRS)	1	1	2
Tb927.1.2330	beta tubulin	1	1	2
Tb927.1.1790	hypothetical protein	1	1	2
Tb927.10.1120	hypothetical protein, conserved	1	1	2
Tb927.10.460	NIMA-related protein kinase (NRKC)	1	1	2
Tb927.11.4350	hypothetical protein, conserved	1	1	2
Tb927.2.2260	PIK-related	1	1	2
Tb927.4.220	retrotransposon hot spot protein (RHS2, pseudogene), putative	1	1	2
Tb927.6.2880	Kinesin-like but poor match	1	1	2
Tb927.8.3180	hypothetical protein, conserved	1	1	2
Tb927.8.5330	MCP2, binds to tRNAs and assists tRNA synthetases	1	1	2
Tb927.8.800	hypothetical protein, conserved	1	1	2
Tb927.9.1710	hypothetical protein, conserved	1	1	2
Tb927.9.3920	40S ribosomal protein S7	1	1	2
Tb927.11.7610	gp63-1 surface protease pseudogene	1	1	2
Tb927.9.9420	hypothetical protein, conserved	1	1	2
Tb927.10.14950	ZC3H40	1	1	2
Tb927.10.3760	vacuolar ATP synthase subunit d, putative	1	1	2
Tb927.11.1890	hypothetical protein, conserved	1	1	2
Tb927.11.5440	malic enzyme	1	1	2
Tb927.2.4240	GTP binding protein, putative	1	1	2
Tb927.3.3630	EF-TS Mitochondrial elongation factor ts	1	1	2
Tb927.4.3370	hypothetical protein, conserved	1	1	2
Tb927.5.2380	hydrolase, alpha/beta fold family, putative	1	1	2
Tb927.6.1810	hypothetical protein, conserved	1	1	2
Tb927.6.4430	homoserine kinase, putative (HK)	1	1	2

Tb927.6.4920	S-adenosylmethionine synthetase, putative (METK1)	1	1	2
Tb927.7.2020	retrotransposon hot spot protein 7 (RHS7), putative	1	1	2
Tb927.8.5860	Mitchondrial LSU ribosomal protein 50S ribosomal protein L17, putative	1	1	2
Tb927.9.15190	60S ribosomal protein L15	1	1	2
Tb09.v4.0013	retrotransposon hot spot (RHS) protein	1	1	2
Tb927.2.3400	hypothetical protein, copurified with splicing complex	1	1	2
Tb927.3.3610	PEX7 peroxisomal targeting signal type 2 receptor	1	1	2
Tb927.11.16080	hypothetical protein, conserved	1	1	2
Tb927.5.550	vacuolar ATP synthase, putative	1	1	2
Tb927.11.2260	eIF4E1	1	1	2
Tb927.5.2940	stress-induced protein sti1, putative	1	1	2
Tb927.8.8330	calpain, putative,cysteine peptidase, putative	1	1	2
Tb927.10.1900	DNA topoisomerase IA, putative	1	1	2
Tb927.9.6290	arginine kinase (AK)	1	1	2
Tb927.11.500	UBP1	1	1	2
Tb927.8.7980	vacuolar-type H( )-translocating pyrophosphatase (TVP1)	1	1	2

Supplementary table 2: Proteins that co-purified with the *EP 3'-UTR* detected by DL-QMS

In this table the enrichment obtained using the method developed in the present dissertation is compared to a previous purification done in the laboratory by Cornelia Klein. The last two columns in this table correspond to proteins found in membrane bound polyribosomes for procyclic form trypanosomes and the number of times these peptides were found in previous purifications, respectively.

Accession	**Description**	**3SBP/ No SBP**	**Coverage**	**PC meb polys Peptides**	**no times found before**
Tb927.11.3600	40S ribosomal protein S4	300	25,27	0	0
Tb927.7.7420	ATP synthase alpha chain, mitochondrial precursor,ATP synthase F1, alpha subunit	300	9,76	11	5
Tb927.10.12840	mitochondrial carrier protein, MCP12	300	18,42	0	0
Tb927.3.2150	protein phosphatase 2C, putative	292	9,39	0	0
Tb927.11.11360	guanine nucleotide-binding protein (TRACK)	277	22,33	15	4
Tb927.10.4310	prohibitin 2, putative (PHB2)	255	15,93	0	0
Tb927.5.3400	calcium-translocating P-type ATPase,calcium pump	230	29,28	0	0
Tb927.5.1210	short-chain dehydrogenase, putative	214	18,97	0	0
Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putative;with=GeneDB:LmjF36.2950	206	5,25	7	1
Tb927.11.16280	60S ribosomal protein L2 L8	197	37,31	10	9
Tb927.9.5730	nucleosome assembly protein-like protein (28G16.410)	195	4,50	0	0
Tb927.9.15150	60S ribosomal protein L5	189	8,44	12	6

Tb927.10.3660	aspartate aminotransferase	173	6,20	0	1
Tb927.11.2050	60S acidic ribosomal subunit	157	71,91	0	0
Tb927.7.4900	5'-3' exonuclease XRNA, putative,exoribonuclease 1, putative (XRNA)	147	3,74	0	2
Tb927.4.2850	hypothetical protein, conserved no domains	141	4,11	0	0
Tb927.9.4680	ATP-dependent DEAD box helicase, putative (1L12.525)	135	15,59	8	6
Tb927.6.1520	aquaporin 3, putative (AQP1)	135	13,71	0	0
Tb927.10.8520	glucose transporter, putative	129	4,35	0	0
Tb927.10.8490	glucose transporter, putative	129	4,35	0	0
Tb927.10.8500	glucose transporter, putative	129	4,35	0	0
Tb927.7.3040	hypothetical protein, conserved ARM and WD40 repeat	122	8,35	0	2
Tb927.11.9920	polyubiquitin, putative	114	38,10	2	3
Tb927.3.3130	hypothetical protein, conserved SAM (DNA-bindng?) domain, no yeast match	108	2,19	0	0
Tb927.9.15460	calcium motive p-type ATPase, putative	106	5,09	0	0
Tb927.10.8170	nuclear pore complex protein (NUP155), putative,nucleoporin, putative	101	14,87	0	0
Tb927.11.14190	hypothetical protein, conserved	90	3,46	2	1
Tb927.8.1870	Golgi/lysosome glycoprotein 1 (tGLP1)	88	11,36	0	2
Tb927.9.8740	RNA-binding protein (DRBD3)	84	7,03	2	6
Tb927.10.14830	mitochondrial carrier protein, MCP5b	83	37,13	0	0
Tb927.10.8270	eukaryotic translation initiation factor 3 subunit 8, putative	78	3,38	13	2
Tb927.11.11460	hypothetical protein, conserved	73	5,78	0	1
Tb927.9.4210	fatty acyl CoA synthetase 3 (ACS3)	67	5,70	0	1
Tb927.10.4430	pumillo RNA binding protein PUF1 (PUF1)	66	3,85	0	1
Tb927.2.4400	hypothetical protein, conserved	65	1,95	0	1
Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD], glycosomal	61	16,67	7	6
Tb927.4.590	hypothetical protein, conserved	59	7,33	0	0
Tb927.6.2790	L-threonine 3-dehydrogenase, putative	57	21,69	7	2
Tb927.10.1100	60S ribosomal protein L9	51	24,34	8	6
Tb927.6.4690	60S ribosomal protein L9	51	22,99	0	1
Tb927.6.3750	heat shock 70 kDa protein, mitochondrial precursor, putative	51	38,36	0	0
Tb927.7.2190	hypothetical protein, conserved	50	7,52	0	2
Tb927.1.3070	hypothetical protein, conserved	50	7,02	0	1
Tb927.6.4800	hypothetical protein, conserved	46	4,31	0	1
Tb927.11.1900	T-complex protein 1, beta subunit, putative	44	31,00	16	1
Tb927.3.5050	60S ribosomal protein L4	38	16,84	23	11
Tb927.10.8940	hypothetical protein, conserved	37	18,73	0	1
Tb927.4.5200	nucleoporin (NUP54/57), putative (TbNup62)	35	8,58	0	0
Tb927.4.4490	multidrug resistance protein E,p-glycoprotein (MRPE)	31	1,25	0	0
Tb927.2.3780	translation initiation factor IF-2, putative (28H13.240)	31	4,92	2	3
Tb927.10.3990	DHH1 (DHH1)	26	14,78	6	7

Tb927.11.13180	hypothetical protein, conserved	26	7,14	0	3
Tb927.11.10760	kinesin-like protein, putative	26	8,10	0	0
Tb927.7.5020	60S ribosomal protein L19	25	18,08	0	0
Tb927.2.450	retrotransposon hot spot protein 4 (RHS4), putative (3B10.180)	24	14,47	0	1
Tb927.3.3150	hypothetical protein, conserved	24	4,08	0	0
Tb927.10.3940	40S ribosomal protein S3A	22	42,58	14	7
Tb927.10.3930	40S ribosomal protein S3A	22	39,84	0	0
Tb927.11.330	Nucleoporin (TbMlp-1)	22	10,67	0	0
Tb927.3.5370	hypothetical protein, conserved	21	13,10	0	2
Tb927.9.9290	Polyadenylate-binding protein 1 (Poly(A)-binding protein 1) (PABP 1) (PABP1)	21	14,84	25	4
Tb927.5.520	stomatin-like protein, putative	19	10,36	0	2
Tb927.5.440	trans-sialidase, putative	17	6,58	0	0
Tb927.5.4420	nucleolar RNA helicase II, putative,nucleolar RNA helicase Gu, putative	16	10,92	0	5
Tb927.10.4570	elongation factor 2	14	29,79	0	0
Tb927.2.100	retrotransposon hot spot protein 1 (RHS1), putative (3B10.5)	13	2,41	0	1
Tb927.11.10790	40S ribosomal protein SA	12	18,44	8	11
Tb927.11.10910	40S ribosomal protein SA	12	16,25	0	0
Tb927.9.11600	Gim5B protein,glycosomal membrane protein (gim5B)	12	12,03	0	2
Tb927.9.11580	Gim5A protein,glycosomal membrane protein (gim5A)	12	11,93	0	0
Tb927.8.3750	nucleolar protein, putative	12	6,00	11	3
Tb927.9.8880	actin A	11	23,40	0	0
Tb927.1.2340	alpha tubulin	11	61,20	18	22
Tb927.11.14020	RNA-binding protein (NRBD2)	11	22,94	12	6
Tb927.11.14000	RNA-binding protein (NRBD1)	11	18,32	0	1
Tb927.8.8050	Nucleoporin (TbNup75)	11	7,31	0	0
Tb927.4.3950	cytoskeleton-associated protein CAP5.5, putative,cysteine peptidase	11	11,14	8	2
Tb927.11.7460	glucose-regulated protein 78, putative,luminal binding protein 1 (BiP), putative	10	23,89	15	10
Tb927.11.2650	heat shock protein 84, putative, mit HSp90	10	22,44	2	3
Tb927.10.12500	P-type H -ATPase, putative	10	7,89	0	0
Tb927.10.12510	P-type H -ATPase, putative	10	6,52	0	0
Tb927.9.6230	arginine kinase (AK)	10	28,65	0	4
Tb927.9.6290	arginine kinase (AK)	10	27,53	9	1
Tb927.9.6170	arginine kinase (AK) in cytosol and flagellum	10	26,24	0	1
Tb927.10.6400	chaperonin HSP60, mitochondrial precursor (HSP60)	9	73,84	15	7
Tb927.3.1380	ATP synthase beta chain, mitochondrial precursor,ATP synthase F1, beta subunit	9	22,74	15	4
Tb927.1.2370	beta tubulin	9	77,15	0	0
Tb927.9.10310	mitochondrial carrier protein, MCP11	9	13,88	0	1
Tb927.11.16760	T-complex protein 1, alpha subunit, putative (TCP-1-alpha)	9	10,30	18	1
Tb927.10.1060	T-complex protein 1, delta subunit, putative (TCP-1-delta)	8	13,25	17	2
Tb927.10.2100	elongation factor 1-alpha,EF-1-	8	43,88	9	6

	alpha (TEF1)				
Tb927.9.8070	60S ribosomal protein L10	8	10,80	11	9
Tb927.11.11680	2-oxoglutarate dehydrogenase E2 component, putative	8	13,58	10	6
Tb927.11.11330	heat shock protein 70	7	31,88	35	24
Tb927.11.15990	nucleoporin Nup109 (TbNup109)	7	13,39	0	0
Tb927.3.4760	dynamin, putative,vacuolar sortin protein 1, putative	7	8,33	0	0
Tb927.11.6440	hypothetical protein, conserved, often in TAP MS	7	13,15	0	13
Tb927.9.2470	nucleolar protein (NOP86)	6	12,42	9	4
Tb927.11.3240	T-complex protein 1, zeta subunit, putative (TCP-1-zeta)	6	12,68	14	6
Tb927.10.540	ATP-dependent DEAD/H RNA helicase, putative,DEAD box RNA helicase, putative	6	6,88	0	1
Tb927.10.12700	pyruvate dehydrogenase E1 alpha subunit, putative	6	14,29	12	4
Tb927.8.1500	hypothetical protein, conserved	6	6,99	0	4
Tb927.10.10930	heat shock protein, putative	6	39,77	0	0
Tb927.9.11270	T-complex protein 1, eta subunit, putative,t- complex protein 1 (eta subunit)	6	13,38	11	2
Tb927.2.470	retrotransposon hot spot protein 4 (RHS4), putative (3B10.190)	6	14,07	0	1
Tb927.3.3180	Nucleoporin (TbNup98)	6	54,33	0	1
Tb927.1.120	retrotransposon hot spot protein 4 (RHS4), putative	6	25,70	0	1
Tb927.9.5900	glutamate dehydrogenase (GDH)	6	19,86	2	9
Tb927.10.14180	protein transport protein Sec13, putative,cytosolic coat protein, putative	6	5,88	0	2
Tb927.2.2520	voltage-dependent anion-selective channel (25N14.5)	6	22,22	0	0
Tb927.11.17000	AIR9	6	13,55	11	4
Tb927.9.10770	Polyadenylate-binding protein 2 (Poly(A)-binding protein 2)	5	15,50	24	13
Tb927.11.1450	2-oxoglutarate dehydrogenase E1 component, putative	5	16,47	27	4
Tb927.11.9780	hypothetical protein, conserved	5	4,09	0	0
Tb927.9.12510	ATP-dependent DEAD/H RNA helicase, DED1	5	3,95	5	6
Tb927.6.4770	MKT1	5	4,63	2	5
Tb927.10.7570	dihydrolipoamide acetyltransferase E2 subunit, putative	5	8,65	8	3
Tb927.7.2300	Nucleoporin (TbNup132)	5	13,05	0	0
Tb927.2.1330	retrotransposon hot spot protein (RHS, pseudogene), putative	5	3,65	0	1
Tb927.8.1330	60S ribosomal protein L7a	5	10,14	13	8
Tb927.3.1790	pyruvate dehydrogenase E1 beta subunit, putative	5	11,78	7	2
Tb927.11.980	Nucleoporin (TbNup158)	5	30,39	0	0
Tb927.2.1170	retrotransposon hot spot protein 5 (RHS5), putative (25N24.105)	5	7,85	0	1
Tb927.10.7060	nucleoporin interacting component (NUP93), putative	5	13,36	0	0
Tb927.6.4280	glyceraldehyde 3-phosphate dehydrogenase, glycosomal (GAPDH)	5	29,81	0	10
Tb927.9.12550	glycerol kinase, glycosomal	5	8,40	9	12

	(glk1)				
Tb927.9.12570	glycerol kinase, glycosomal (glk1)	5	8,40	0	2
Tb927.9.12630	glycerol kinase, glycosomal (glk1)	5	8,40	0	0
Tb927.9.12610	glycerol kinase, glycosomal (glk1)	5	8,40	0	0
Tb927.11.13520	hypothetical protein, conserved	4	2,63	0	0
Tb927.6.2640	importin alpha subunit, putative (TbKap60)	4	6,00	4	4
Tb927.10.9650	hypothetical protein, conserved	4	10,95	0	0
Tb927.9.14240	Nucleoporin (Nup82)	4	21,14	0	0
Tb927.11.2950	Nucleoporin (TbNup89)	4	30,03	0	0
Tb927.5.1060	mitochondrial processing peptidase, beta subunit, putative	4	16,77	2	2
Tb927.11.11080	Nucleoporin (TbNup149)	4	37,46	0	0
Tb927.6.1870	eukaryotic translation initiation factor 4e, putative	4	6,56	2	3
Tb927.11.6280	pyruvate phosphate dikinase (PPDK)	4	10,19	23	4
Tb927.6.4090	chaperonin HSP60, mitochondrial precursor, putative (HSP60)	4	3,88	0	0
Tb927.11.11090	Nucleoporin (TbNup140)	4	2,46	0	0
Tb927.2.4230	NUP-1 protein, putative (28H13.465)	3	24,49	0	1
Tb927.2.240	retrotransposon hot spot protein 5 (RHS5), putative (3B10.75)	3	8,16	0	0
Tb927.2.380	retrotransposon hot spot protein 2 (RHS2), putative (3B10.145)	3	2,53	0	1
Tb927.1.190	retrotransposon hot spot protein (RHS, pseudogene), putative	3	2,41	0	0
Tb927.2.400	retrotransposon hot spot protein 2 (RHS2), putative (3B10.155)	3	2,53	0	0
Tb927.2.280	retrotransposon hot spot protein 2 (RHS2), putative (3B10.95)	3	2,53	0	0
Tb927.11.9980	2-oxoglutarate dehydrogenase E1 component, putative	3	25,67	36	3
Tb927.8.3950	hypothetical protein, conserved	3	8,21	0	0
Tb927.11.3120	nucleolar GTP-binding protein 1 (NOG1)	3	4,27	6	2
Tb927.7.2680	ZC3H22	3	12,15	0	0
Tb927.10.1510	NOT1 (NOT1)	3	3,44	0	3
Tb927.4.4940	hypothetical protein, conserved	3	5,08	0	0
Tb927.6.4440	RBP42 (binds energy metabolism RNA CDSs)	3	33,52	2	11
Tb927.9.1410	hypothetical protein, conserved	3	14,89	0	1
Tb927.8.4820	eukaryotic translation initation factor 4 gamma, putative	3	5,47	5	2
Tb927.11.6630	3-methylcrotonoyl-CoA carboxylase beta subunit, putative	2	27,29	0	0
Tb927.4.4310	Nucleoporin (TbNup64)	2	13,16	0	1
Tb927.11.3980	mitochondrial processing peptidase alpha subunit, putative	2	9,07	0	0
Tb927.10.14550	ATP-dependent DEAD/H RNA helicase, DBP1	2	21,36	3	10
Tb927.11.6600	hypothetical protein, conserved	2	6,04	0	0
Tb927.9.6460	hypothetical protein, conserved	2	4,21	0	0
Tb927.4.5350	3-methylcrotonyl-CoA carboxylase, pseudogene	2	15,63	0	0
Tb927.3.3300	hypothetical protein, conserved	2	3,56	0	2

Tb927.8.6970	3-methylcrotonyl-CoA carboxylase alpha subunit, putative	2	16,52	0	0
Tb927.8.7100	acetyl-CoA carboxylase, putative	1	24,90	0	1
Tb927.5.2530	hypothetical protein, conserved	1	1,83	0	0

Supplementary table 3: Proteins enriched in the *EP 3'-UTR* purification

Proteins detected by mass-spectrometry. The ratio obtained between peptides found in the 3SBPs-purification compared to the control purification (without SBPs) of four independent replicates (1 to 4) is shown in the table. The table is arranged by largest ratio.

Accession	Description	Replicates (ratio 3 SBPs/No SBP)			
		1	2	3	4
Tb927.1.2400	alpha tubulin	34,0	30,0	1,2	1,0
Tb927.7.710	heat shock 70 kDa protein, putative (HSP70.4)	1,0	1,0	34,0	0,1
Tb927.1.2390	beta tubulin	29,0	28,0	1,0	1,0
Tb927.11.5500	mitochondrial RNA binding protein 1 KRIPP1 (PPR)	13,0	1,0	1,0	1,0
Tb927.9.12610	glycerol kinase, glycosomal (glk1)	1,0	11,0	1,1	1,0
Tb927.11.9980	2-oxoglutarate dehydrogenase E1 component, putative	2,6	10,0	2,2	0,3
Tb927.4.4380	vacuolar-type proton translocating pyrophosphatase 1, putative (PPase1)	10,0	1,0	1,0	0,2
Tb927.9.4420	hypothetical protein, conserved	1,0	1,0	9,0	1,0
Tb927.5.4330	dihydrolipoamide branched chain transacylase, putative	1,0	1,0	9,0	1,0
Tb927.3.1010	hypothetical protein, conserved, Trypanosoma-specific	9,0	1,0	3,0	1,0
Tb927.11.3490	hypothetical protein, conserved, no domains, no good BLASTp matches	1,0	1,0	9,0	0,3
Tb927.8.2630	Kinesin KIN-C. Trypanosome-specific kinesin family 2	9,0	0,3	7,0	0,1
Tb927.9.6230	arginine kinase (AK)	8,3	1,2	5,7	0,8
Tb927.5.4040	Mitchondrial SSU ribosomal associated (coiled coil)	8,0	1,0	3,0	1,0
Tb927.4.3680	protein phosphatase 2C, putative	8,0	1,0	2,0	1,0
Tb927.3.3300	hypothetical protein, conserved	2,0	1,0	8,0	0,3
Tb927.9.13380	phosphoinositide-binding protein, putative	2,5	0,3	8,0	0,5
Tb927.2.4130	enoyl-CoA hydratase/Enoyl-CoA isomerase/3-hydroxyacyl-CoA dehydrogenase, putative	3,0	1,0	8,0	0,3
Tb927.2.2970	mitochondrial carrier protein, MCP13	8,0	3,0	1,3	0,3
Tb927.2.4400	Mitchondrial SSU ribosomal protein	5,0	0,6	8,0	0,1
Tb927.11.5060	Mitchondrial SSU ribosomal protein	7,0	1,0	1,0	1,0
Tb927.4.2080	CC2D is a FAZ-ER protein, also present on the basal bodies.	7,0	1,0	4,0	1,0
Tb927.11.7710	Gp63-1 surface protease homolog, putative	7,0	3,0	4,0	1,0
Tb927.9.9310	hypothetical protein, conserved	7,0	1,0	1,0	1,0
Tb927.1.1620	ATP-dependent DEAD/H RNA helicase, putative, no clear yeast homologue	1,0	1,0	7,0	1,0
Tb927.7.270	ribosome biogenesis protein, putative	7,0	1,0	6,0	1,0
Tb927.9.8820	hypothetical protein, conserved, no yeast	7,0	0,7	3,0	0,4

	or human match				
Tb927.10.10850	AGO1	7,0	1,0	5,0	0,3
Tb927.11.13520	hypothetical protein, conserved	1,0	1,0	7,0	0,3
Tb927.10.11760	PUF6	7,0	1,0	1,8	0,2
Tb927.11.14730	metalloprotease, putative,cell division protein FtsH homologue, putative	5,0	1,0	7,0	0,1
Tb927.9.12200	60S ribosomal protein L31	6,0	5,0	1,0	1,7
Tb927.7.3050	Mitchondrial SSU ribosomal associated	6,0	1,0	1,0	1,0
Tb927.2.4550	FtsJ cell division protein, putative (30M24.305)	4,0	1,0	6,0	1,0
Tb927.11.14980	Mitchondrial LSU ribosomal protein	6,0	1,0	1,0	1,0
Tb927.10.12510	P-type H -ATPase, putative	6,0	1,3	1,4	1,0
Tb927.9.12730	chaperone protein DNAj, endoplasmatic reticulum	1,5	1,0	6,0	1,0
Tb927.8.6390	lysophospholipase, putative,alpha/beta hydrolase, putative (TbLysoPLA)	1,0	1,0	6,0	1,3
Tb927.2.5530	POMP22	6,0	1,0	1,0	1,0
Tb927.6.1250	Mitchondrial SSU ribosomal protein S29	6,0	1,0	1,0	1,0
Tb927.5.2530	hypothetical protein, conserved	3,0	1,0	6,0	1,0
Tb927.3.3130	POMP24	6,0	3,0	1,1	0,4
Tb927.4.5350	3-methylcrotonyl-CoA carboxylase, pseudogene	1,7	1,0	6,0	0,2
Tb927.11.11460	POMP9	5,5	4,0	2,0	0,1
Tb927.7.6090	hypothetical protein, conserved	5,0	1,0	1,0	1,0
Tb927.10.3840	60S ribosomal protein L18	1,0	1,0	1,0	5,0
Tb927.8.2160	multidrug resistance protein A,p-glycoprotein (PGPA)	5,0	1,0	1,7	1,0
Tb927.11.6510	40S ribosomal protein S21	1,0	1,0	1,0	5,0
Tb927.11.6000	Mitchondrial LSU ribosomal protein	5,0	1,0	1,0	1,0
Tb927.4.4210	ATP-dependent zinc metallopeptidase, putative,metallo-peptidase, Clan MA(E) Family M41	1,0	1,0	5,0	1,0
Tb927.1.2670	flagellar protein PF16	5,0	4,0	5,0	1,0
Tb927.2.2950	hypothetical protein, conserved	5,0	1,0	1,0	1,0
Tb927.10.7090	alternative oxidase (AOX)	5,0	1,0	1,0	1,0
Tb927.2.5980	ATP-dependent Clp protease subunit, heat shock protein 104 (HSP104)	5,0	1,0	1,2	1,0
Tb927.9.6920	hypothetical protein, conserved	3,0	1,0	5,0	1,0
Tb927.4.4670	hypothetical protein, conserved	5,0	1,0	1,3	1,0
Tb927.11.9080	hypothetical protein, conserved	4,0	1,0	5,0	1,0
Tb927.1.2570	coatomer beta subunit (beta-coP)	1,0	1,0	5,0	1,0
Tb927.1.1700	hypothetical protein, conserved	1,0	1,0	5,0	1,0
Tb927.10.14330	hypothetical protein, conserved	1,0	1,0	5,0	1,0
Tb927.6.1020	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (Rhodesain)	1,0	1,0	1,0	5,0
Tb927.7.3970	hypothetical protein, conserved	1,0	1,0	5,0	1,0
Tb927.7.5110	hypothetical protein, conserved	5,0	1,0	1,0	1,0
Tb927.9.12070	hypothetical protein, conserved	1,0	1,0	5,0	1,0
Tb927.9.15330	hypothetical protein, conserved	1,0	1,0	5,0	1,0
Tb927.9.7540	calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative	5,0	1,0	1,0	1,0
Tb927.7.1790	Adenine phosphoribosyltransferase, putative	1,0	1,0	1,0	5,0

Tb927.10.15650	tRNA pseudouridine synthase A-like protein	5,0	1,0	1,0	1,0
Tb927.10.180	ATP synthase F1 subunit gamma protein, putative	1,7	5,0	1,8	0,8
Tb927.11.510	UBP2	5,0	4,0	0,8	1,3
Tb927.7.5930	Protein Associated with Differentiation (TbPAD1)	1,7	5,0	1,7	0,6
Tb927.10.8170	Nucleoporin (NUP155)	1,7	1,1	5,0	0,5
Tb927.4.1850	hypothetical protein, conserved	5,0	1,0	0,3	1,0
Tb927.11.10370	glycosyl hydrolase-like protein	1,3	1,0	5,0	0,3
Tb927.3.2050	hypothetical protein, conserved, no domains, poly(Q) tracts, K-specific	5,0	1,0	2,0	0,3
Tb927.7.4290	Nuclear migration protein NudC (animals), 46% identity	5,0	1,0	2,0	0,3
Tb927.8.1420	acyl-CoA dehydrogenase, mitochondrial precursor, putative	3,5	1,0	5,0	0,3
Tb927.4.1540	POMP27, NAD-dependent epimerase, aldehyde reductase, one putative TM domain	5,0	0,3	1,0	0,2
Tb927.3.3150	hypothetical protein, conserved	5,0	1,0	3,0	0,2
Tb927.6.1430	hypothetical protein, conserved	5,0	1,0	1,8	0,2
Tb927.7.6850	trans-sialidase (TbTS)	5,0	1,0	1,0	0,1
Tb927.7.4500	hypothetical protein, conserved	5,0	5,0	2,3	0,1
Tb927.6.2010	hypothetical protein, conserved	4,7	1,0	2,5	0,3
Tb927.5.2930	Mitochondrial ATP synthase subunit, putative	4,3	0,8	2,2	0,8
Tb927.8.7100	acetyl-CoA carboxylase, putative	1,6	1,0	4,2	0,8
Tb927.7.3940	mitochondrial carrier protein, MCP16	3,0	4,0	1,7	1,7
Tb927.8.5200	Mitchondrial SSU ribosomal associated	1,0	1,0	4,0	1,0
Tb927.11.3120	nucleolar GTP-binding protein 1 (NOG1)	4,0	1,0	1,0	1,0
Tb927.2.3180	TbPPR1 mitochondrial RNA binding protein 1	4,0	1,0	1,0	1,0
Tb927.10.1100	60S ribosomal protein L9	4,0	1,0	1,0	2,0
Tb927.10.380	PPR5 mitochondrial RNA binding protein 1	4,0	1,0	1,0	1,0
Tb927.9.2900	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.8.3110	Mitchondrial SSU ribosomal protein S9	4,0	1,0	1,0	1,0
Tb927.2.5240	PRP19-like protein, putative (TbPRP19)	4,0	1,0	1,0	1,0
Tb927.3.970	Mitchondrial SSU ribosomal protein	4,0	1,0	1,0	1,0
Tb927.8.3170	Mitchondrial LSU ribosomal protein	4,0	1,0	1,0	1,0
Tb927.7.7450	GTP-binding protein, putative	3,0	1,0	4,0	1,0
Tb927.5.1790	Mitchondrial SSU ribosomal protein	4,0	1,0	1,0	1,0
Tb927.4.3300	mitochondrial ATP-dependent zinc metallopeptidase, putative	4,0	1,0	3,0	1,0
Tb927.11.15850	kinetoplast poly(A) polymerase complex 1 subunit	4,0	1,0	1,0	1,0
Tb927.4.760	AP-1 adapter complex gamma subunit, putative	1,0	1,0	4,0	1,0
Tb927.11.8870	mitochondrial DEAD box protein,KREH1 (KREH1)	4,0	1,0	1,3	1,0
Tb927.3.3460	hypothetical protein, conserved (SDH5 in T cruzi)	4,0	1,0	3,0	1,0
Tb927.10.8270	eIF3 subunit 8, putative	4,0	1,0	3,0	1,0
Tb927.3.1910	hypothetical protein, conserved, histone RNA binding domain 259-326	2,0	1,0	4,0	1,0
Tb927.11.10560	eIF4G4	1,0	1,0	4,0	1,0
Tb927.10.13730	60S ribosomal protein L7	4,0	1,0	1,0	1,0

Tb927.10.14700	hypothetical protein, conserved, no human or yeast homologue	1,0	1,0	4,0	3,0
Tb927.3.4600	hypothetical protein, conserved	4,0	1,0	1,0	1,0
Tb927.7.4220	hypothetical protein, conserved	4,0	1,0	1,0	1,0
Tb927.10.70	retrotransposon hot spot (RHS), putative, (fragment)	4,0	1,0	1,3	1,0
Tb927.7.2410	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.10.9810	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.10.5760	adenylate kinase, putative	1,0	1,0	1,0	4,0
Tb927.10.14320	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.10.1540	ZC3H30	1,0	1,0	4,0	1,0
Tb927.10.2550	malate dehydrogenase-related	1,0	1,0	4,0	1,0
Tb927.10.9660	CRN/SYF3 part of splicing PRP19 complex, putative	4,0	1,0	1,0	1,0
Tb927.11.12880	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.11.14970	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.11.4700	prostaglandin f synthase	1,0	1,0	4,0	1,0
Tb927.11.9530	14-3-3-I protein	1,0	1,0	1,0	4,0
Tb927.4.1790	60S ribosomal protein L3	4,0	1,0	1,0	1,0
Tb927.6.2550	TRRM2	1,0	1,0	4,0	1,0
Tb927.7.2390	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.8.1980	hypothetical protein, conserved	1,0	1,0	4,0	1,0
Tb927.9.11850	structural maintenance of chromosome 1 (SMC1)	1,0	1,0	4,0	1,0
Tb927.10.5880	Proteophosphoglycan, putative	3,0	1,0	4,0	1,0
Tb927.7.5340	hypothetical protein, conserved	3,0	1,0	4,0	1,0
Tb927.6.1900	U3/U14 snoRNA-associated small subunit rRNA processing protein, putative	4,0	1,0	1,5	1,0
Tb927.5.1580	ZC3H13	1,8	4,0	3,0	1,0
Tb927.3.1940	hypothetical protein, possible component of cytochorome oxidase complex	4,0	1,0	3,0	1,0
Tb927.11.9710	60S ribosomal protein L10a	1,1	0,8	4,0	1,0
Tb927.9.13990	DRBD2	4,0	1,0	1,3	0,8
Tb927.3.5340	Hsc70-interacting protein (Hip), putative	1,0	1,0	0,8	4,0
Tb927.8.1890	cytochrome c1	4,0	0,8	1,5	1,0
Tb927.4.2000	ruvB-like DNA helicase, putative,ATP-dependent DNA helicase, putative	4,0	1,0	0,7	1,0
Tb927.9.14160	rieske iron-sulfur protein, mitochondrial precursor (RISP)	1,6	4,0	1,6	0,6
Tb927.8.3150	T-complex protein 1, gamma subunit, putative (TCP-1-gamma)	4,0	0,8	0,5	0,5
Tb927.11.2600	hypothetical protein, conserved	3,0	1,0	4,0	0,3
Tb927.6.1470	hypothetical protein, conserved	4,0	1,0	1,0	0,3
Tb927.7.5820	Monooxygenase, putative	1,5	1,0	4,0	0,3
Tb927.2.3370	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase, putative (10C8.485)	3,0	1,0	4,0	0,3
Tb927.9.15460	calcium motive p-type ATPase, putative	1,7	0,3	4,0	0,3
Tb927.1.2990	PPR2	4,0	1,0	1,0	0,3
Tb927.2.100	retrotransposon hot spot protein 1 (RHS1), putative	4,0	1,0	0,3	1,0
Tb927.11.4320	hypothetical protein, conserved, PF02622 DUF179	1,0	1,0	4,0	0,3
Tb927.10.12660	PUF2	0,3	1,0	4,0	1,0
Tb927.11.15760	GPI transamidase subunit Tta1 (TTA1)	4,0	1,0	1,0	0,3

Tb927.7.3550	hypothetical protein, conserved, no yeast or human match	2,0	1,0	4,0	0,3
Tb927.5.780	hypothetical protein, conserved	1,0	1,0	4,0	0,3
Tb927.6.4210	aldehyde dehydrogenase, putative (ALDH)	4,0	1,0	0,3	0,3
Tb927.3.3940	DRBD11	1,2	4,0	0,8	0,2
Tb927.10.5770	valosin-containing protein homolog,Transitional endoplasmic reticulum ATPase, putative (VCP)	4,0	1,0	0,3	0,2
Tb927.8.650	cation-transporting ATPase, putative	3,0	1,0	4,0	0,1
Tb927.7.990	chaperone protein DNAj, putative	3,7	1,0	1,0	0,1
Tb927.6.4320	hypothetical protein, conserved	3,5	1,0	2,3	0,4
Tb927.10.3990	DHH1	3,5	0,3	1,0	1,0
Tb927.11.1250	kinetoplast poly(A) polymerase complex 1 subunit, MIT ssu-associated	3,5	1,0	1,0	0,2
Tb927.10.10920	heat shock protein 83, HSP83	3,4	0,7	0,8	1,0
Tb927.7.2670	ZC3H21	2,3	1,0	3,3	0,3
Tb927.9.11120	Mitchondrial SSU ribosomal associated	3,0	1,0	1,0	1,0
Tb927.11.540	ABC transporter, putative	3,0	1,0	1,0	1,0
Tb927.9.7170	Mitchondrial LSU ribosomal protein	3,0	1,0	1,0	1,0
Tb927.10.6850	MItochondrial SSU S18 or kinetoplast poly(A) polymerase complex 1 subunit	3,0	1,0	1,0	1,0
Tb927.11.15430	U5 small nuclear ribonucleoprotein U5-116K, putative	3,0	1,0	1,0	1,0
Tb927.7.5230	lanosterol synthase	3,0	1,0	1,0	1,0
Tb927.10.1450	hypothetical protein, conserved, in bilobe	1,0	1,0	3,0	1,0
Tb927.3.4920	LETM1 and EF-hand domain-containing protein 1, inner mitochondrial membrane?	3,0	1,0	1,0	1,0
Tb927.11.11690	vacuolar ATP synthase subunit b, putative,v- ATPase B subunit,vacuolar proton pump B subunit	1,5	1,0	3,0	1,0
Tb927.5.1510	Mitchondrial SSU ribosomal protein	3,0	1,0	1,0	1,0
Tb927.9.5840	tryparedoxin peroxidase (TRYP1)	1,0	1,0	1,0	3,0
Tb927.11.6170	protein transport protein Sec31, putative,cytosolic coat protein, putative	1,0	1,0	3,0	1,0
Tb927.11.9940	hypothetical protein, conserved	1,0	1,0	1,0	3,0
Tb927.9.8070	60S ribosomal protein L10	2,2	1,0	3,0	1,8
Tb927.11.10150	Mitchondrial SSU ribosomal protein	3,0	1,0	1,0	1,0
Tb927.7.2400	MCP1 Arc1p homologue, assists aa tRNA synthetases	1,0	1,0	1,0	3,0
Tb927.7.4310	hypothetical protein, conserved	3,0	1,0	1,0	1,0
Tb927.6.4560	Mitchondrial SSU ribosomal protein	3,0	1,0	1,0	1,0
Tb927.8.7650	amino acid transporter, putative	1,0	1,0	1,0	3,0
Tb927.9.15380	NADH-ubiquinone oxidoreductase complex I subunit, putative,NDUFA9 subunit, putative	1,0	1,0	3,0	1,0
Tb927.10.560	40S ribosomal protein S11	1,0	1,0	1,0	3,0
Tb927.8.560	GEM1, putative	1,5	1,0	3,0	1,0
Tb927.11.4910	hypothetical protein, conserved,predicted ankyrin repeat family protein	1,0	1,0	1,0	3,0
Tb927.9.6510	Mitchondrial SSU ribosomal associated	3,0	1,0	1,0	1,0
Tb927.11.5400	signal recognition particle 54 kDa (SRP54)	3,0	1,0	1,3	1,0
Tb927.10.10360	microtubule-associated protein, putative	1,0	3,0	1,0	1,0
Tb927.11.11480	Trichohyalin, putative	1,0	1,0	3,0	1,0
Tb927.10.470	choline dehydrogenase, putative	3,0	1,0	3,0	1,0

Tb927.11.7290	pantothenate kinase subunit, putative	1,0	1,0	3,0	1,0
Tb927.1.2410	beta tubulin, pseudogene	3,0	1,0	1,0	1,0
Tb927.10.6320	NOC3, nuclear export of ribosomes, assoc with NRG1	1,0	1,0	3,0	1,0
Tb927.10.13510	zinc metallopeptidase, putative	3,0	1,0	3,0	1,0
Tb927.9.9630	hypothetical protein, conserved	1,0	3,0	1,3	1,0
Tb927.11.5970	phosphoinositide-specific phospholipase C, putative	3,0	1,0	1,0	1,0
Tb927.7.4970	glutamine synthetase, putative	1,0	1,0	1,0	3,0
Tb927.9.2590	hypothetical protein, conserved	1,0	3,0	1,0	1,0
Tb927.3.2600	ATP-dependent DEAD/H RNA helicase, putative, no clear yeast homologue	1,0	1,0	3,0	1,0
Tb927.3.3580	lipophosphoglycan biosynthetic protein, putative (LPG3)=GRP94 ER chaperone	1,0	1,0	3,0	1,0
Tb927.11.1680	vesicular-fusion protein SEC18, putative	3,0	1,0	1,0	1,0
Tb927.10.14510	hypothetical protein, conserved	3,0	1,0	1,0	1,0
Tb927.7.3980	immunodominant antigen, putative,tc40 antigen-like	1,0	1,0	3,0	1,0
Tb927.7.570	prefoldin, putative	1,0	1,0	1,0	3,0
Tb927.7.6460	FG-GAP repeat protein, putative,intergrin alpha chain protein, putative	1,0	1,0	3,0	1,0
Tb927.9.7110	hypothetical protein, conserved	1,0	1,0	1,0	3,0
Tb927.2.1080	retrotransposon hot spot protein 5 (RHS5), putative	1,0	3,0	1,0	1,0
Tb927.4.410	CAF40	3,0	1,0	1,3	1,0
Tb927.10.6630	ATP-dependent DEAD/H RNA helicase HEL64, putative (cytosolic)	3,0	1,0	1,3	1,0
Tb927.11.8990	cation transporter, putative	3,0	1,0	3,0	1,0
Tb927.10.4610	dolicholphosphate-mannose synthase, putative	1,0	1,0	1,0	3,0
Tb927.11.14190	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.11.3130	glycosomal transporter (GAT2)	3,0	1,0	1,0	1,0
Tb927.2.2210	hypothetical protein, conserved	3,0	1,0	1,0	1,0
Tb927.7.1290	hypothetical protein, conserved.	3,0	1,0	1,0	1,0
Tb927.9.2450	electron transport protein SCO1/SCO2, putative	1,0	1,0	3,0	1,0
Tb927.11.1840	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.11.370	repressor activator protein 1 (RAP1)	3,0	1,0	1,0	1,0
Tb927.3.4680	RAB GDP dissociation inhibitor alpha, putative	1,0	1,0	3,0	1,0
Tb927.4.5110	KKT8 Kinetochore protein	1,0	1,0	3,0	1,0
Tb927.8.3970	oxidoreductase, putative	1,0	1,0	3,0	1,0
Tb927.10.15410	glycosomal malate dehydrogenase (gMDH)	1,0	1,0	1,0	3,0
Tb927.9.8160	chaperone protein DNAj, putative	3,0	1,0	3,0	1,0
Tb927.11.530	RBP3	3,0	1,0	1,0	1,0
Tb927.11.13090	EF1 gamma, elongation factor 1 gamma, putative	1,0	1,0	1,2	3,0
Tb927.9.5040	cAMP-specific phosphodiesterase (PDEB1)	1,0	1,0	3,0	1,0
Tb927.11.9570	hypothetical protein, conserved	3,0	1,0	3,0	1,0
Tb927.11.16730	dihydrolipoyl dehydrogenase (GCVL-2)	2,5	1,0	1,8	3,0
Tb927.8.1150	KKT9 Kinetochore protein	3,0	1,0	1,5	1,0
Tb10.v4.0052	microtubule-associated protein 2s	3,0	1,0	1,0	1,0
Tb927.1.2730	hypothetical protein, conserved	1,0	1,0	3,0	1,0

Tb927.10.12860	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.10.14630	fibrillarin, putative	1,0	1,0	3,0	1,0
Tb927.10.3010	hypothetical protein, conserved, in bilobe	1,0	1,0	3,0	1,0
Tb927.10.7500	fibrillarin (NOP1)	1,0	1,0	3,0	1,0
Tb927.10.7810	Homologue of yeast ESF1,nucleolar protein involved in pre-rRNA processing	1,0	1,0	3,0	1,0
Tb927.10.8870	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.10.9780	ATP-dependent DEAD/H RNA helicase, putative Mak5, ribosome biogenesis?	1,0	1,0	3,0	1,0
Tb927.10.9890	hypothetical protein, conserved, no domains	3,0	1,0	1,0	1,0
Tb927.11.12830	acyl-CoA binding protein, putative	1,0	1,0	3,0	1,0
Tb927.11.3550	NPA3/XAB1 homologue, interacts with XPA and with RNA pol II	1,0	1,0	3,0	1,0
Tb927.11.9490	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.2.5210	3-oxoacyl-ACP reductase, putative	1,0	1,0	3,0	1,0
Tb927.3.2490	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.3.5020	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.3.5250	ZC3H8	1,0	1,0	3,0	1,0
Tb927.4.3670	hypothetical protein, conserved	3,0	1,0	1,0	1,0
Tb927.4.920	hypothetical protein, conserved	1,0	1,0	1,0	3,0
Tb927.5.2500	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.5.980	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.6.2140	Mitochondrial RNA binding complex I component, putative	1,0	1,0	3,0	1,0
Tb927.7.1780	Adenine phosphoribosyltransferase, putative	1,0	1,0	1,0	3,0
Tb927.7.4440	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.7.6620	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.7.700	hypothetical protein, conserved	1,0	1,0	3,0	1,0
Tb927.8.3310	acetyltransferase, putative	1,0	1,0	3,0	1,0
Tb927.8.5040	hypothetical protein, conserved	3,0	1,0	1,0	1,0
Tb927.8.7170	inositol polyphosphate 1-phosphatase, putative	1,0	1,0	3,0	1,0
Tb927.9.8680	cytochrome c oxidase assembly factor, putative	3,0	1,0	1,0	1,0
Tb927.9.4910	protein kinase, putative	3,0	1,0	1,0	1,0
Tb927.10.1490	Possible splicing factor	1,0	1,0	3,0	1,0
Tb927.5.1460	hypothetical protein, conserved	1,0	3,0	1,0	1,0
Tb927.4.1300	Amidinotransferase superfamily protein	1,0	3,0	1,2	2,0
Tb927.11.760	protein phosphatase 2C, putative	1,0	1,0	1,0	3,0
Tb927.10.4560	EF2 elongation factor 2	3,0	1,6	0,9	1,0
Tb927.11.8060	hypothetical protein, conserved	1,0	1,0	3,0	0,8
Tb927.2.4090	hypothetical protein, conserved	3,0	1,0	3,0	0,8
Tb927.10.2730	hypothetical protein, conserved	3,0	1,0	0,7	0,8
Tb927.8.5460	flagellar calcium-binding protein,44 kDa calflagin, (Tb-44)	2,3	3,0	1,0	0,7
Tb927.6.4000	small glutamine-rich tetratricopeptide repeat protein, putative	3,0	1,0	0,4	3,0
Tb927.9.8950	CAAX prenyl protease 1, putative,metallo-peptidase, Clan M- Family M48	1,3	3,0	3,0	0,3
Tb927.3.4580	hypothetical protein, conserved	3,0	1,0	3,0	0,3
Tb927.8.1190	hypothetical protein, conserved	3,0	1,0	0,3	1,0
Tb927.10.4880	hypothetical protein, possible component	1,0	0,3	3,0	1,0

	of cytochorome oxidase complex				
Tb927.7.5700	pATOM36	1,7	0,3	1,0	3,0
Tb927.7.3500	glutathione-S-transferase/glutaredoxin, putative	3,0	0,3	0,8	3,0
Tb927.11.11590	eIF3E eukaryotic translation initiation factor, putative	3,0	0,3	0,8	1,0
Tb927.6.2300	adenosine kinase, putative	3,0	1,0	0,8	0,3
Tb927.11.12230	heat shock protein HslU2, in mitochondrion	3,0	0,3	1,6	1,0
Tb927.11.870	Mitchondrial LSU ribosomal protein	3,0	1,0	1,0	0,3
Tb927.4.1930	EIF3D	1,0	1,0	0,3	3,0
Tb927.4.1270	ruvB-like DNA helicase, putative	3,0	1,0	0,7	0,3
Tb927.9.13520	hypothetical protein, conserved	3,0	1,0	0,3	1,0
Tb927.5.3010	MRB complex protein	1,0	1,0	3,0	0,3
Tb927.11.9780	hypothetical protein, conserved	3,0	0,3	2,7	0,3
Tb927.10.9900	ABC1 protein, putative	1,0	1,0	3,0	0,3
Tb927.2.2440	proteasome regulatory non-ATPase subunit 6 (RPN6)	3,0	1,0	0,3	0,3
Tb927.10.4850	hypothetical protein, conserved	3,0	1,0	1,0	0,3
Tb927.9.5890	solanesyl-diphosphate synthase, putative (28G16.440)	3,0	0,3	1,2	0,3
Tb927.3.2230	succinyl-CoA synthetase alpha subunit, putative	3,0	0,3	0,7	1,7
Tb927.6.5070	hypothetical protein, conserved	3,0	1,0	2,2	0,3
Tb927.11.14250	T-complex protein 1, epsilon subunit, putative (TCP-1-epsilon)	3,0	1,0	0,9	0,3
Tb927.11.3240	T-complex protein 1, zeta subunit, putative (TCP-1-zeta)	3,0	0,5	1,1	0,2
Tb927.9.2650	POMP2	3,0	1,0	1,0	0,2
Tb927.10.520	Mitochondrial ATP synthase subunit, putative	2,0	1,0	3,0	0,2
Tb927.11.5560	hypothetical protein, conserved	1,3	3,0	0,8	0,2
Tb927.10.2770	eIF5	3,0	1,0	0,2	1,0
Tb927.11.13230	TbVAP, flagellar attachment zone	3,0	1,0	1,0	0,2
Tb927.10.8080	hypothetical protein, conserved	3,0	1,0	1,0	0,2
Tb927.11.10140	hypothetical protein, possible component of cytochorome oxidase complex	3,0	1,0	3,0	0,2
Tb927.9.8740	DRBD3 (PTB1)	3,0	0,2	1,1	1,5
Tb927.4.5200	nucleoporin (NUP54/57, TbNup62)	1,3	1,0	3,0	0,2
Tb927.11.1980	ZC3H41	3,0	0,2	0,7	0,2
Tb927.9.4190	fatty acyl CoA syntetase 1 (ACS1)	1,0	3,0	1,3	0,1
Tb927.5.520	stomatin-like protein, putative	2,8	0,2	2,0	0,2
Tb927.4.590	hypothetical protein, quinoprotein alchohol dehydrogenase-like domain	2,8	0,4	0,9	0,1
Tb927.11.7380	glycerol-3-phosphate dehydrogenase (FAD-dependent), mitochondrial	2,8	1,0	2,0	0,1
Tb927.8.1270	hypothetical protein, conserved	2,0	0,3	2,7	0,3
Tb927.1.4100	cytochrome oxidase subunit IV (COXIV)	2,7	1,2	2,3	0,2
Tb927.11.7460	BiP	1,2	2,7	0,7	0,1
Tb927.11.1450	2-oxoglutarate dehydrogenase E1 component, putative	2,4	2,0	2,7	1,0
Tb927.9.5900	glutamate dehydrogenase (GDH)	2,6	1,0	0,9	0,8
Tb927.3.3560	hypothetical protein, conserved	2,5	1,0	1,0	1,0
Tb927.11.3300	hypothetical protein, conserved, HA tagged is in mitochondrion, poly(Q), rich in E, K.	1,0	1,0	2,5	1,0
Tb927.9.5320	nucleolar RNA binding protein, putative	2,5	1,0	2,0	1,0

	(28G16.220)				
Tb927.11.10690	hypothetical protein, conserved	2,5	1,0	0,8	0,6
Tb927.11.2650	heat shock protein 84, putative, mit HSp90	2,5	0,7	1,0	0,4
Tb927.5.1520	heat shock protein HslU1, in mitochondrion	2,5	1,0	1,2	0,3
Tb927.8.6580	succinate dehydrogenase flavoprotein, SDH1	2,5	1,0	1,0	0,3
Tb927.4.1920	GPI transamidase, putative (TbGPI16)	2,5	1,0	1,0	0,3
Tb927.7.2640	hypothetical protein, conserved	2,5	0,2	1,0	0,3
Tb927.6.1870	eIF4E4	2,5	0,3	1,2	0,2
Tb927.5.3400	calcium-translocating P-type ATPase, calcium pump	2,1	1,1	2,4	0,7
Tb927.8.1740	Mitochondrial tRNA import	2,4	1,0	1,2	0,1
Tb927.10.4430	PUF1	2,4	0,4	1,3	0,1
Tb927.10.12840	mitochondrial carrier protein, MCP12	1,7	1,4	1,3	2,3
Tb927.9.2620	hypothetical protein, conserved	2,3	1,0	1,0	1,0
Tb927.11.4980	ATP-dependent DEAD/H RNA helicase, putative Dbp9, ribosome assembly?	2,3	1,0	1,5	1,0
Tb927.1.1560	vesicular-fusion protein nsf, putative,N-ethylmaleimide sensitive factor (NsF)	1,0	1,0	2,3	1,0
Tb927.10.510	LOK1=POMP19 Mitochondrial membrane formation	2,3	1,0	0,3	0,3
Tb927.10.8190	T-complex protein 1, theta subunit, (TCP-1-theta)	2,3	1,0	0,8	0,3
Tb927.7.2240	hypothetical protein, conserved	2,3	1,0	0,6	0,3
Tb927.10.1060	T-complex protein 1, delta subunit, putative (TCP-1-delta)	2,3	0,3	0,9	0,3
Tb927.11.15990	Nucleoporin (TbNup109)	2,3	0,8	0,8	1,3
Tb927.4.3950	cytoskeleton-associated protein CAP5.5, putative,cysteine peptidase	1,8	1,1	2,3	0,1
Tb927.6.2790	L-threonine 3-dehydrogenase, putative	1,0	0,4	1,0	2,2
Tb927.5.1210	short-chain dehydrogenase, putative	2,2	0,9	1,3	1,5
Tb927.8.890	small GTP-binding protein Rab1, putative	1,0	1,0	1,0	2,0
Tb927.3.5240	Mitchondrial SSU ribosomal associated KRIPP8 (PPR)	2,0	1,0	1,0	1,0
Tb927.3.5610	ribosomal protein L3 mitochondrial, putative	2,0	1,0	1,0	1,0
Tb927.10.15390	kinesin, clathrin-associated, Trypanosome-specific kinesin family 2	2,0	1,0	1,0	1,0
Tb927.5.4420	nucleolar RNA helicase 2 human, DDX50 Mouse	2,0	1,0	1,1	1,0
Tb927.3.4720	dynamin, putative,vacuolar sortin protein 1, putative	1,2	1,0	2,0	1,0
Tb927.6.3890	replication factor C, subunit 2, putative	1,0	1,0	2,0	1,0
Tb927.3.1710	Mitchondrial LSU ribosomal protein	2,0	1,0	1,0	1,0
Tb927.11.13470	hypothetical protein, conserved	1,0	1,0	2,0	1,0
Tb927.10.14180	protein transport protein Sec13, putative	2,0	0,9	0,9	1,0
Tb927.2.2130	small GTP-binding protein RAB6, putative (25N14.200)	2,0	1,0	0,8	1,2
Tb927.9.4680	eIF4A1	2,0	1,3	0,7	1,4
Tb927.10.2320	hypothetical protein, conserved	2,0	0,8	0,7	1,0
Tb927.10.14160	aquaporin 3, putative	2,0	0,7	0,7	1,0
Tb927.11.3790	hypothetical protein, conserved	0,6	0,9	1,0	2,0
Tb927.10.540	ATP-dependent DEAD/H RNA helicase, DDX39-like	2,0	0,6	1,3	0,6
Tb927.11.15370	hypothetical protein, conserved (TbKap123)	2,0	2,0	0,9	0,4

Tb927.8.4500	eIF4G5	2,0	1,0	1,3	0,3
Tb927.10.11220	procyclic form surface glycoprotein (PSSA-2)	2,0	1,0	1,0	0,3
Tb927.5.890	oligosaccharyl transferase subunit, putative	2,0	0,3	1,0	0,3
Tb927.10.12330	ZC3H34	2,0	0,3	1,0	1,0
Tb927.8.1870	Golgi/lysosome glycoprotein 1 (tGLP1)	2,0	1,7	1,8	0,3
Tb927.10.15310	hypothetical protein, conserved, linked to melarsoprol resistance, no yeast/human by NCBI	0,3	0,3	2,0	0,7
Tb927.5.1300	vacuolar proton translocating ATPase subunit A, putative	2,0	1,0	1,0	0,3
Tb927.3.3270	ATP-dependent phosphofructokinase (TbPFK)	2,0	0,3	1,0	0,3
Tb927.6.3630	Sphingosine-1-phosphate lyase	1,6	1,2	2,0	0,3
Tb927.11.4210	hypothetical protein, conserved	2,0	1,0	0,3	0,3
Tb927.6.1550	POMP30 leucine-rich repeat protein (LRRP)	1,0	1,0	2,0	0,3
Tb927.11.6600	hypothetical protein, conserved	1,7	0,3	2,0	1,0
Tb927.9.11220	Mitochondrial tRNA import	1,5	1,0	2,0	0,2
Tb927.8.8050	Nucleoporin (TbNup75)	2,0	0,8	1,6	0,2
Tb927.11.10760	KIN-D kinesin, associated with sub-pellicular microtubules, essential	2,0	1,0	2,0	0,2
Tb927.10.2240	NTF2-like domain, in Y14 TAP	2,0	1,0	0,7	0,1
Tb11.02.5420	NADPH--cytochrome p450 reductase, putative (CPR)	2,0	1,0	1,0	0,1
Tb927.10.2900	importin beta-1 subunit, putative	2,0	2,0	1,4	0,1
Tb927.4.2850	hypothetical protein, conserved	1,0	1,0	2,0	0,1
Tb927.9.2470	nucleolar protein (NOP86)	1,4	1,1	2,0	0,1
Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putative	1,9	1,1	0,6	1,0
Tb927.7.2680	ZC3H22	1,9	1,0	1,2	0,2
Tb927.8.7480	hypothetical protein, conserved, no clear yeast or human matches	1,9	1,0	1,5	1,0
Tb927.5.1060	mitochondrial processing peptidase, beta subunit, MMPbeta	1,9	0,7	1,2	0,9
Tb927.11.7780	POMP16	1,9	0,8	0,7	0,8
Tb927.7.7430	ATP synthase F1, alpha subunit	1,9	1,2	1,4	1,0
Tb927.9.10310	mitochondrial carrier protein, MCP11	1,9	1,0	1,4	1,3
Tb927.10.14780	protein kinase, putative,mitogen-activated protein kinase kinase kinase, putative	1,0	1,0	1,9	0,3
Tb927.9.9290	PABP1	1,9	0,8	1,1	0,3
Tb927.7.5020	60S ribosomal protein L19	1,2	1,1	0,9	1,8
Tb927.11.16760	T-complex protein 1, alpha subunit, putative (TCP-1-alpha)	1,8	0,8	0,9	0,7
Tb927.11.11680	2-oxoglutarate dehydrogenase E2 component, putative	1,3	1,8	1,4	1,3
Tb927.10.12960	ras-related protein rab-5,small GTPase, putative (RAB5A)	1,3	1,0	1,8	1,1
Tb927.11.6210	sterol 14-alpha-demethylase (CYP51)	1,8	1,0	0,6	0,4
Tb927.9.1380	hypothetical protein, conserved	0,3	0,3	1,8	1,0
Tb927.9.6460	hypothetical protein, conserved	1,8	0,8	1,6	0,4
Tb927.10.6640	COP-coated vesicle membrane protein erv25 precursor,	1,0	1,0	1,0	1,8
Tb927.11.11360	guanine nucleotide-binding protein beta subunit- like protein (TRACK)	1,1	0,8	0,9	1,8
Tb927.8.2460	hypothetical protein, conserved	0,3	1,0	1,8	0,8

Tb927.10.13800	hypothetical protein, conserved, no yeast or human match	1,2	0,3	1,8	0,2
Tb927.6.3050	aldehyde dehydrogenase family, putative	1,8	1,0	1,8	0,1
Tb927.10.11390	60S ribosomal protein L6	1,2	0,8	1,0	1,7
Tb927.10.14710	40S ribosomal protein S2	1,1	1,1	1,0	1,7
Tb927.8.4820	eIF4G3	1,7	1,6	1,0	0,2
Tb927.10.600	Mitchondrial LSU ribosomal protein	1,7	1,0	1,0	1,0
Tb927.10.3640	hypothetical protein, conserved	1,0	1,0	1,7	1,3
Tb927.6.4200	Mitchondrial LSU ribosomal protein	1,7	1,0	1,0	1,0
Tb927.8.7530	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor, putative	1,0	1,7	1,0	1,0
Tb427tmp.160.5200	0	1,0	1,0	1,7	1,0
Tb927.9.3280	acidocalcisomal exopolyphosphatase, putative (3C4.175)	1,0	1,0	1,7	1,0
Tb927.5.3640	Mitchondrial SSU ribosomal protein	1,7	1,0	1,0	1,0
Tb927.7.3440	EF hand protein	1,3	0,9	1,7	0,9
Tb927.11.14090	hypothetical protein, conserved, 2 very weak RRMs	1,7	0,8	1,3	0,8
Tb927.8.6970	3-methylcrotonyl-CoA carboxylase alpha subunit, putative	1,5	0,5	1,7	0,8
Tb927.8.8310	chaperone protein DNAj, putative	1,0	1,0	1,7	0,5
Tb927.11.1900	T-complex protein 1, beta subunit, putative (TCP-1-beta)	1,7	0,5	1,2	1,0
Tb927.10.14550	DED1-1, ATP-dependent DEAD/H RNA helicase,	1,7	1,0	1,3	0,4
Tb927.11.6430	hypothetical protein, conserved	1,0	1,0	1,7	0,3
Tb927.7.2140	ZC3H18	1,7	0,3	1,6	1,0
Tb927.10.4980	ubiquitin-like protein DSK2, putative (DSK2)	1,0	1,0	0,3	1,7
Tb927.11.2300	ERF1 eukaryotic peptide chain release factor subunit 1, putative	0,5	1,0	1,7	0,3
Tb927.11.13180	POMP10	1,4	1,7	1,3	0,2
Tb927.10.3660	aspartate aminotransferase	1,7	1,0	1,0	0,2
Tb927.9.11150	hypothetical protein, conserved	1,1	1,2	1,7	0,2
Tb927.5.440	trans sialidase, putative	1,7	1,0	1,3	0,1
Tb927.7.210	proline dehydrogenase	1,4	0,5	1,7	0,1
Tb927.11.6280	pyruvate phosphate dikinase (PPDK)	1,0	1,0	1,6	1,0
Tb927.3.1840	3-oxo-5-alpha-steroid 4-dehydrogenase, putative	1,6	0,8	1,3	1,3
Tb927.3.1380	ATP synthase F1, alpha subunit	1,3	0,8	1,6	0,8
Tb927.1.3070	hypothetical protein, conserved, poly(Q), poly(H)	1,2	1,0	1,6	0,3
Tb927.11.330	Nucleoporin (TbMlp-1)	1,6	0,9	1,4	0,4
Tb927.3.1120	GTP-binding nuclear protein rtb2, putative (rtb2)	1,3	1,1	1,6	1,3
Tb927.8.1330	60S ribosomal protein L7a	1,0	1,0	1,0	1,6
Tb927.3.3310	60S ribosomal protein L13	0,9	0,8	1,6	1,0
Tb927.9.8410	chaperone protein DNAj, putative	1,3	1,2	1,6	0,6
Tb927.9.3760	poly(A) export protein, putative (TbGLE2)	1,0	1,0	1,6	1,0
Tb927.7.4900	XRNA	1,0	1,0	1,6	1,0
Tb927.3.5050	60S ribosomal protein L4	1,5	0,9	0,9	1,3
Tb927.3.4380	Tob55, SAM50	1,5	1,0	1,0	1,0
Tb927.9.7590	60S ribosomal protein L11	1,0	1,0	1,0	1,5
Tb927.6.3930	Mitchondrial LSU ribosomal protein	1,5	1,0	1,0	1,0

Tb927.9.15360	40S ribosomal protein S6	1,5	1,3	1,0	1,2
Tb927.8.580	hypothetical protein, conserved	1,5	1,0	1,0	1,0
Tb927.11.17000	AIR9	1,5	1,0	1,3	1,0
Tb927.11.4770	retrotransposon hot spot protein (RHS, pseudogene), putative	1,0	1,0	1,5	1,0
Tb927.9.5730	nucleosome assembly protein-like protein	1,5	1,0	1,0	1,0
Tb927.6.140	retrotransposon hot spot protein 5 (RHS5), putative	1,0	1,0	1,5	1,0
Tb927.6.2080	Mitchondrial SSU ribosomal associated KRIPP22 (PPR)	1,5	1,0	1,0	1,0
Tb927.11.10910	40S ribosomal protein SA	1,5	0,9	0,8	1,0
Tb927.11.3980	mitochondrial processing peptidase alpha subunit, MMPalpha	1,5	0,8	1,3	0,9
Tb927.6.1090	proteasome regulatory ATPase subunit 3 (RPT3)	1,5	1,0	0,8	1,0
Tb927.8.4810	prohibitin 1 (PHB1)	1,5	1,2	1,2	0,6
Tb927.11.2340	hypothetical protein, conserved	1,0	1,1	1,5	0,6
Tb927.7.6260	hypothetical protein, conserved	1,5	0,7	1,3	0,4
Tb927.6.1770	kinesin	1,5	1,0	1,3	0,3
Tb927.6.1500	1-Alkyl-dihydroxyacetonephosphate synthase	0,5	1,0	1,5	0,3
Tb927.6.4740	importin-alpha re-exporter protein, putative	1,5	1,0	0,3	0,2
Tb927.10.6880	glyceraldehyde 3-phosphate dehydrogenase, cytosolic (GAP)	1,5	0,2	0,8	1,0
Tb927.11.7140	cell cycle sequence binding phosphoprotein CSB)II	1,0	1,0	1,5	0,2
Tb927.8.3950	hypothetical protein, conserved	1,3	1,2	1,5	0,5
Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative	1,3	1,5	1,1	1,0
Tb927.10.13500	60S ribosomal protein L10a	1,0	1,0	1,0	1,5
Tb927.9.12630	glycerol kinase, glycosomal (glk1)	1,4	0,1	1,0	1,0
Tb927.9.11270	T-complex protein 1, eta subunit, putative, (TCP-1-eta)	1,4	0,4	1,0	0,7
Tb927.9.11600	Gim5B protein,glycosomal membrane protein (gim5B)	1,0	1,0	1,0	1,4
Tb927.8.6160	40S ribosomal protein S8	1,1	1,1	1,4	1,0
Tb927.8.1960	NOT11	1,0	1,0	1,4	1,0
Tb927.2.2520	voltage-dependent anion-selective channel	1,3	0,9	1,0	1,4
Tb927.11.10510	ubiquinone biosynthesis methyltransferase, putative	0,9	1,4	0,8	1,4
Tb927.9.4310	tricarboxylate carrier, putative	1,3	1,0	1,4	0,8
Tb927.7.2190	hypothetical protein, conserved	1,0	1,0	1,4	0,8
Tb927.9.3630	hypothetical protein, conserved	1,0	1,0	1,4	0,6
Tb927.9.12510	DED1-2, ATP-dependent DEAD/H RNA helicase, DED1-2	1,0	1,0	1,4	0,3
Tb927.6.890	hypothetical protein, conserved	1,4	1,3	1,4	0,3
Tb927.1.860	hypothetical protein, conserved	1,4	0,7	1,2	0,3
Tb927.7.900	hypothetical protein, conserved	1,4	0,6	0,7	0,1
Tb927.4.4310	Nucleoporin (TbNup64)	1,3	0,8	1,4	0,1
Tb927.11.10790	40S ribosomal protein SA	1,0	1,0	1,0	1,4
Tb927.11.16280	60S ribosomal protein L2 L8	1,1	1,0	1,4	0,9
Tb927.10.12700	pyruvate dehydrogenase E1 alpha subunit, putative	1,4	1,0	0,7	0,4
Tb927.7.1730	60S ribosomal protein L7	1,0	1,0	1,0	1,3
Tb927.11.6230	pretranslocation protein, alpha subunit, SEC61	1,3	1,0	1,0	1,3

Tb927.4.2070	antigenic protein, putative	1,0	1,0	1,3	1,0
Tb927.10.15520	signal recognition particle protein, putative	1,0	1,0	1,3	1,0
Tb927.10.13780	glycogen synthase kinase 3 (GSK3)	1,0	1,0	1,3	1,0
Tb927.5.3810	orotidine-5-phosphate decarboxylase/orotate phosphoribosyltransferase, OMPDCase-OPRTase	1,0	1,0	1,3	1,0
Tb927.6.2780	U3 small nuclear ribonucleoprotein (snRNP), putative	1,3	1,0	1,3	1,0
Tb927.10.10700	splicing factor Prp31	1,3	1,0	1,3	1,0
Tb927.8.5810	mitochondrial carrier protein, MCP24	1,3	1,0	1,3	1,0
Tb927.8.4330	small GTP-binding protein Rab11 (RAB11)	1,0	1,0	1,0	1,3
Tb927.11.3600	40S ribosomal protein S4	1,3	1,3	1,0	1,0
Tb927.10.190	40S ribosomal protein S6	1,3	1,0	1,0	1,0
Tb927.7.4180	Elongase 3	1,3	1,0	1,3	1,0
Tb927.10.12710	heat shock protein, HSP110	1,0	1,0	1,3	1,0
Tb927.10.4060	hypothetical protein, conserved	1,0	1,0	1,3	1,0
Tb927.10.2010	hexokinase (HK1)	1,0	1,0	1,3	1,0
Tb927.8.3530	glycerol-3-phosphate dehydrogenase [NAD], glycosomal	1,3	1,0	1,2	1,3
Tb927.11.720	hypothetical protein, conserved	1,0	1,0	1,3	1,0
Tb927.11.7520	hypothetical protein, conserved	1,0	1,0	1,3	1,0
Tb927.10.15830	hypothetical protein, conserved	1,3	1,0	1,0	1,0
Tb927.5.3410	Mitchondrial LSU ribosomal protein	1,3	1,0	1,0	1,0
Tb927.7.2110	KKT11 Kinetochore protein	1,0	1,0	1,3	1,0
Tb927.3.640	hypothetical protein, conserved	1,3	1,0	1,3	1,0
Tb927.8.6080	POMP42	1,3	1,0	1,0	1,0
Tb927.11.14460	ADP-ribosylation factor GTPase activating protein 1, putative	1,0	1,0	1,3	1,0
Tb927.7.1110	asparagine synthetase a, putative	1,0	1,0	1,3	1,0
Tb927.1.4830	Phospholipase A1	1,0	1,0	0,8	1,3
Tb927.4.2880	Nucleoporin (TbNup225)	1,3	0,9	1,3	0,7
Tb927.6.4440	RBP42	1,3	0,7	1,0	0,8
Tb927.9.9660	Archaic translocase of the outer mitochondrial membrane ATOM	1,3	1,1	1,3	0,6
Tb927.11.6870	14-3-3 protein	1,3	1,0	0,6	0,8
Tb927.10.3810	hypothetical protein, in UPF1-TAP	0,5	0,8	1,3	1,0
Tb927.5.4380	kinetoplastid-specific phospho-protein phosphatase, putative	0,5	1,0	1,3	1,0
Tb927.9.13970	hypothetical protein, conserved	1,3	1,0	0,3	1,0
Tb927.5.1810	lysosomal/endosomal membrane protein p67 (p67)	1,3	0,3	1,0	0,8
Tb927.3.2100	hypothetical protein, conserved	1,0	0,3	1,0	1,3
Tb927.8.5640	Homologue of T. cruzi Complex II subunit SDH6	1,3	0,3	1,3	0,3
Tb11.1790	retrotransposon hot spot protein (RHS, pseudogene), putative	1,0	0,3	1,3	1,0
Tb927.3.3540	Nucleoporin (TbNup53b)	0,8	0,3	1,3	0,3
Tb927.11.4540	nucleoporin 48 (TbNup48)	1,3	0,7	1,0	0,3
Tb927.8.750	nucleolar RNA-binding protein, putative	1,3	1,0	1,0	0,3
Tb10.v4.0053	hypothetical protein	0,3	0,3	1,3	1,0
Tb927.8.6250	hypothetical protein, conserved	1,1	1,1	1,3	0,1
Tb927.11.6630	3-methylcrotonoyl-CoA carboxylase beta subunit, putative	1,0	1,1	1,3	0,5

Tb927.10.14820	mitochondrial carrier protein, MCP5a	1,3	1,1	1,1	1,0
Tb927.10.8940	hypothetical protein, conserved	1,0	1,0	1,3	0,8
Tb927.3.3180	Nucleoporin (TbNup98)	0,9	0,2	1,3	0,8
Tb927.6.4090	chaperonin HSP60, mitochondrial precursor	1,1	0,8	1,3	0,3
Tb927.8.1340	60S ribosomal protein L7a	1,3	1,1	0,8	1,0
Tb927.5.1610	60S ribosomal protein L13a	1,2	0,7	1,0	1,3
Tb927.10.3930	40S ribosomal protein S3A	1,3	1,0	1,1	1,2
Tb927.10.7060	nucleoporin interacting component (NUP93), putative	1,2	0,8	1,3	0,2
Tb927.6.5040	60S ribosomal protein L15	1,0	1,0	1,0	1,3
Tb927.5.3980	Mitchondrial LSU ribosomal protein	1,3	1,0	1,0	1,0
Tb927.10.2290	chaperone protein DNAj, endoplasmatic reticulum	1,0	1,0	1,3	1,0
Tb927.10.10160	hypothetical protein, conserved	1,0	1,0	1,3	1,0
Tb927.11.5450	malic enzyme	1,0	1,0	1,3	1,0
Tb927.11.8100	hypothetical protein, conserved	1,0	1,0	1,3	1,0
Tb927.10.2980	19S proteasome regulatory subunit, (RPN11)	1,0	1,0	1,3	1,0
Tb927.10.8440	glucose transporter 1B (THT1-)	1,3	1,0	0,8	1,0
Tb427.11.01.v4	hypothetycal prot conserved	1,0	0,8	1,3	1,0
Tb927.11.11090	Nucleoporin (TbNup140)	1,0	0,8	1,3	1,0
Tb927.11.11080	Nucleoporin (TbNup149)	0,6	1,3	0,9	0,6
Tb927.7.1750	60S ribosomal protein L7	1,3	1,0	0,5	1,0
Tb927.6.4800	hypothetical protein, conserved	1,0	1,0	1,3	0,3
Tb927.6.1440	Mitchondrial LSU ribosomal protein	1,3	0,3	1,0	1,0
Tb927.8.6660	paraflagellar rod protein PFC1	0,5	1,0	1,3	0,3
Tb927.7.5940	Protein Associated with Differentiation (TbPAD2)	1,3	0,2	1,0	1,0
Tb927.11.2610	hypothetical protein, conserved, no domains, no yeast or human match	1,3	0,7	1,1	0,2
Tb927.10.14830	mitochondrial carrier protein, MCP5b	1,0	1,0	1,0	1,2
Tb927.2.4230	Nucleoporin NUP-1 protein, putative	1,2	0,7	1,2	0,7
Tb927.10.9650	hypothetical protein, conserved	1,0	1,2	0,9	0,6
Tb927.11.6440	hypothetical protein, conserved, Bromodomain and poly(Q), often in TAP MS	1,2	0,1	1,2	0,1
Tb927.11.2950	Nucleoporin (TbNup89)	0,8	1,2	0,9	0,3
Tb927.7.2300	Nucleoporin (TbNup132)	1,2	1,1	0,8	0,6
Tb927.10.8030	Mitochondrial ATP synthase subunit, putative	1,0	1,0	1,0	1,2
Tb927.10.6400	chaperonin HSP60, mitochondrial precursor	1,1	1,0	1,2	0,5
Tb927.10.16170	potassium voltage-gated channel, putative	1,2	1,0	1,0	0,3
Tb927.6.2640	importin alpha subunit, putative (TbKap60)	1,2	0,8	1,1	0,3
Tb927.11.2370	MEX67	1,0	1,0	1,2	0,3
Tb927.11.15040	chaperonin HSP60, mitochondrial precursor	1,2	0,3	0,9	0,1
Tb927.11.980	Nucleoporin (TbNup158)	1,2	0,9	0,9	0,9
Tb927.10.2100	EF1-alpha elongation factor 1-alpha, (TEF1)	1,0	1,0	1,0	1,2
Tb927.10.15760	hypothetical protein, conserved, in flagellar proteomes	1,2	1,0	1,0	0,9
Tb927.10.4310	prohibitin 2, putative (PHB2)	1,1	0,8	1,2	0,8

Tb927.10.11540	40S ribosomal protein S3	1,1	0,8	1,0	1,2
Tb927.10.1510	NOT1	1,0	1,0	1,2	1,0
Tb927.11.14020	RNA-binding protein (NRBD2)	1,0	1,0	1,0	1,2
Tb927.11.10780	Voltage-dependent anion channel (VDAC), putative	1,2	0,3	0,3	0,3
Tb927.8.6150	40S ribosomal protein S8	1,0	1,0	1,0	1,1
Tb10.v4.0247	s-adenosyl-L-methionine-c-24-delta-sterol-methy lt ransferase a, putatives	1,1	0,8	1,0	1,0
Tb927.8.1570	hypothetical protein, conserved	1,1	1,0	0,9	0,5
Tb927.11.2050	60S acidic ribosomal subunit	1,1	0,7	0,8	1,1
Tb927.9.1410	hypothetical protein, conserved	0,9	1,1	0,8	0,3
Tb927.9.10770	PABP2	1,1	0,8	1,0	0,5
Tb927.8.3750	nucleolar protein (Nop56 homologue)	1,0	1,0	1,1	1,0
Tb927.10.7570	dihydrolipoamide acetyltransferase E2 subunit, putative	1,1	0,4	1,0	0,6
Tb927.10.2110	EF1-alpha elongation factor 1-alpha, (TEF1)	1,1	0,9	1,0	1,0
Tb927.9.15150	60S ribosomal protein L5	1,1	1,0	1,1	1,1
Tb927.11.14000	RNA-binding protein (NRBD1)	1,1	1,0	0,7	1,0
Tb927.9.14240	Nucleoporin (Nup82)	1,0	1,0	0,8	0,1
Tb927.9.3990	40S ribosomal protein S7	1,0	1,0	1,0	1,0
Tb927.11.680	60S ribosomal protein L21e	1,0	1,0	1,0	1,0
Tb927.4.1860	40S ribosomal protein S19	1,0	1,0	1,0	1,0
Tb927.10.8430	40S ribosomal protein S12	1,0	1,0	1,0	1,0
Tb927.11.9320	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.5370	40S ribosomal protein S10	1,0	1,0	1,0	1,0
Tb927.11.4300	60S ribosomal protein L18	1,0	1,0	1,0	1,0
Tb927.7.2340	40S ribosomal protein S15	1,0	1,0	1,0	1,0
Tb927.11.6300	40S ribosomal protein S5	1,0	1,0	1,0	1,0
Tb927.6.720	60S ribosomal protein L14	1,0	1,0	1,0	1,0
Tb927.4.2180	60S ribosomal protein L35a	1,0	1,0	1,0	1,0
Tb927.6.4980	40S ribosomal protein S14	1,0	1,0	1,0	1,0
Tb927.7.320	RBP8	1,0	1,0	1,0	1,0
Tb927.10.7330	40S ribosomal protein S24E	1,0	1,0	1,0	1,0
Tb927.10.5340	40S ribosomal protein S18	1,0	1,0	1,0	1,0
Tb927.10.5610	40S ribosomal protein S9	1,0	1,0	1,0	1,0
Tb927.10.14090	transporter, putative	1,0	1,0	1,0	1,0
Tb927.3.680	cytochrome P450, putative	1,0	1,0	1,0	1,0
Tb927.6.2210	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.3940	40S ribosomal protein S3A	1,0	1,0	1,0	1,0
Tb927.10.7620	mitochondrial ATP-dependent zinc metallopeptidase	1,0	1,0	1,0	1,0
Tb927.5.1780	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.6030	60S ribosomal protein L12	1,0	1,0	1,0	1,0
Tb927.9.15210	60S ribosomal protein L36	1,0	1,0	1,0	1,0
Tb927.7.5170	60S ribosomal protein L23a or L25	1,0	1,0	1,0	1,0
Tb927.11.3230	60S ribosomal protein L44	1,0	1,0	1,0	1,0
Tb927.11.6200	60S ribosomal protein L28	1,0	1,0	1,0	1,0
Tb927.11.4820	60S ribosomal protein L17	1,0	1,0	1,0	1,0
Tb927.11.9720	40S ribosomal protein S27	1,0	1,0	1,0	1,0

Tb927.3.5130	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.240	40S ribosomal protein S33	1,0	1,0	1,0	1,0
Tb927.7.540	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.2.5910	40S ribosomal protein S13	1,0	1,0	1,0	1,0
Tb927.10.1080	40S ribosomal protein S23	1,0	1,0	1,0	1,0
Tb927.3.2440	serine/threonine-protein kinase, putative,protein kinase, putative	1,0	1,0	1,0	1,0
Tb927.3.4190	endosomal integral membrane protein, putative	1,0	1,0	1,0	1,0
Tb927.5.450	NADH-ubiquinone oxidoreductase, mitochondrial, putative	1,0	1,0	1,0	1,0
Tb927.7.5970	protein associated with differentiation 5, PAD5	1,0	1,0	1,0	1,0
Tb927.10.13280	GCN1 homologue, possibly involved in translation control	1,0	1,0	1,0	1,0
Tb927.8.3060	cytosolic leucyl aminopeptidase, putative,metallo-peptidase, Clan MF, Family M17	1,0	1,0	1,0	1,0
Tb927.9.11470	60S ribosomal protein L27a	1,0	1,0	1,0	1,0
Tb927.10.10610	protein tyrosine phosphatase, putative	1,0	1,0	1,0	1,0
Tb927.11.1630	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.5.4120	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.5.960	MSP1, Mitochondrial protein involved in sorting of proteins in the mitochondria, putative	1,0	1,0	1,0	1,0
Tb927.6.1520	aquaporin 3, putative (AQP1)	1,0	1,0	1,0	1,0
Tb927.6.1880	aspartyl-tRNA synthetase, putative	1,0	1,0	1,0	1,0
Tb927.7.2330	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.3430	Mitchondrial LSU ribosome-associated cyclophilin type peptidyl-prolyl cis-trans isomerase	1,0	1,0	1,0	1,0
Tb927.9.4230	fatty acyl CoA synthetase 4 (ACS4)	1,0	1,0	1,0	1,0
Tb927.9.6620	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.14370	60S ribosomal protein L26	1,0	1,0	1,0	1,0
Tb927.11.15900	60S ribosomal protein L27	1,0	1,0	1,0	1,0
Tb927.1.420	retrotransposon hot spot protein 5 (RHS5), putative	1,0	1,0	1,0	1,0
Tb927.11.11780	acyl-CoA dehydrogenase, putative	1,0	1,0	1,0	1,0
Tb927.11.1850	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.6720	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.9750	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.3220	RNA polymerase-associated protein CTR9, putative	1,0	1,0	1,0	1,0
Tb927.3.3520	POMP25	1,0	1,0	1,0	1,0
Tb927.4.2040	ALBA3	1,0	1,0	1,0	1,0
Tb927.5.2140	UPF1	1,0	1,0	1,0	1,0
Tb927.6.4500	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.4470	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.5960	protein associated with differentiation 4, PAD4	1,0	1,0	1,0	1,0
Tb927.8.6650	DRBD13	1,0	1,0	1,0	1,0
Tb927.8.6820	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.2530	mitochondrial RNA binding complex 1 subunit	1,0	1,0	1,0	1,0
Tb927.5.3360	Mitchondrial LSU ribosomal protein L2,	1,0	1,0	1,0	1,0

	putative				
Tb927.9.11880	Mitchondrial SSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.11.11820	40S ribosomal protein S17	1,0	1,0	1,0	1,0
Tb927.7.1040	40S ribosomal protein S16	1,0	1,0	1,0	1,0
Tb927.10.6050	clathrin heavy chain (CHC)	1,0	1,0	1,0	1,0
Tb927.10.14010	RP2 basal body protein, required for axoneme formation	1,0	1,0	1,0	1,0
Tb927.10.5460	60S ribosomal protein L24	1,0	1,0	1,0	1,0
Tb927.11.8200	40S ribosomal protein S26	1,0	1,0	1,0	1,0
Tb927.6.4370	eIF3 subunit 7-like protein	1,0	1,0	1,0	1,0
Tb927.8.7340	trans-sialidase, putative,neuraminidase, putative	1,0	1,0	1,0	1,0
Tb927.9.15420	60S ribosomal protein L32	1,0	1,0	1,0	1,0
Tb927.10.10570	histone H2B	1,0	1,0	1,0	1,0
Tb927.10.12290	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.10.2610	hypothetical protein, conserved, DUF1935 domain	1,0	1,0	1,0	1,0
Tb927.10.2840	40S ribosomal protein S25	1,0	1,0	1,0	1,0
Tb927.10.6370	60S ribosomal protein L37a	1,0	1,0	1,0	1,0
Tb927.10.8040	adaptin complex 1 subunit, putative,beta-adaptin, fragment (BAD1)	1,0	1,0	1,0	1,0
Tb927.11.10260	hypothetical protein, conserved, no known domains	1,0	1,0	1,0	1,0
Tb927.11.3380	Ran-binding protein 1, putative	1,0	1,0	1,0	1,0
Tb927.4.3060	hypothetical protein, conserved, PLP dependent transferases like domain	1,0	1,0	1,0	1,0
Tb927.7.4550	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.8.1200	vacuolar-type Ca2 -ATPase 2 (TbA2)	1,0	1,0	1,0	1,0
Tb927.8.2760	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.8.6980	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.4280	glyceraldehyde 3-phosphate dehydrogenase, glycosomal (GAPDH)	1,0	1,0	1,0	1,0
Tb927.11.6140	40S ribosomal protein S15A	1,0	1,0	1,0	1,0
Tb927.7.2820	histone H2A	1,0	1,0	1,0	1,0
Tb927.9.1850	60S ribosomal protein L35	1,0	1,0	1,0	1,0
Tb927.9.2220	SUMO1/Ulp2, putative	1,0	1,0	1,0	1,0
Tb927.9.7690	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.8200	PES1	1,0	1,0	1,0	1,0
Tb927.10.1550	proteasome regulatory non-ATP-ase subunit 5,19S proteasome regulatory subunit (RPN5)	1,0	1,0	1,0	1,0
Tb927.4.3850	WDR12	1,0	1,0	1,0	1,0
Tb927.8.1510	ATP-dependent DEAD/H RNA helicase, putative Dbp3	1,0	1,0	1,0	1,0
Tb927.10.6910	Sterol methyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.3.3320	60S ribosomal protein L13	1,0	1,0	1,0	1,0
Tb927.1.190	retrotransposon hot spot protein (RHS, pseudogene)	1,0	1,0	1,0	1,0
Tb927.11.6040	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.6250	Mitochondrial ATP synthase subunit, putative	1,0	1,0	1,0	1,0
Tb927.11.14170	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.4110	60S ribosomal protein L30	1,0	1,0	1,0	1,0

Tb927.10.8910	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.9220	proteasome regulatory non-ATP-ase subunit 2 (RPN2)	1,0	1,0	1,0	1,0
Tb927.9.14200	hypothetical protein, possible component of cytochorome oxidase complex	1,0	1,0	1,0	1,0
Tb927.9.15090	cytosolic coat protein, putative	1,0	1,0	1,0	1,0
Tb927.3.5520	26S proteasome regulatory non-ATPase subunit (RPN1)	1,0	1,0	1,0	1,0
Tb927.4.4910	3,2-trans-enoyl-CoA isomerase, mitochondrial precursor, putative	1,0	1,0	1,0	1,0
Tb927.10.2810	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4650	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.7.2170	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.6300	Mitchondrial SSU ribosomal protein S5	1,0	1,0	1,0	1,0
Tb927.11.4130	ubiquitin-like protein, putative	1,0	1,0	1,0	1,0
Tb927.1.2370	beta tubulin	1,0	1,0	1,0	1,0
Tb927.10.14620	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.5430	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.2190	RAPTOR-like (TOR complexes)	1,0	1,0	1,0	1,0
Tb927.1.4310	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.4420	ABC transporter, putative	1,0	1,0	1,0	1,0
Tb927.10.1970	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.2880	calcium channel protein, putative	1,0	1,0	1,0	1,0
Tb927.10.3100	Glycerol-3-phosphate acyltransferase	1,0	1,0	1,0	1,0
Tb927.10.5350	IAD-4 inner arm dynein heavy chain	1,0	1,0	1,0	1,0
Tb927.10.6330	KKT1 kinetochore protein	1,0	1,0	1,0	1,0
Tb927.10.740	structural maintenance of chromosome 4 (SMC4)	1,0	1,0	1,0	1,0
Tb927.10.8450	glucose transporter,glucose transporter 1E (THT1E)	1,0	1,0	1,0	1,0
Tb927.10.8780	P-loop nucleoside hydrolase domain, like a human adenylate kinase domain protein	1,0	1,0	1,0	1,0
Tb927.11.10520	KKT2 kinetochore protein, tyrosine kinase, putative	1,0	1,0	1,0	1,0
Tb927.11.15450	hypothetical protein	1,0	1,0	1,0	1,0
Tb927.11.3100	POMP13, Trypanosome-specific, putative TM domain at Cterminus	1,0	1,0	1,0	1,0
Tb927.11.5820	hypothetical protein, conserved, probably in mitochondrion	1,0	1,0	1,0	1,0
Tb927.11.6350	AAA ATPase, putative	1,0	1,0	1,0	1,0
Tb927.2.1260	ESAG4 pseudogene	1,0	1,0	1,0	1,0
Tb927.2.2370	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.3080	fatty acid desaturase, putative,oleate desaturase, putative	1,0	1,0	1,0	1,0
Tb927.2.5870	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.4030	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.1500	RNA editing associated helicase 2 (REH2) MRB 1 subunit	1,0	1,0	1,0	1,0
Tb927.4.2530	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.5020	RNA polymerase IIA largest subunit (RPB1)	1,0	1,0	1,0	1,0
Tb927.5.1120	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.3510	structural maintenance of chromosome 3 (SMC3)	1,0	1,0	1,0	1,0
Tb927.6.870	myotubularin, putative	1,0	1,0	1,0	1,0

Tb927.6.920	helicase, putative	1,0	1,0	1,0	1,0
Tb927.7.220	CDP-diacylglycerol synthetase, putative	1,0	1,0	1,0	1,0
Tb927.7.4170	Elongase 2	1,0	1,0	1,0	1,0
Tb927.7.5280	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.7400	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.2200	terbinafine resistance locus protein (yip1), putative	1,0	1,0	1,0	1,0
Tb927.8.2820	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.3630	folate transporter, ESAG10, putative	1,0	1,0	1,0	1,0
Tb927.8.4950	kinesin	1,0	1,0	1,0	1,0
Tb927.8.6620	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.12700	phospholipase A1, putative	1,0	1,0	1,0	1,0
Tb927.9.1770	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.3680	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.5700	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.9450	ZC3H28	1,0	1,0	1,0	1,0
Tb927.9.9740	AMP deaminase, putative	1,0	1,0	1,0	1,0
Tb927.9.9810	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.6740	PUF10	1,0	1,0	1,0	1,0
Tb927.11.14960	PUF7	1,0	1,0	1,0	1,0
Tb927.11.460	hypothetical protein, conserved,predicted WD40 repeat protein	1,0	1,0	1,0	1,0
Tb927.8.3520	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.1370	rRNA biogenesis protein, putative	1,0	1,0	1,0	1,0
Tb927.1.2430	histone H3	1,0	1,0	1,0	1,0
Tb927.1.450	retrotransposon hot spot protein (RHS, pseudogene), putative	1,0	1,0	1,0	1,0
Tb927.10.15660	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.6790	BOP1/Erb1p	1,0	1,0	1,0	1,0
Tb927.4.1080	V-type ATPase, A subunit, putative	1,0	1,0	1,0	1,0
Tb927.4.3890	ATP-dependent DEAD/H RNA helicase	1,0	1,0	1,0	1,0
Tb927.5.1560	ATP-dependent DEAD/H RNA helicase, putative, no clear yeast homologue	1,0	1,0	1,0	1,0
Tb927.5.4230	histone H4	1,0	1,0	1,0	1,0
Tb927.6.5090	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.13350	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.12430	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.3280	60S ribosomal protein L38	1,0	1,0	1,0	1,0
Tb927.11.2120	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.4890	ribosomal protein L11, putative	1,0	1,0	1,0	1,0
Tb927.8.5490	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.720	phosphoglycerate kinase (PGKA)	1,0	1,0	1,0	1,0
Tb927.10.15170	NRG1, nucleolar regulator of GPEET expression	1,0	1,0	1,0	1,0
Tb927.10.1560	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.3790	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.6090	tRNA pseudouridine synthase A, putative	1,0	1,0	1,0	1,0
Tb927.10.7630	hypothetical protein, conserved,transportin2- like protein	1,0	1,0	1,0	1,0
Tb927.10.9330	hypothetical protein, conserved, partial PSP1 superfamily (signal peptidase-like)	1,0	1,0	1,0	1,0

Tb927.11.16530	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.6360	60S ribosomal protein L24	1,0	1,0	1,0	1,0
Tb927.11.8040	Mitochondrial LSU-associated	1,0	1,0	1,0	1,0
Tb927.11.8050	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.5020	acyl-CoA oxidase, putative	1,0	1,0	1,0	1,0
Tb927.2.510	retrotransposon hot spot protein 4 (RHS4), putative	1,0	1,0	1,0	1,0
Tb927.2.6240	adenosine transporter 2 (TbNT5)	1,0	1,0	1,0	1,0
Tb927.3.4080	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.1610	VDAC-like	1,0	1,0	1,0	1,0
Tb927.4.2790	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.3070	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.3840	nucleolar protein, putative	1,0	1,0	1,0	1,0
Tb927.4.4600	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.4.4720	KRIPP7/TbPPR7 Kinetoplast ribosomal PPR-repeat containing protein	1,0	1,0	1,0	1,0
Tb927.5.4020	hypothetical protein no clear yeast or human matches	1,0	1,0	1,0	1,0
Tb927.7.3060	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.3380	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.650	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.2600	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.3300	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.9.10560	POMP6	1,0	1,0	1,0	1,0
Tb927.9.14410	RNA 3'-terminal phosphate cyclase-like protein	1,0	1,0	1,0	1,0
Tb927.9.4200	fatty acyl CoA synthetase 2 (ACS2)	1,0	1,0	1,0	1,0
Tb927.9.8290	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.2.5610	POMP22	1,0	1,0	1,0	1,0
Tb927.6.960	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	1,0	1,0	1,0	1,0
Tb927.1.1130	glycerol-3-phosphate dehydrogenase (FAD-dependent), putative	1,0	1,0	1,0	1,0
Tb927.1.1530	protein kinase, putative	1,0	1,0	1,0	1,0
Tb927.1.2120	calpain, putative,cysteine peptidase, Clan CA, family C2, putative	1,0	1,0	1,0	1,0
Tb927.1.3450	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.710	phosphoglycerate kinase (PGKB)	1,0	1,0	1,0	1,0
Tb927.10.10170	hypothetical protein, conserved,predicted WD40 repeat protein	1,0	1,0	1,0	1,0
Tb927.10.10280	microtubule-associated protein, putative	1,0	1,0	1,0	1,0
Tb927.10.11310	intraflagellar transport protein IFT55/IFT57	1,0	1,0	1,0	1,0
Tb927.10.11340	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.12740	ZC3H35	1,0	1,0	1,0	1,0
Tb927.10.12930	hypothetical protein, conservedn no domains or good BLASTp hits	1,0	1,0	1,0	1,0
Tb927.10.13040	GRESAG 4, putative receptor-type adenylate cyclase	1,0	1,0	1,0	1,0
Tb927.10.13290	ethanolamine phosphotransferase (EPT)	1,0	1,0	1,0	1,0
Tb927.10.14730	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.10.1500	methionyl-tRNA synthetase, putative (MetRS)	1,0	1,0	1,0	1,0
Tb927.10.1660	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.2020	hexokinase (HK2)	1,0	1,0	1,0	1,0

Tb927.10.3080	methionine biosynthetic protein, putative	1,0	1,0	1,0	1,0
Tb927.10.3330	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.430	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.4900	TPR-repeat-containing chaperone protein DNAJ, putative,TPR repeat protein	1,0	1,0	1,0	1,0
Tb927.10.5030	40S ribosomal protein S27+ ubiquitin	1,0	1,0	1,0	1,0
Tb927.10.7230	hypothetical protein, conserved, 7 nucleoside diphostate kinase domains	1,0	1,0	1,0	1,0
Tb927.10.760	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.8920	TbGRP ras-like small GTPase	1,0	1,0	1,0	1,0
Tb927.10.9200	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.9800	60S ribosomal protein L22	1,0	1,0	1,0	1,0
Tb927.11.1010	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.11.10870	32 kDa ER-associated protein (ERAP32)	1,0	1,0	1,0	1,0
Tb927.11.11470	Mitchondrial SSU ribosomal associated KRIPP14 (PPR)	1,0	1,0	1,0	1,0
Tb927.11.11630	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.11.11740	membrane-bound acid phosphatase, putative	1,0	1,0	1,0	1,0
Tb927.11.13250	eIF-2-gamma	1,0	1,0	1,0	1,0
Tb927.11.13270	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.13360	AAA ATPase, putative	1,0	1,0	1,0	1,0
Tb927.11.13750	small GTPase, putative,ras-related rab-4 (RAB4)	1,0	1,0	1,0	1,0
Tb927.11.13890	RNA ligase asociated with SSU	1,0	1,0	1,0	1,0
Tb927.11.14330	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.15530	C-14 sterol reductase, putative	1,0	1,0	1,0	1,0
Tb927.11.15750	AMP deaminase, putative	1,0	1,0	1,0	1,0
Tb927.11.2670	Nucleoporin (TbNup59)	1,0	1,0	1,0	1,0
Tb927.11.3320	ras-like small GTPase, putative (TbGTR)	1,0	1,0	1,0	1,0
Tb927.11.3750	NADH-cytochrome b5 reductase, putative (B5R)	1,0	1,0	1,0	1,0
Tb927.11.3940	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4180	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4330	hypothetical protein, conserved, no clear yeast or human matches	1,0	1,0	1,0	1,0
Tb927.11.4680	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4850	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.5420	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.5510	dynein light chain p28, axonemal, putative	1,0	1,0	1,0	1,0
Tb927.11.8320	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.2230	hypothetical protein, conserved, no domains, Kineto-specific	1,0	1,0	1,0	1,0
Tb927.2.2940	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.3800	mRNA processing protein, putative GAP1 MRB complex protein	1,0	1,0	1,0	1,0
Tb927.2.450	retrotransposon hot spot protein 4 (RHS4), putative	1,0	1,0	1,0	1,0
Tb927.2.5270	IAD-3 inner arm dynein heavy chain	1,0	1,0	1,0	1,0
Tb927.2.5280	trans-sialidase, putative (30J2.90)	1,0	1,0	1,0	1,0
Tb927.2.5810	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.2080	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.3260	hypothetical protein, conserved, kinetoplastid-specific, poly(E) repeats	1,0	1,0	1,0	1,0

Tb927.3.3820	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.4040	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.4120	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.4390	dihydrolipoamide dehydrogenase, putative (GCVL-1)	1,0	1,0	1,0	1,0
Tb927.3.4640	POMP26	1,0	1,0	1,0	1,0
Tb927.3.5490	flagellar transport protein, putative (PIFTB2)	1,0	1,0	1,0	1,0
Tb927.4.1600	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.1660	mitochondrial carrier protein, MCP6	1,0	1,0	1,0	1,0
Tb927.4.1970	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.2280	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.2600	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.5300	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.5.1020	disulfide isomerase, putative	1,0	1,0	1,0	1,0
Tb927.5.1710	ribonucleoprotein p18, mitochondrial precursor, putative	1,0	1,0	1,0	1,0
Tb927.5.2060	cell division control protein CDC5f, putative part of PRP19 complex	1,0	1,0	1,0	1,0
Tb927.5.2150	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.3020	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.3220	signal peptidase type I, putative	1,0	1,0	1,0	1,0
Tb927.6.2170	co-chaperone GrpE, putative	1,0	1,0	1,0	1,0
Tb927.6.2490	Succinate dehydrogenase complex component (SDH7 in T cruzi)	1,0	1,0	1,0	1,0
Tb927.6.2930	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.3540	zinc-finger protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.3650	ADP-ribosylation factor, putative	1,0	1,0	1,0	1,0
Tb927.6.4990	ATP synthase, epsilon chain, putative	1,0	1,0	1,0	1,0
Tb927.6.580	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.2070	heat shock protein DNAJ, putative	1,0	1,0	1,0	1,0
Tb927.7.4160	Elongase 1	1,0	1,0	1,0	1,0
Tb927.7.4950	NAD(p)-dependent steroid dehydrogenase-like protein	1,0	1,0	1,0	1,0
Tb927.7.5130	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.5250	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.5320	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.5710	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.6200	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.7.6440	v-SNARE like protein	1,0	1,0	1,0	1,0
Tb927.7.6660	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.7.680	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.7.740	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.7.830	telomerase-associated protein, putative	1,0	1,0	1,0	1,0
Tb927.8.1940	endosomal integral membrane protein, putative	1,0	1,0	1,0	1,0
Tb927.8.2240	tryptophanyl-tRNA synthetase, putative	1,0	1,0	1,0	1,0
Tb927.8.2540	Acetyl-CoA acetyltransferase	1,0	1,0	1,0	1,0
Tb927.8.2910	mannosyl-oligosaccharide 1,2-alpha-mannosidase IB, ER quality control	1,0	1,0	1,0	1,0
Tb927.8.4150	hypothetical protein, conserved	1,0	1,0	1,0	1,0

Tb927.8.4770	small GTP-binding protein Rab18 (TbRAB18)	1,0	1,0	1,0	1,0
Tb927.8.5280	Mitchondrial SSU ribosomal associated TbMRPS34	1,0	1,0	1,0	1,0
Tb927.8.5370	hypothetical protein, conserved, no yeast or human match	1,0	1,0	1,0	1,0
Tb927.8.570	proteasome regulatory non-ATP-ase subunit 10	1,0	1,0	1,0	1,0
Tb927.8.6200	tubulin folding cofactor D, putative	1,0	1,0	1,0	1,0
Tb927.8.7120	Squalene synthase	1,0	1,0	1,0	1,0
Tb927.8.760	nucleolar RNA-binding protein (Nopp44/46)	1,0	1,0	1,0	1,0
Tb927.9.12710	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.14070	short-chain dehydrogenase, putative	1,0	1,0	1,0	1,0
Tb927.9.15010	NADH-ubiquinone oxidoreductase complex I subunit, putative	1,0	1,0	1,0	1,0
Tb927.9.2560	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.2670	POMP3	1,0	1,0	1,0	1,0
Tb927.9.3620	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.3640	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.9.3820	syntaxin, putative	1,0	1,0	1,0	1,0
Tb927.9.5280	Mitchondrial SSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.9.7830	mitochondrial tRNA import complex, putative	1,0	1,0	1,0	1,0
Tb927.9.9710	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.1.90	retrotransposon hot spot protein (RHS, pseudogene), putative	1,0	1,0	1,0	1,0
Tb927.10.13860	GPI-anchor transamidase subunit 8 (GPI8)	1,0	1,0	1,0	1,0
Tb927.10.13990	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.15330	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.15710	mitochondrial carrier protein, MCP7	1,0	1,0	1,0	1,0
Tb927.10.3210	delta-1-pyrroline-5-carboxylate dehydrogenase, putative	1,0	1,0	1,0	1,0
Tb927.10.4280	Mitochondrial cytochrome bc1 complex component, Cytochrome bd ubiquinol oxidase domain	1,0	1,0	1,0	1,0
Tb927.10.5220	hypothetical protein, possible component of cytochorome oxidase complex	1,0	1,0	1,0	1,0
Tb927.10.5640	TbGemin2	1,0	1,0	1,0	1,0
Tb927.10.660	2-oxoisovalerate dehydrogenase alpha subunit, putative	1,0	1,0	1,0	1,0
Tb927.10.8720	NOT10	1,0	1,0	1,0	1,0
Tb927.10.8730	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.1030	KKT7 Kinetochore protein	1,0	1,0	1,0	1,0
Tb927.11.12890	POMP17	1,0	1,0	1,0	1,0
Tb927.11.14270	protein kinase, putative	1,0	1,0	1,0	1,0
Tb927.11.15230	cytosolic coat protein, putative	1,0	1,0	1,0	1,0
Tb927.11.15870	hypothetical protein, conserved, no human or yeast homologue	1,0	1,0	1,0	1,0
Tb927.11.16110	FG-GAP repeat protein, putative,intergrin alpha chain protein, putative	1,0	1,0	1,0	1,0
Tb927.11.16810	dynein light intermediate chain D1bLIC, putative	1,0	1,0	1,0	1,0
Tb927.11.4490	long-chain-fatty-acid-CoA ligase, putative,acyl-CoA synthetase	1,0	1,0	1,0	1,0
Tb927.11.5520	triosephosphate isomerase (TIM)	1,0	1,0	1,0	1,0

Tb927.11.9820	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.9900	phytoene synthase, putative, complex I	1,0	1,0	1,0	1,0
Tb927.2.4830	TFIIF-stimulated CTD phosphatase, putative	1,0	1,0	1,0	1,0
Tb927.2.5930	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.3950	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.2760	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.4570	hypothetical protein, conserved, no domains, no yeast or human match	1,0	1,0	1,0	1,0
Tb927.5.1130	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.1930	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.3190	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.770	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.1140	dolichyl-P-Man:GDP-Man5GlcNAc2-PP-dolichyl alpha-1,2-mannosyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.6.1990	hypothetical protein, conserved, no yeast or human match	1,0	1,0	1,0	1,0
Tb927.6.2560	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.3510	tRNA modification enzyme, putative	1,0	1,0	1,0	1,0
Tb927.6.3720	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.4070	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.4080	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.7.2500	proteasome regulatory ATPase subunit 1	1,0	1,0	1,0	1,0
Tb927.7.3180	Mu-adaptin 1, putative,adaptor complex AP-1 medium subunit, putative	1,0	1,0	1,0	1,0
Tb927.7.3370	intraflagellar transport protein IFT74	1,0	1,0	1,0	1,0
Tb927.7.3880	protein kinase, putative	1,0	1,0	1,0	1,0
Tb927.7.4980	ZC3H23 POMP35	1,0	1,0	1,0	1,0
Tb927.7.5080	ATP-NAD kinase-like protein	1,0	1,0	1,0	1,0
Tb927.7.6930	ATPase, putative	1,0	1,0	1,0	1,0
Tb927.8.1290	hypothetical protein, conserved, no domains	1,0	1,0	1,0	1,0
Tb927.8.3580	mitochondrial chaperone BCS1, putative	1,0	1,0	1,0	1,0
Tb927.8.3770	mitogen-activated protein kinase, putative	1,0	1,0	1,0	1,0
Tb927.8.4050	flagellar membrane protein that interacts with FLA1, similar to FLA3	1,0	1,0	1,0	1,0
Tb927.8.4610	small GTP-binding protein Rab1 (Trab1)	1,0	1,0	1,0	1,0
Tb927.8.5560	NADH-ubiquinone oxidoreductase complex I subunit, putative	1,0	1,0	1,0	1,0
Tb927.8.6360	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.10450	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.11540	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.13580	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.13650	ADP-ribosylation factor, putative	1,0	1,0	1,0	1,0
Tb927.9.14080	vesicle-associated membrane protein, putative	1,0	1,0	1,0	1,0
Tb927.9.15000	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.7180	adenosine monophosphate deaminase, putative,AMP deaminase, putative	1,0	1,0	1,0	1,0
Tb927.9.9150	GTP-binding protein, putative	1,0	1,0	1,0	1,0
Tb927.1.5030	leucine-rich repeat protein (LRRP)	1,0	1,0	1,0	1,0
Tb927.10.14200	syntaxin 5	1,0	1,0	1,0	1,0

Tb927.10.14530	proteasome regulatory non-ATPase subunit 8,26S proteasome regulatory subunit (Rpn8)	1,0	1,0	1,0	1,0
Tb927.10.14860	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.15430	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.15720	19S proteasome regulatory subunit (RPN9)	1,0	1,0	1,0	1,0
Tb927.10.4050	serine palmitoyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.10.4640	eIF-3 subunit L	1,0	1,0	1,0	1,0
Tb927.10.4760	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.5300	eIF-6	1,0	1,0	1,0	1,0
Tb927.10.7100	delta-4 fatty acid desaturase	1,0	1,0	1,0	1,0
Tb927.11.15150	1-acyl-sn-glycerol-3-phosphate acyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.11.16670	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.2690	succinyl-coA:3-ketoacid-coenzyme A transferase, mitochondrial precursor, putative	1,0	1,0	1,0	1,0
Tb927.11.2750	POMP12	1,0	1,0	1,0	1,0
Tb927.11.3570	aminopeptidase, putative,metallo-peptidase, Clan MA(E) Family M1	1,0	1,0	1,0	1,0
Tb927.11.3860	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4160	hypothetical protein, conserved,predicted C2 domain protein	1,0	1,0	1,0	1,0
Tb927.11.5110	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.5760	hypothetical protein, conserved, small unknown domain, SET domain	1,0	1,0	1,0	1,0
Tb927.3.3850	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.3.4820	acyltransferase, putative	1,0	1,0	1,0	1,0
Tb927.6.1040	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	1,0	1,0	1,0	1,0
Tb927.7.1220	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.2980	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.3810	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.4940	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.6050	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.7040	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.8120	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.3400	endo-beta-N-acetylglucosaminidase, putative	1,0	1,0	1,0	1,0
Tb927.9.7800	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.13620	NADH-ubiquinone oxidoreductase complex I subunit, NDUFA9 subunit, putative	1,0	1,0	1,0	1,0
Tb927.10.16150	ATP-dependent zinc metallopeptidase, putative,metallo-peptidase, Clan MA(E) Family M41	1,0	1,0	1,0	1,0
Tb927.10.1860	hypothetical protein, conserved, tryp specific	1,0	1,0	1,0	1,0
Tb927.10.3520	protease regulatory ATPase subunit 4, putative (RPT4)	1,0	1,0	1,0	1,0
Tb927.10.4040	3-Ketosphinganine reductase	1,0	1,0	1,0	1,0
Tb927.11.14910	protein phosphatase 2C, putative	1,0	1,0	1,0	1,0
Tb927.11.3270	squalene monooxygenase, putative	1,0	1,0	1,0	1,0
Tb927.2.4210	glycosomal phosphoenolpyruvate carboxykinase (PEPCK)	1,0	1,0	1,0	1,0
Tb927.3.1080	POMP23	1,0	1,0	1,0	1,0

Tb927.4.2890	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.730	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.2570	guide RNA associated protein, GAP2	1,0	1,0	1,0	1,0
Tb927.7.7130	TWY1 homologue, tRNA modification	1,0	1,0	1,0	1,0
Tb927.7.7470	GRESAG 4, putative receptor-type adenylate cyclase	1,0	1,0	1,0	1,0
Tb927.8.4890	endoplasmic reticulum oxidoreductin, Ero1, oxidises PDI	1,0	1,0	1,0	1,0
Tb927.9.10520	hypothetical protein, possible component of cytochorome oxidase complex	1,0	1,0	1,0	1,0
Tb927.9.6090	PTP1-interacting protein, 39 kDa PIP39	1,0	1,0	1,0	1,0
Tb927.10.15680	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.2890	enolase	1,0	1,0	1,0	1,0
Tb927.10.5400	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.770	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.1070	glycosomal transporter (GAT3)	1,0	1,0	1,0	1,0
Tb927.11.14700	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.4280	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.5220	chaperone protein DNAj, putative	1,0	1,0	1,0	1,0
Tb927.2.5800	sedoheptulose-1,7-bisphosphatase (SBPase)	1,0	1,0	1,0	1,0
Tb927.3.3450	ADP-ribosylation factor-like protein 3A, putative	1,0	1,0	1,0	1,0
Tb927.3.750	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.4.4350	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.2080	guanosine monophosphate reductase, putative	1,0	1,0	1,0	1,0
Tb927.6.2360	adenosine kinase, putative	1,0	1,0	1,0	1,0
Tb927.9.3170	cytochrome oxidase subunit V (COXV)	1,0	1,0	1,0	1,0
Tb927.2.3990	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.2290	U5 splicing factor U5-200K, putative or BRR2 homologue	1,0	1,0	1,0	1,0
Tb927.8.2770	IP3 inositol trisphosphate receptor.	1,0	1,0	1,0	1,0
Tb927.9.15290	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.1720	Phosphatidylglycerophosphate synthase	1,0	1,0	1,0	1,0
Tb927.9.4500	HSP70, endoplasmatic reticulum, HSP70.a	1,0	1,0	1,0	1,0
Tb927.10.14150	hypothetical protein, conserved, related to yeast BFR1	1,0	1,0	1,0	1,0
Tb927.10.13250	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.9890	signal recognition particle receptor alpha subunit, putative	1,0	1,0	1,0	1,0
Tb927.5.900	oligosaccharyl transferase subunit, putative	1,0	1,0	1,0	1,0
Tb927.6.3500	RME8 endosomal trafficking protein, clathrin-associated	1,0	1,0	1,0	1,0
Tb927.8.5760	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.5770	Elongator-like Protein 3a ELP3a	1,0	1,0	1,0	1,0
Tb927.9.10010	Sec63 homologue, chaperone protein DNAj	1,0	1,0	1,0	1,0
Tb927.9.1780	sec1 family transport protein, putative (SLY1)	1,0	1,0	1,0	1,0
Tb927.9.11110	PRP8 protein homologue,U5 snRNA-associated splicing factor	1,0	1,0	1,0	1,0
Tb927.10.5620	fructose-bisphosphate aldolase, glycosomal (ALD)	1,0	1,0	1,0	1,0
Tb927.1.1930	TbTOR4 = TOR-like 2	1,0	1,0	1,0	1,0

Tb927.5.3800	glutamine hydrolysing (not ammonia-dependent) carbomoyl phosphate synthase, putative	1,0	1,0	1,0	1,0
Tb927.8.1590	TOM1, HECT ubiquitin-protein ligase involved in mRNA export from nucleus	1,0	1,0	1,0	1,0
Tb927.9.6170	arginine kinase (AK) in cytosol and flagellum	1,0	1,0	1,0	1,0
Tb927.10.7710	40S ribosomal protein S8	1,0	1,0	1,0	1,0
Tb927.11.5300	kinesin, Kif13-3, predicted centromere-associated	1,0	1,0	1,0	1,0
Tb927.11.6540	hypothetical protein, conserved, kinetoplastid-specific	1,0	1,0	1,0	1,0
Tb927.2.370	retrotransposon hot spot protein 1 (RHS1), putative	1,0	1,0	1,0	1,0
Tb927.3.1590	MRB complex protein mitochondrial RNA binding complex 1 subunit	1,0	1,0	1,0	1,0
Tb927.3.4500	fumarate hydratase, class I (FHc)	1,0	1,0	1,0	1,0
Tb927.4.630	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.5.1090	threonyl-tRNA synthetase, putative	1,0	1,0	1,0	1,0
Tb927.5.1470	NADH-cytochrome b5 reductase, putative (B5R)	1,0	1,0	1,0	1,0
Tb927.5.3920	PEX6 peroxisome assembly protein, AAA ATPase	1,0	1,0	1,0	1,0
Tb927.5.590	protein phosphatase 1, regulatory subunit, putative	1,0	1,0	1,0	1,0
Tb927.5.920	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.1300	protein disulfide isomerase,ERp72-like, cofactor wth calnexin, putative	1,0	1,0	1,0	1,0
Tb927.7.1360	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.1920	paraflagellar rod protein PFC5	1,0	1,0	1,0	1,0
Tb927.7.2660	ZC3H20	1,0	1,0	1,0	1,0
Tb927.7.2700	NADH-cytochrome b5 reductase, putative (B5R)	1,0	1,0	1,0	1,0
Tb927.7.3080	hypothetical protein, conserved, kinetoplastid-specific	1,0	1,0	1,0	1,0
Tb927.7.4390	threonine synthase, putative	1,0	1,0	1,0	1,0
Tb927.7.7030	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.7460	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.8.2030	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.11050	4E-IP, 4E-interacting protein, putative	1,0	1,0	1,0	1,0
Tb927.9.11910	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.9.6450	PEX16, putative	1,0	1,0	1,0	1,0
Tb927.9.9870	MCAK-like kinesin, putative	1,0	1,0	1,0	1,0
Tb927.6.1920	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.10.8660	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.11.5290	mitochondrial carrier protein, MCP9	1,0	1,0	1,0	1,0
Tb927.10.6810	guanylate kinase, putative	1,0	1,0	1,0	1,0
Tb927.7.3030	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.4710	Mitchondrial LSU ribosomal protein	1,0	1,0	1,0	1,0
Tb927.9.11580	Gim5A protein,glycosomal membrane protein (gim5A)	1,0	1,0	1,0	1,0
Tb927.9.6100	TFIIF-stimulated CTD phosphatase, in UPF1-TAP	1,0	1,0	1,0	1,0
Tb927.7.3040	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.6.4770	MKT1	1,0	1,0	1,0	1,0
Tb927.9.7470	purine nucleoside transporter NT10	1,0	1,0	1,0	1,0

Tb927.11.14900	coatomer epsilon subunit, epsilon-COP	1,0	1,0	1,0	1,0
Tb927.10.2640	intraflagellar transport protein IFT81	1,0	1,0	1,0	1,0
Tb927.11.16160	ATP binding protein-like protein	1,0	1,0	1,0	1,0
Tb927.11.3770	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.2.4980	hypothetical protein, conserved	1,0	1,0	1,0	1,0
Tb927.7.1500	Thiomodification of cytiosolic tRNAs (NBP 2)	1,0	1,0	1,0	1,0
Tb11.02.5400	cystathionine beta-synthase, putative	1,0	1,0	1,0	1,0
Tb927.10.14930	ZC3H39	1,0	1,0	1,0	1,0
Tb927.9.8880	actin A	1,0	1,0	1,0	0,9
Tb927.9.8850	actin A	0,9	0,9	0,9	1,0
Tb927.4.3590	EF1 beta, translation elongation factor 1-beta, putative	1,0	1,0	0,9	1,0
Tb927.11.4900	guanine nucleotide-binding beta subunit-like protein,G-protein (beta)-like protein	1,0	1,0	0,9	1,0
Tb927.10.8490	glucose transporter, putative	1,0	1,0	1,0	0,8
Tb927.9.6060	2Fe-2S iron-sulfur cluster binding domain containing protein, POMP4	1,0	1,0	0,8	1,0
Tb927.2.3780	IF2 translation initiation factor IF-2, putative	1,0	1,0	0,8	1,0
Tb927.2.5060	GTP binding protein, putative	1,0	1,0	0,8	1,0
Tb927.3.1790	pyruvate dehydrogenase E1 beta subunit, putative	0,9	1,0	1,0	0,8
Tb927.7.7420	ATP synthase F1, alpha subunit	1,0	1,0	1,0	0,8
Tb927.11.11520	PEX11 glycosomal membrane protein	1,0	1,0	0,8	1,0
Tb927.8.6110	hypothetical protein, conserved	1,0	1,0	1,0	0,8
Tb927.6.1000	cysteine peptidase precursor, Clan CA, family C1, Cathepsin L-like (CP)	1,0	1,0	1,0	0,8
Tb927.10.1170	intraflagellar transport protein IFT172	1,0	1,0	0,8	1,0
Tb927.4.1800	Mitochondrial LSU ribosomal protein L3	1,0	0,8	0,9	0,9
Tb927.10.2560	mitochondrial malate dehydrogenase (mMDH)	1,0	0,8	1,0	1,0
Tb927.10.15180	nucleosome assembly protein, putative	1,0	1,0	1,0	0,8
Tb927.6.4690	60S ribosomal protein L9	0,8	1,0	1,0	1,0
Tb927.1.3110	soluble N-ethylmaleimide sensitive factor (NSF) attachment protein, putative	1,0	1,0	0,8	1,0
Tb927.4.1170	ankyrin, putative	1,0	1,0	0,8	1,0
Tb927.7.1970	retrotransposon hot spot protein 7 (RHS7), putative	1,0	1,0	0,8	1,0
Tb927.10.4570	EF2 elongation factor 2	1,0	1,0	1,0	0,7
Tb927.8.1500	hypothetical protein, conserved	1,0	1,0	0,7	1,0
Tb927.9.12570	glycerol kinase, glycosomal (glk1)	1,0	1,0	1,0	0,7
Tb927.11.3030	phosphoribosylpyrophosphate synthetase, putative (PRS)	1,0	1,0	0,7	1,0
Tb927.11.8880	hypothetical protein, conserved	0,8	1,0	0,7	1,0
Tb927.3.1920	NOT5	1,0	1,0	0,6	1,0
Tb927.5.930	NADH-dependent fumarate reductase (FRDg)	1,0	1,0	0,6	1,0
Tb927.11.13080	hypothetical protein, conserved	1,0	1,0	0,7	0,6
Tb927.10.1070	cdc2- like protein kinase (CRK1)	1,0	1,0	1,0	0,6
Tb927.9.9550	hypothetical protein, conserved, no yeast or human match	1,0	1,0	1,0	0,6
Tb927.11.5140	ubiquitin carboxyl-terminal hydrolase, Clan CA cysteine peptidase, family C12, putative	1,0	1,0	0,6	1,0

Tb927.11.670	epsinR	1,0	1,0	1,0	0,6
Tb927.3.3030	hypothetical protein, conserved	0,6	0,6	1,0	1,0
Tb927.10.9430	phosphoribosylpyrophosphate synthetase, putative (PRS)	1,0	1,0	0,6	1,0
Tb927.10.9440	NADH dehydrogenase (54 NDH2)	1,0	1,0	1,0	0,6
Tb927.1.2330	beta tubulin	0,7	0,5	0,8	1,0
Tb927.10.13360	EF-Tu Mitochondrial elongation factor Tu	0,5	1,0	1,0	0,8
Tb927.11.14750	hypothetical protein, conserved, PSP1 C-terminal region domain, no yeast or human match	0,5	1,0	1,0	1,0
Tb927.8.4450	RBP11	0,5	1,0	1,0	1,0
Tb927.9.12500	POMP7	0,5	1,0	1,0	1,0
Tb927.6.5080	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.8.7160	UDP-Gal or UDP-GlcNAc-dependent glycosyltransferase pseudogene	0,5	1,0	1,0	1,0
Tb927.1.1790	hypothetical protein	0,5	1,0	1,0	1,0
Tb927.10.1120	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.10.460	NIMA-related protein kinase (NRKC)	0,5	1,0	1,0	1,0
Tb927.11.4350	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.2.2260	PIK-related	0,5	1,0	1,0	1,0
Tb927.4.220	retrotransposon hot spot protein (RHS2, pseudogene), putative	1,0	1,0	0,5	1,0
Tb927.6.2880	Kinesin-like but poor match	0,5	1,0	1,0	1,0
Tb927.8.3180	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.8.5330	MCP2, binds to tRNAs and assists tRNA synthetases	0,5	1,0	1,0	1,0
Tb927.8.800	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.9.1710	hypothetical protein, conserved	0,5	1,0	1,0	1,0
Tb927.9.3920	40S ribosomal protein S7	0,5	1,0	1,0	1,0
Tb927.11.6390	hypothetical protein, conserved, START domain, possible phoshatidylcholine transfer protein	1,0	1,0	1,0	0,5
Tb927.10.10900	heat shock protein 83, HSP83	1,0	1,0	1,0	0,4
Tb927.10.7680	GTPase activating protein, putative	1,0	0,8	0,9	0,4
Tb927.10.15530	ABC transport system ATP-binding protein, putative	1,0	1,0	1,0	0,3
Tb927.9.11380	60S ribosomal protein L23	1,0	1,0	0,3	0,3
Tb927.10.9740	19S proteasome regulatory subunit (RPT6)	1,0	1,0	0,3	1,0
Tb927.7.2550	proteasome regulatory ATPase subunit 5 (RPT5)	1,0	1,0	1,0	0,3
Tb927.3.2150	protein phosphatase 2C, putative	1,0	1,0	1,0	0,3
Tb927.11.15560	Nucleoporin (TbNup53a)	1,0	1,0	0,3	1,0
Tb927.3.1680	hypothetical protein, conserved	1,0	1,0	0,3	1,0
Tb927.10.11270	RBP23	1,0	1,0	1,0	0,3
Tb927.11.10570	Mitchondrial LSU ribosomal protein	0,8	1,0	1,0	0,3
Tb927.9.2320	POMP1 (possible S-Adenosyl methionine methyl transferase	1,0	0,3	0,8	1,0
Tb927.10.730	ATP synthase, putative	1,0	1,0	1,0	0,3
Tb927.11.9920	polyubiquitin, putative	0,5	1,0	1,0	0,3
Tb927.11.9730	60S ribosomal protein L34	0,5	1,0	0,3	0,8
Tb927.11.2410	Flabarin, flagellar membrane protein in Leishmania	1,0	0,3	1,0	1,0
Tb927.9.11000	small GTPase, putative,GTP-binding protein, putative (RAB7)	0,3	1,0	1,0	0,8

Tb927.9.6310	ABC transporter, putative	1,0	1,0	1,0	0,3
Tb927.9.11840	hypothetical protein, conserved	1,0	1,0	1,0	0,3
Tb927.4.1020	Serine palmitoyltransferase	1,0	1,0	1,0	0,3
Tb927.6.3940	hypothetical protein, conserved	1,0	1,0	1,0	0,3
Tb927.10.9910	hypothetical protein, conserved	0,5	1,0	0,3	0,3
Tb927.11.3740	proteasome regulatory ATPase subunit 2 (RPT2)	0,5	1,0	1,0	0,3
Tb927.10.4080	hypothetical protein, conserved	1,0	1,0	1,0	0,3
Tb927.4.4490	multidrug resistance protein E,p-glycoprotein (MRPE)	1,0	1,0	1,0	0,3
Tb927.10.3170	ABC transporter, putative	1,0	1,0	1,0	0,3
Tb927.1.120	retrotransposon hot spot protein 4 (RHS4), putative	1,0	1,0	1,0	0,3
Tb927.7.6800	Mitchondrial LSU ribosomal protein	0,3	1,0	1,0	1,0
Tb927.8.4540	PBP1	1,0	1,0	0,3	1,0
Tb927.8.980	Phosphoacetylglucosamine mutase PAGM, acts as phosphoglucomutase	1,0	1,0	1,0	0,3
Tb927.1.3180	40S ribosomal protein S11	1,0	1,0	1,0	0,3
Tb927.10.13710	hypothetical protein, conserved	1,0	1,0	0,3	1,0
Tb927.4.2240	hypothetical protein, conserved, D-aminoacid aminotransferase domain	1,0	1,0	0,3	1,0
Tb927.7.6890	hypothetical protein, conserved	1,0	1,0	0,3	1,0
Tb927.9.10580	3-demethylubiquinone-9 3-methyltransferase, putative	1,0	1,0	1,0	0,3
Tb927.10.10130	MRB complex component mitochondrial RNA binding complex 1 subunit	1,0	1,0	1,0	0,3
Tb927.11.10020	short-chain dehydrogenase, putative	1,0	1,0	0,3	1,0
Tb927.3.2900	EF-2 alpha, elongation initiation factor 2 alpha subunit,	1,0	1,0	1,0	0,3
Tb927.7.2160	hypothetical protein, conserved, no domains, no yeast or human match	1,0	1,0	1,0	0,3
Tb927.7.2710	NADH-cytochrome b5 reductase, putative	1,0	1,0	1,0	0,3
Tb927.8.3380	electron transfer protein, SDH2N,succinate dehydrogenase complex	1,0	1,0	1,0	0,3
Tb927.4.330	hypothetical protein, conserved	1,0	1,0	1,0	0,3
Tb927.11.4200	ERGIC53 paralogue, lectin-like, ER quality control	1,0	1,0	1,0	0,3
Tb927.6.5000	hypothetical protein, conserved	1,0	1,0	1,0	0,3
Tb927.8.3690	isocitrate dehydrogenase [NADP], mitochondrial precursor, putative (IDH)	1,0	1,0	0,3	1,0
Tb927.10.4130	NADH-ubiquinone oxidoreductase complex I subunit, putative,NDUFA5/B13 subunit, putative	1,0	1,0	1,0	0,3
Tb927.10.5930	protein kinase, putative	1,0	1,0	0,3	1,0
Tb927.11.7610	gp63-1 surface protease pseudogene	0,3	0,3	1,0	1,0
Tb927.9.9420	hypothetical protein, conserved	0,3	1,0	0,3	1,0
Tb927.10.14950	ZC3H40	1,0	1,0	0,3	1,0
Tb927.10.3760	vacuolar ATP synthase subunit d, putative	1,0	1,0	0,3	1,0
Tb927.11.1890	hypothetical protein, conserved	1,0	1,0	0,3	1,0
Tb927.11.5440	malic enzyme	1,0	1,0	1,0	0,3
Tb927.2.4240	GTP binding protein, putative	1,0	1,0	0,3	1,0
Tb927.3.3630	EF-TS Mitochondrial elongation factor ts	1,0	1,0	0,3	1,0
Tb927.4.3370	hypothetical protein, conserved	1,0	1,0	0,3	1,0
Tb927.5.2380	hydrolase, alpha/beta fold family, putative	1,0	1,0	1,0	0,3
Tb927.6.1810	hypothetical protein, conserved	1,0	1,0	1,0	0,3

Tb927.6.4430	homoserine kinase, putative (HK)	1,0	1,0	0,3	1,0
Tb927.6.4920	S-adenosylmethionine synthetase, putative (METK1)	1,0	1,0	0,3	1,0
Tb927.7.2020	retrotransposon hot spot protein 7 (RHS7), putative	1,0	1,0	0,3	1,0
Tb927.8.5860	Mitchondrial LSU ribosomal protein 50S ribosomal protein L17, putative	0,3	1,0	1,0	1,0
Tb927.9.15190	60S ribosomal protein L15	1,0	0,3	1,0	1,0
Tb927.10.12240	short-chain dehydrogenase, putative	1,0	1,0	0,3	0,8
Tb927.3.5370	hypothetical protein, conserved	1,0	0,8	1,0	0,3
Tb09.v4.0013	retrotransposon hot spot (RHS) protein	1,0	0,3	1,0	1,0
Tb927.11.13500	paraflagellar rod protein par1	1,0	1,0	1,0	0,3
Tb927.2.3400	hypothetical protein, copurified with splicing complex	0,3	0,8	1,0	1,0
Tb927.3.3610	PEX7 peroxisomal targeting signal type 2 receptor	1,0	0,3	0,9	0,3
Tb927.11.16080	hypothetical protein, conserved	0,3	1,0	1,0	1,0
Tb927.5.550	vacuolar ATP synthase, putative	1,0	0,3	1,0	1,0
Tb927.5.3970	adenylate kinase, putative (ADKE)	1,0	1,0	1,0	0,3
Tb927.10.6610	chaperone protein DNAj, putative	1,0	0,3	1,0	1,0
Tb927.10.3260	Long-chain-fatty-acid--CoA ligase 5, Acyl-CoA synthetase 5 (LACS 5)	1,0	1,0	1,0	0,3
Tb927.3.4760	dynamin, putative,vacuolar sortin protein 1, putative	1,0	1,0	1,0	0,3
Tb927.8.1610	MSP-B, putative	1,0	1,0	1,0	0,3
Tb927.10.15850	PEX12 peroxisome assembly protein	0,3	0,3	1,0	0,3
Tb927.2.280	retrotransposon hot spot protein 2 (RHS2), putative	1,0	1,0	1,0	0,3
Tb927.8.6410	short-chain dehydrogenase, putative	1,0	1,0	1,0	0,3
Tb927.3.3190	serine/threonine-protein kinase, putative,protein kinase, putative	0,3	1,0	1,0	0,3
Tb927.10.12500	P-type H -ATPase, putative	1,0	1,0	1,0	0,3
Tb927.11.14380	Kinetoplast polyadenylation/uridylation factor 2	1,0	1,0	1,0	0,3
Tb927.8.6640	hypothetical protein, conserved	1,0	1,0	0,8	0,3
Tb927.10.240	PEX14 peroxin 14	1,0	0,3	1,0	1,0
Tb927.11.2130	proteasome regulatory non-ATP-ase subunit 3 (RPN3)	1,0	1,0	0,3	1,0
Tb927.9.12550	glycerol kinase, glycosomal (glk1)	1,0	1,0	1,0	0,3
Tb927.1.2100	calpain-like cysteine peptidase, putative,cysteine peptidase, Clan CA, family C2, putative	1,0	1,0	1,0	0,3
Tb927.11.2260	eIF4E1	1,0	1,0	0,3	1,0
Tb927.5.2940	stress-induced protein sti1, putative	1,0	1,0	0,3	1,0
Tb927.8.8330	calpain, putative,cysteine peptidase, putative	1,0	1,0	1,0	0,3
Tb927.2.5160	chaperone protein DNAj, putative	1,0	1,0	1,0	0,2
Tb927.2.1170	retrotransposon hot spot protein 5 (RHS5), putative	1,0	1,0	1,0	0,2
Tb927.6.850	NOT2	0,5	1,0	0,8	0,2
Tb927.10.7700	ABC transporter, putative	1,0	0,3	1,0	0,2
Tb927.11.2110	hypothetical protein, conserved	0,2	0,3	1,0	0,3
Tb927.9.4210	fatty acyl CoA synthetase 3 (ACS3)	1,0	1,0	0,2	0,3
Tb927.10.5810	hypothetical protein, conserved	1,0	0,2	1,0	1,0
Tb927.6.3750	heat shock 70 kDa protein, mitochondrial precursor, putative	1,0	1,0	1,0	0,2

Tb927.2.1330	retrotransposon hot spot protein (RHS6, pseudogene), putative	1,0	1,0	0,2	0,2
Tb927.2.470	retrotransposon hot spot protein 4 (RHS4), putative	1,0	1,0	1,0	0,2
Tb927.10.2090	EF1-alpha elongation factor 1-alpha, (TEF1)	1,0	1,0	1,0	0,2
Tb927.10.1900	DNA topoisomerase IA, putative	1,0	0,2	1,0	1,0
Tb927.9.6290	arginine kinase (AK)	0,2	1,0	1,0	1,0
Tb927.7.6670	hypothetical protein, conserved	0,7	0,6	1,0	0,1
Tb927.2.3030	ATP-dependent Clp protease subunit, heat shock protein 78 (HSP78)	1,0	1,0	1,0	0,1
Tb927.7.4760	hypothetical protein, conserved	1,0	0,4	1,0	0,1
Tb927.11.500	UBP1	0,2	0,1	1,0	1,0
Tb927.3.1300	hypothetical protein, conserved, no domains, no yeast or human match	1,0	1,0	0,2	0,1
Tb927.8.7980	vacuolar-type H( )-translocating pyrophosphatase (TVP1)	0,1	0,7	0,6	1,0
Tb927.5.1100	PEX5 peroxisome targeting signal 1 receptor	0,8	1,0	0,8	0,1
Tb927.1.180	retrotransposon hot spot protein 1 (RHS1), putative	1,0	1,0	1,0	0,1
Tb927.4.4940	hypothetical protein, conserved, poly(Q), no clear yeast or human matches	1,0	1,0	1,0	0,1
Tb927.11.11330	heat shock protein 70, major HSP70	1,0	0,8	0,0	0,4
Tb927.10.2350	pyruvate dehydrogenase complex E3 binding protein, putative	0,8	0,6	0,8	0,3
Tb927.11.14220	hypothetical protein, conserved, Phyre2 gives 97% probability of RNA-binding domain	0,3	0,3	0,8	0,8