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(54) **DIAGNOSTIC ASSAY FOR *TRYPANOSOMA CRUZI* INFECTION**

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G01N 33/53 (2006.01)

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See application file for complete search history.

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(57) **ABSTRACT**

A sensitive, multicomponent diagnostic test for infection with *T. cruzi*, the causative agent of Chagas disease, including methods of making and methods of use. Also provided is a method for screening *T. cruzi* polypeptides to identify antigenic polypeptides for inclusion as components of the diagnostic test, as well as compositions containing antigenic *T. cruzi* polypeptides.

20 Claims, 22 Drawing Sheets

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Fig. 1A

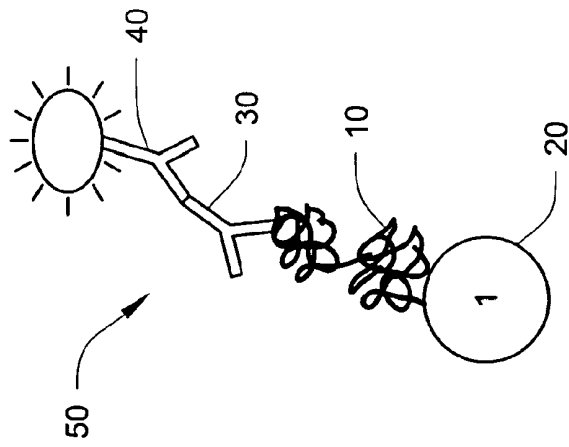


Fig. 1B

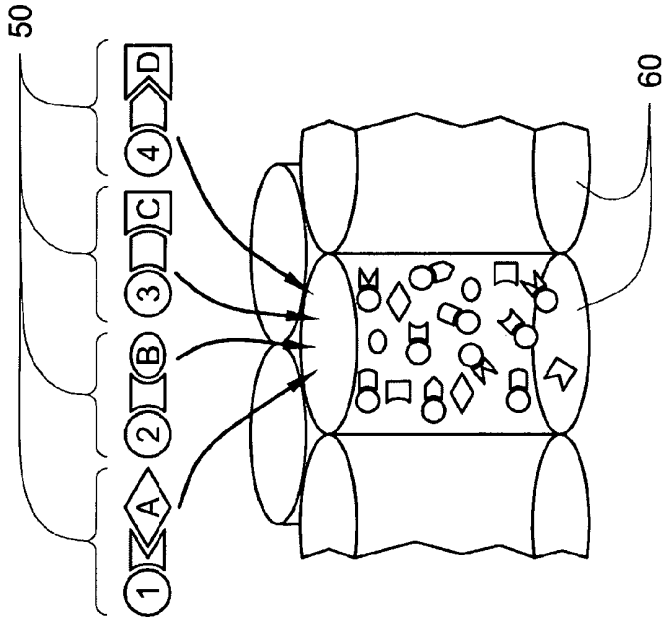
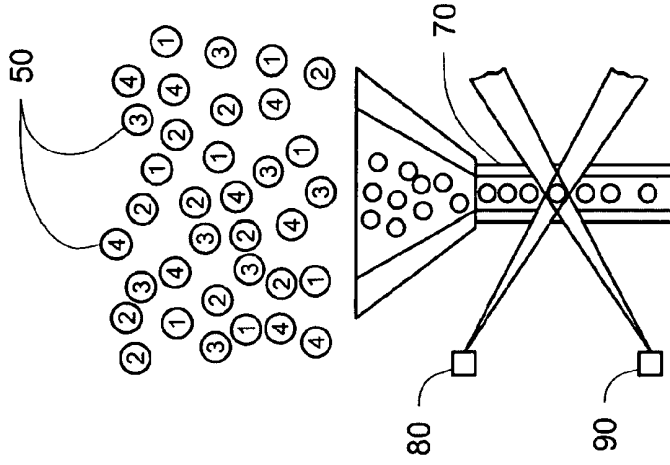


Fig. 1C



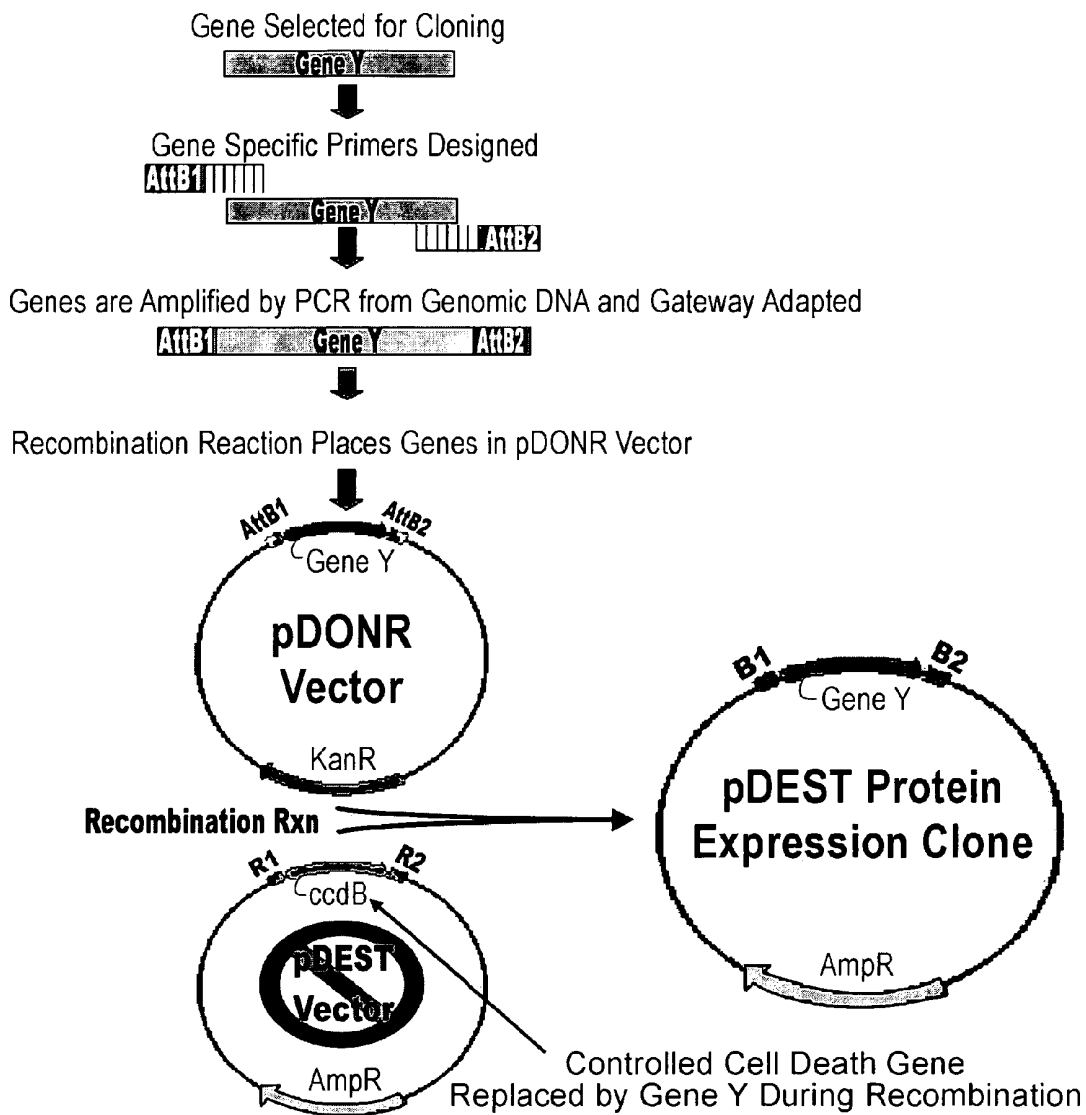


Fig. 2

Fig. 3A

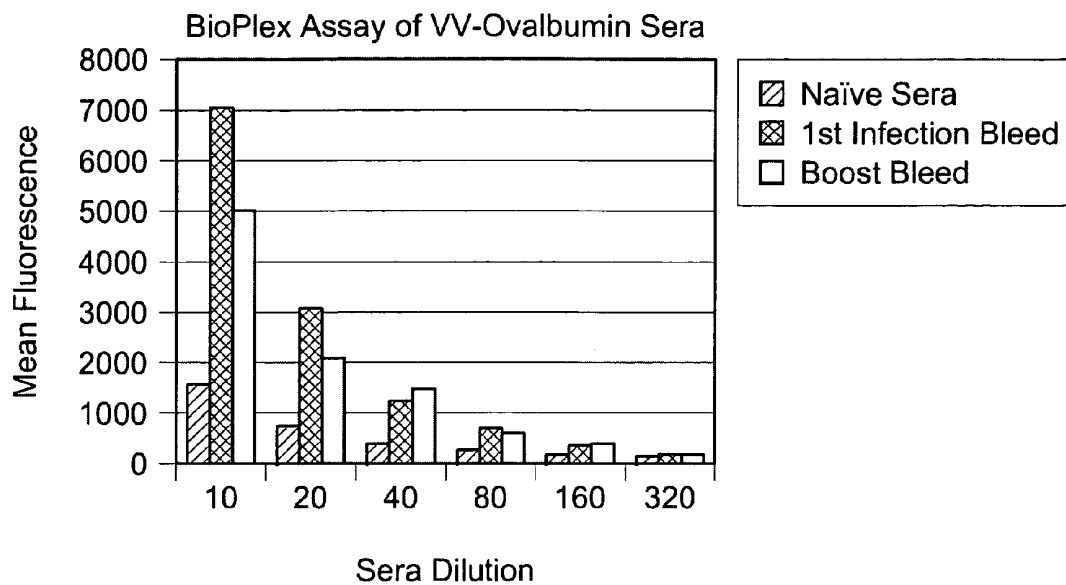


Fig. 3B

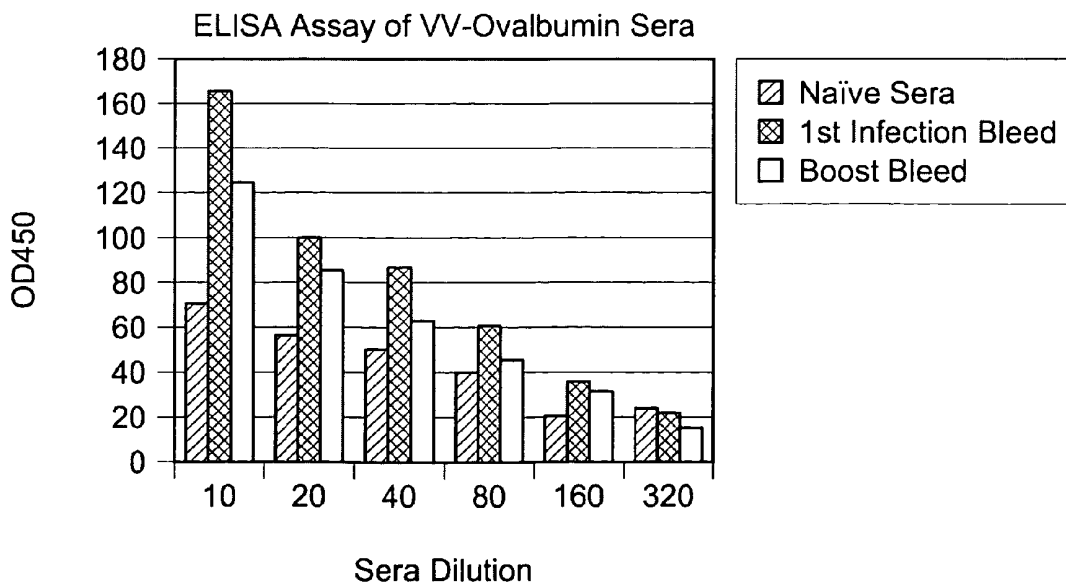


Fig. 4C

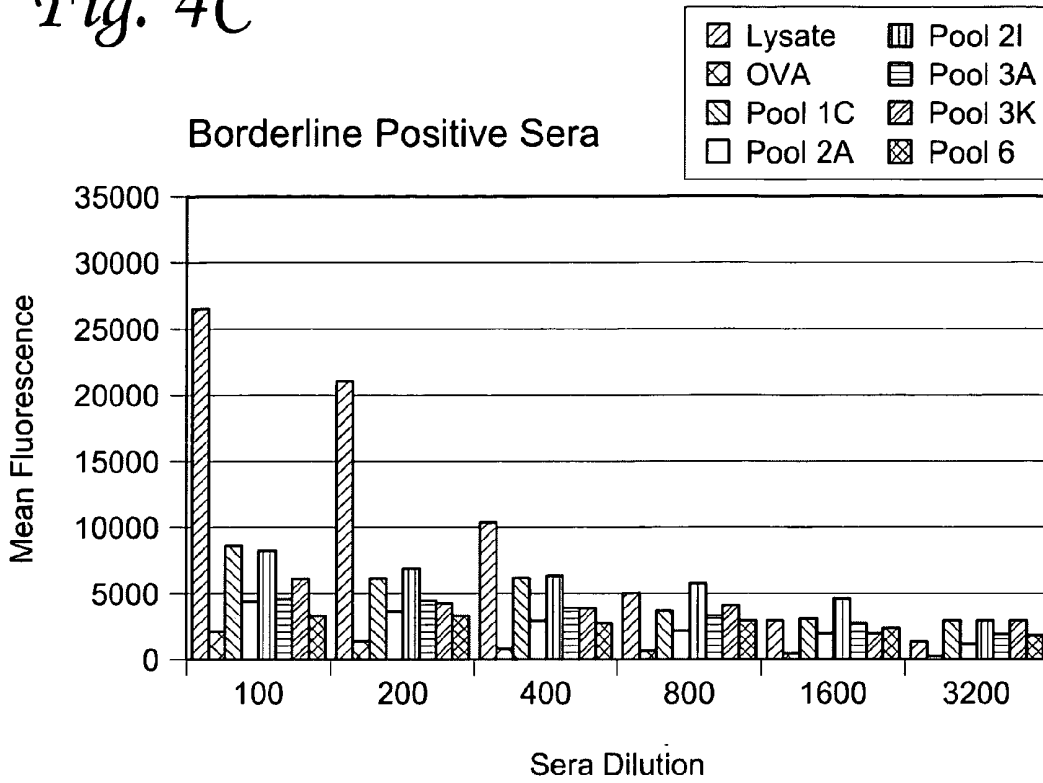


Fig. 4D

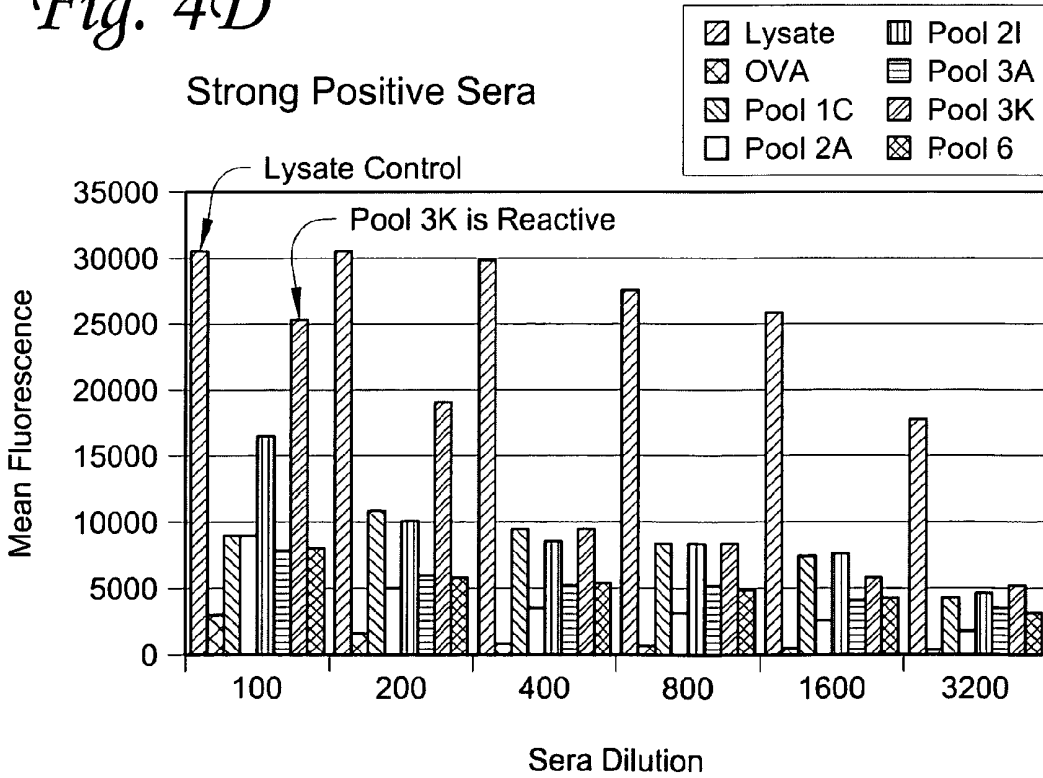


Fig. 5A

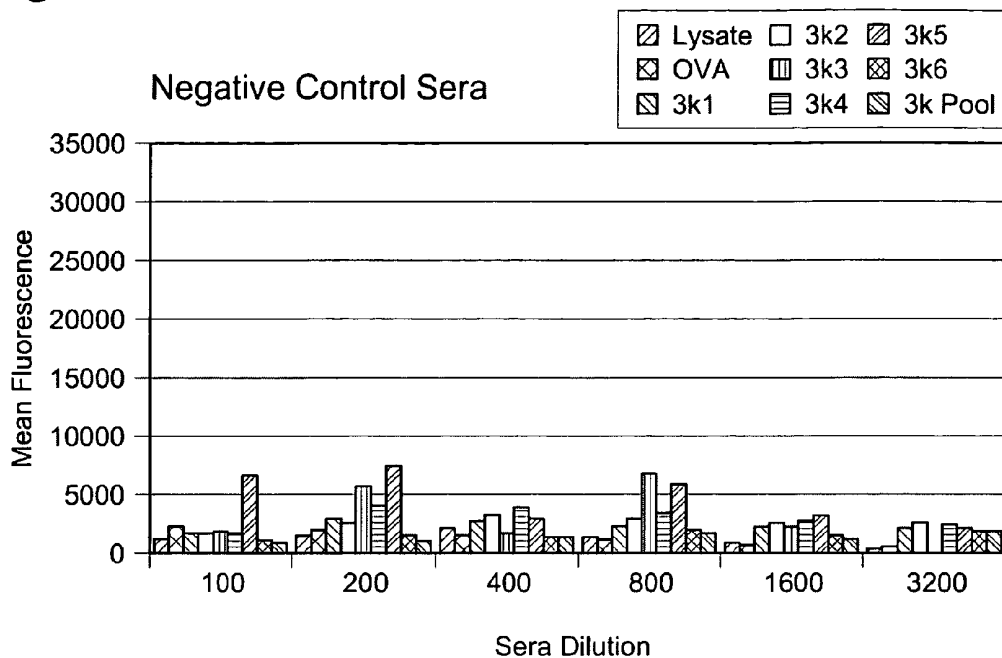


Fig. 5B

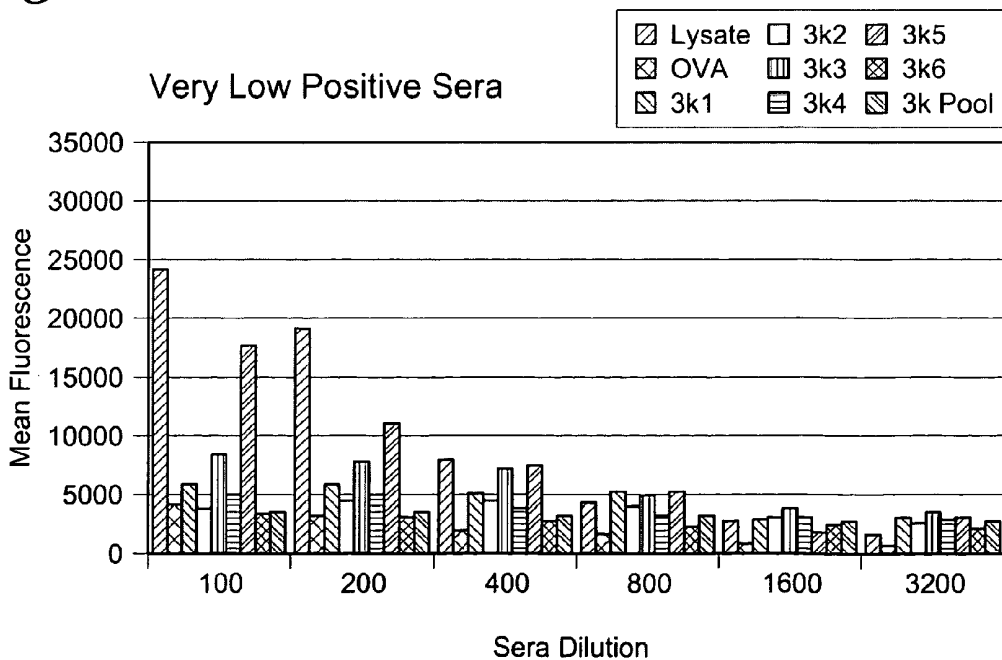


Fig. 5C

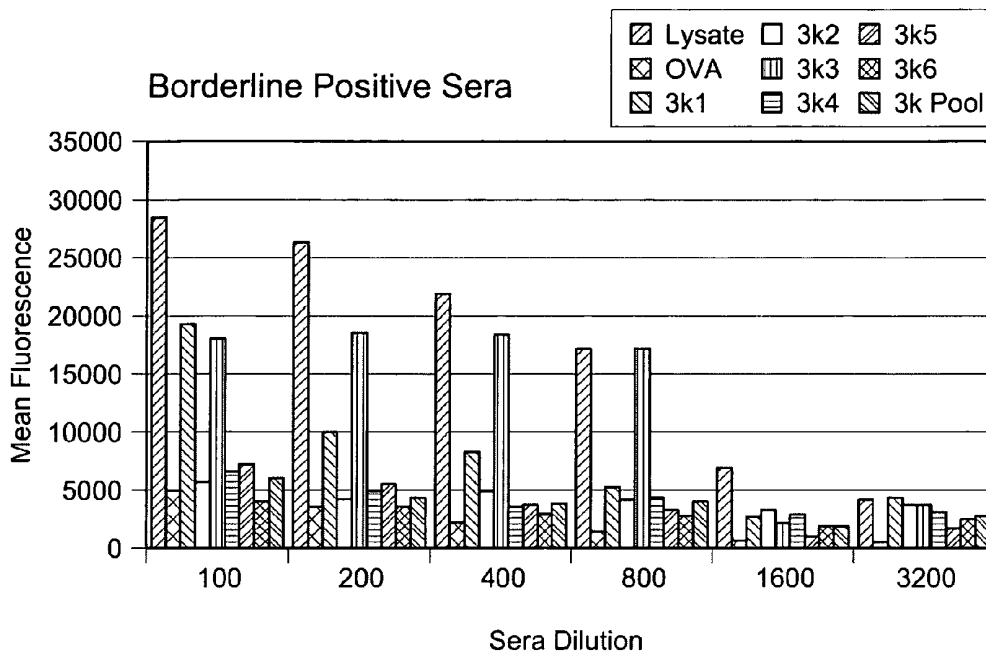


Fig. 5D

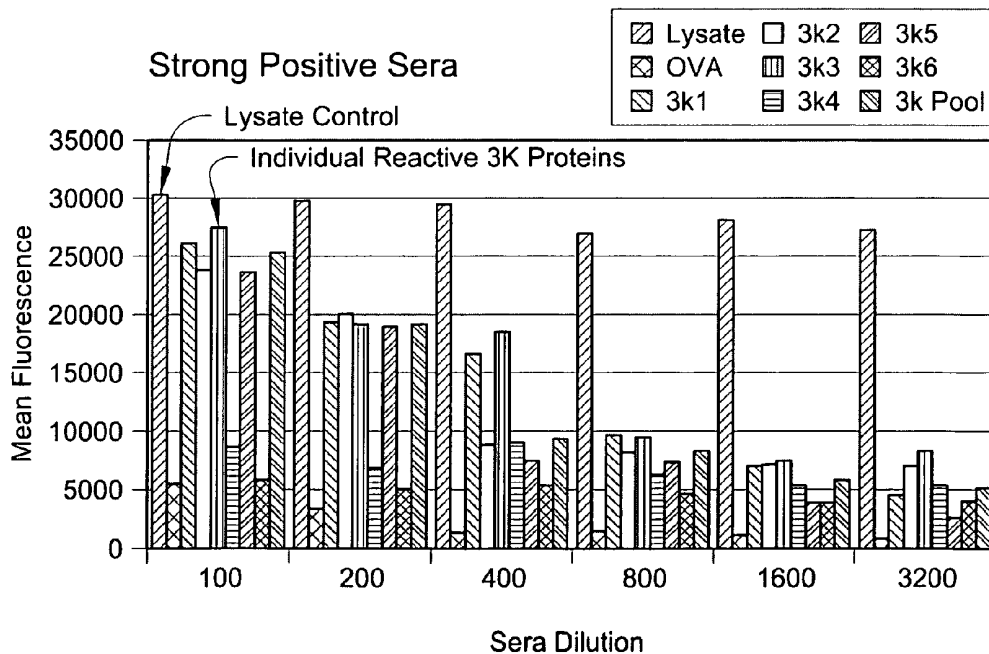


Fig. 6A

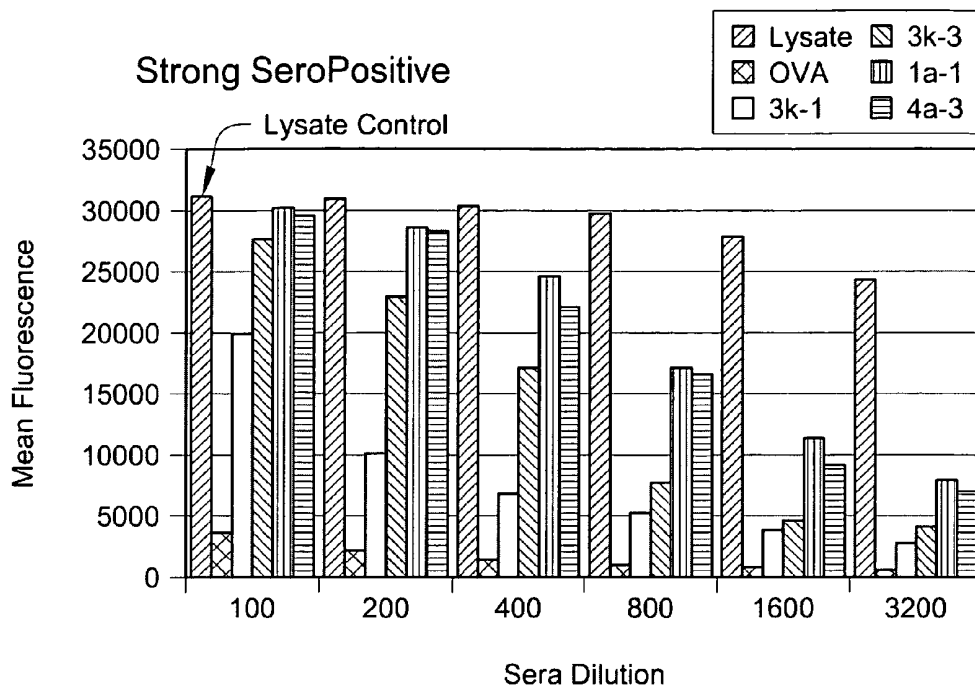


Fig. 6B

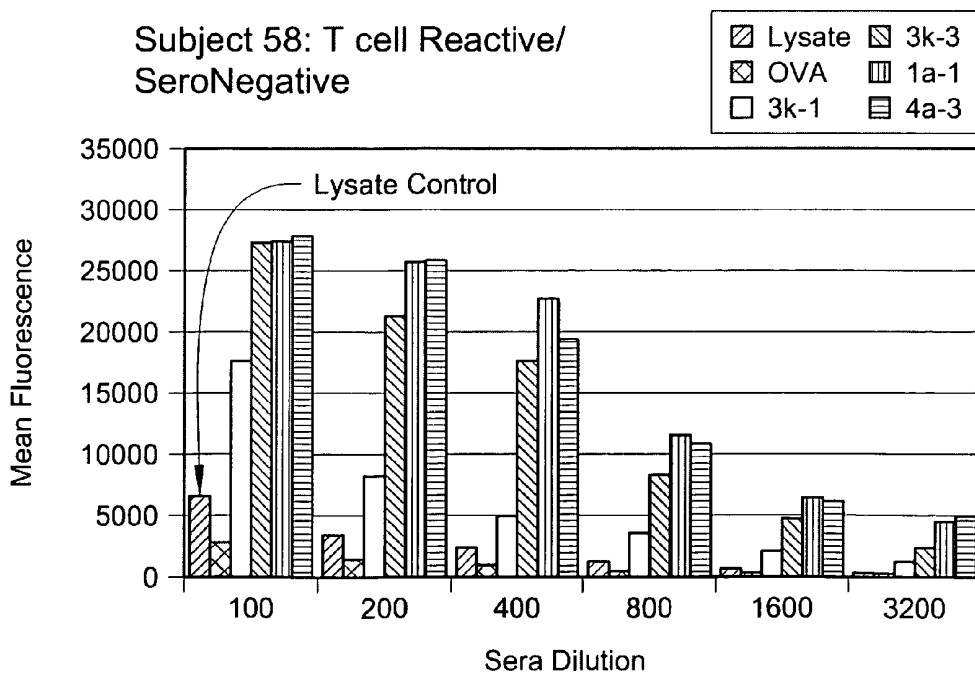


Fig. 6C

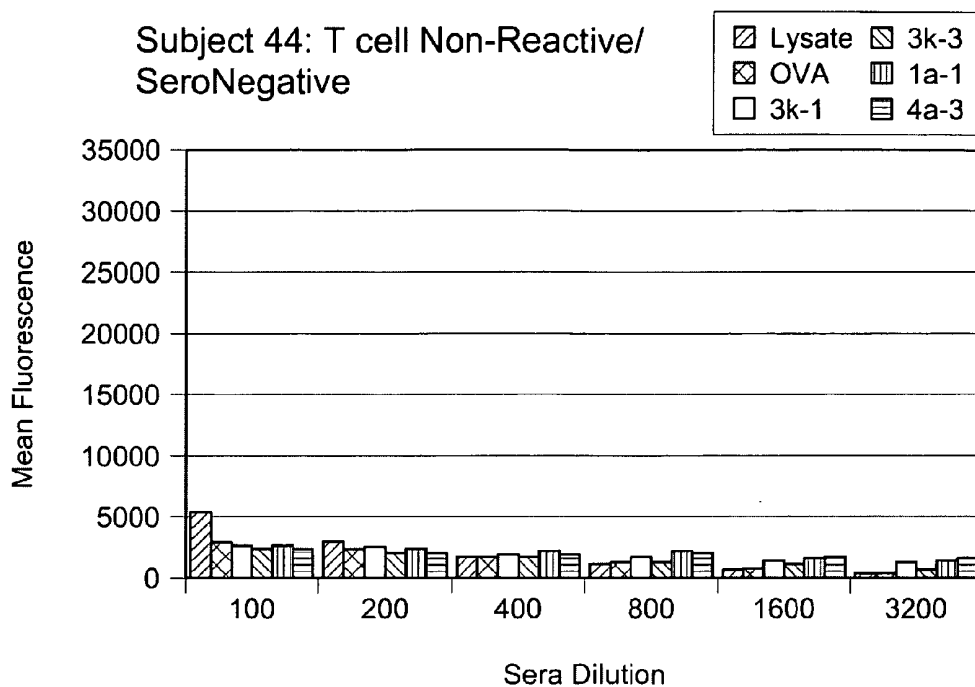
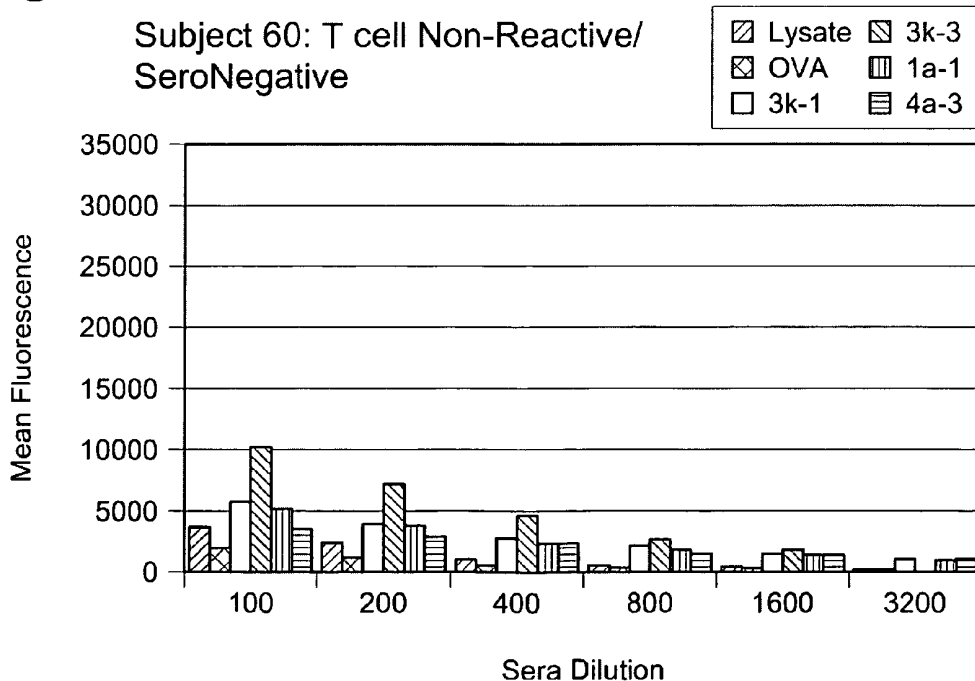


Fig. 6D



Selection of Diagnostic Proteins

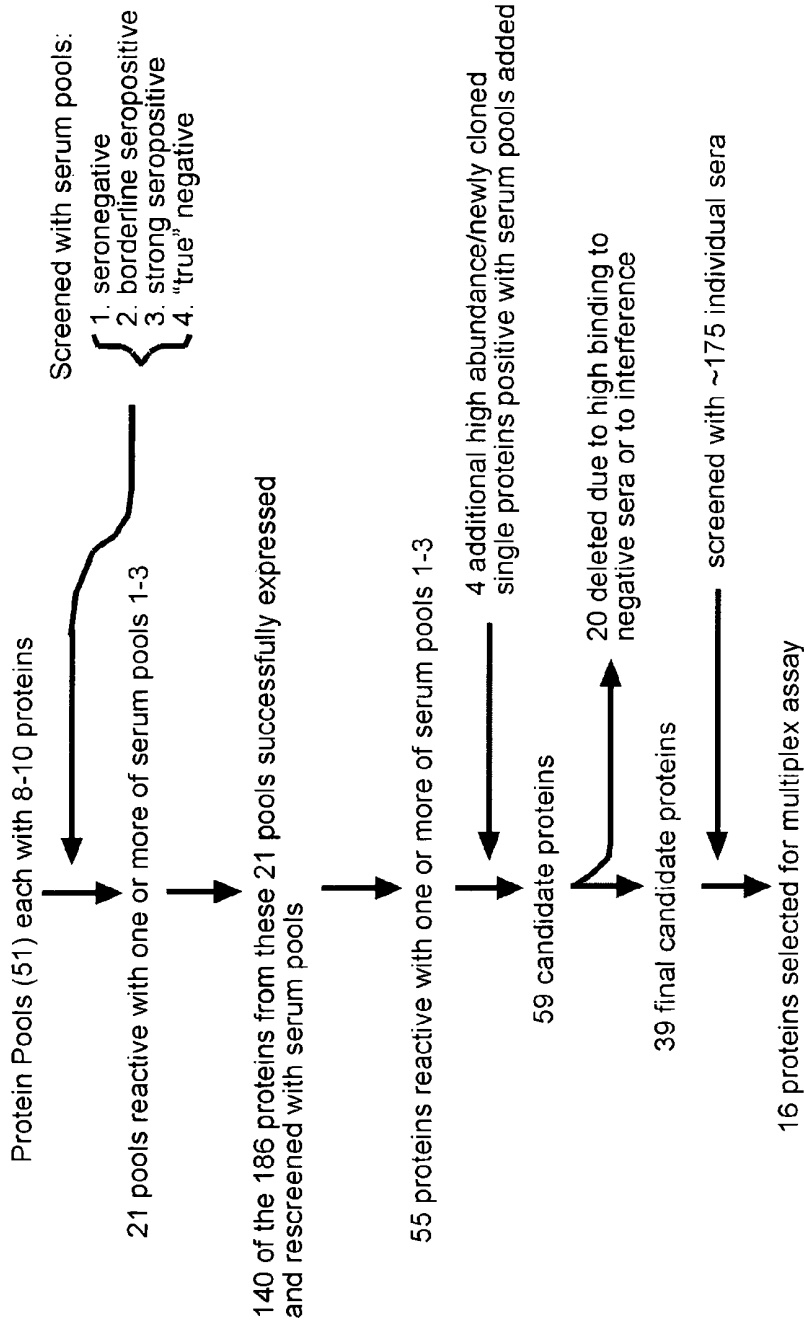


Fig. 7

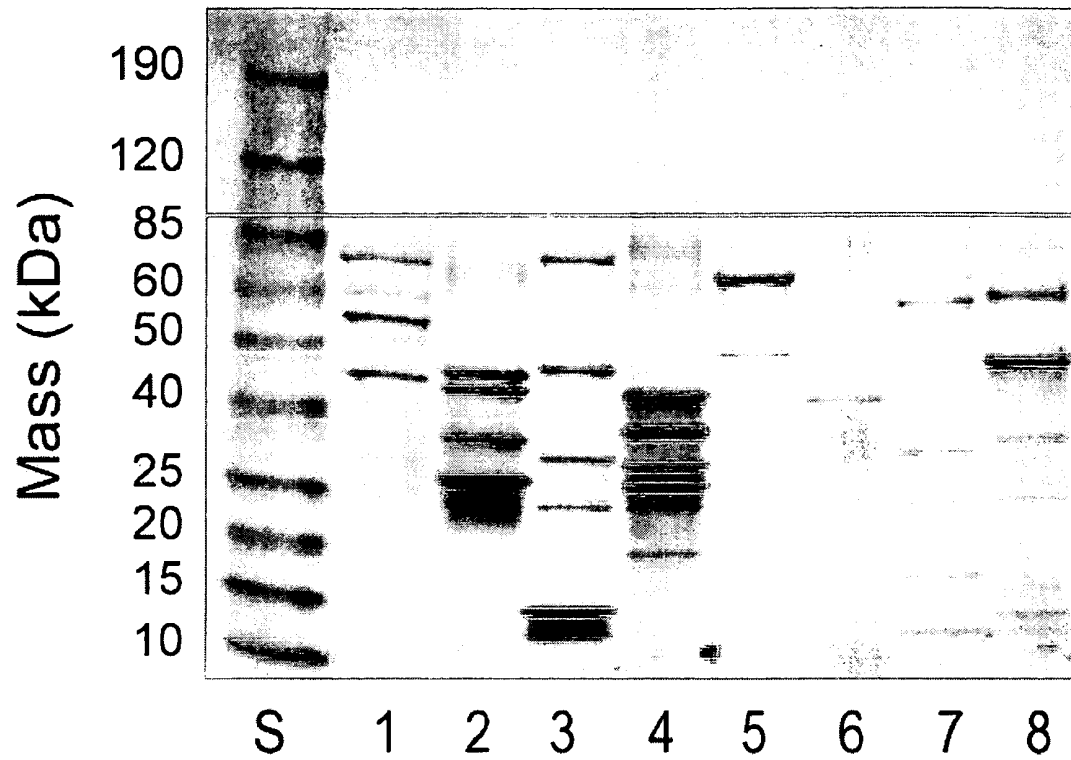


Fig. 8

Fig. 9

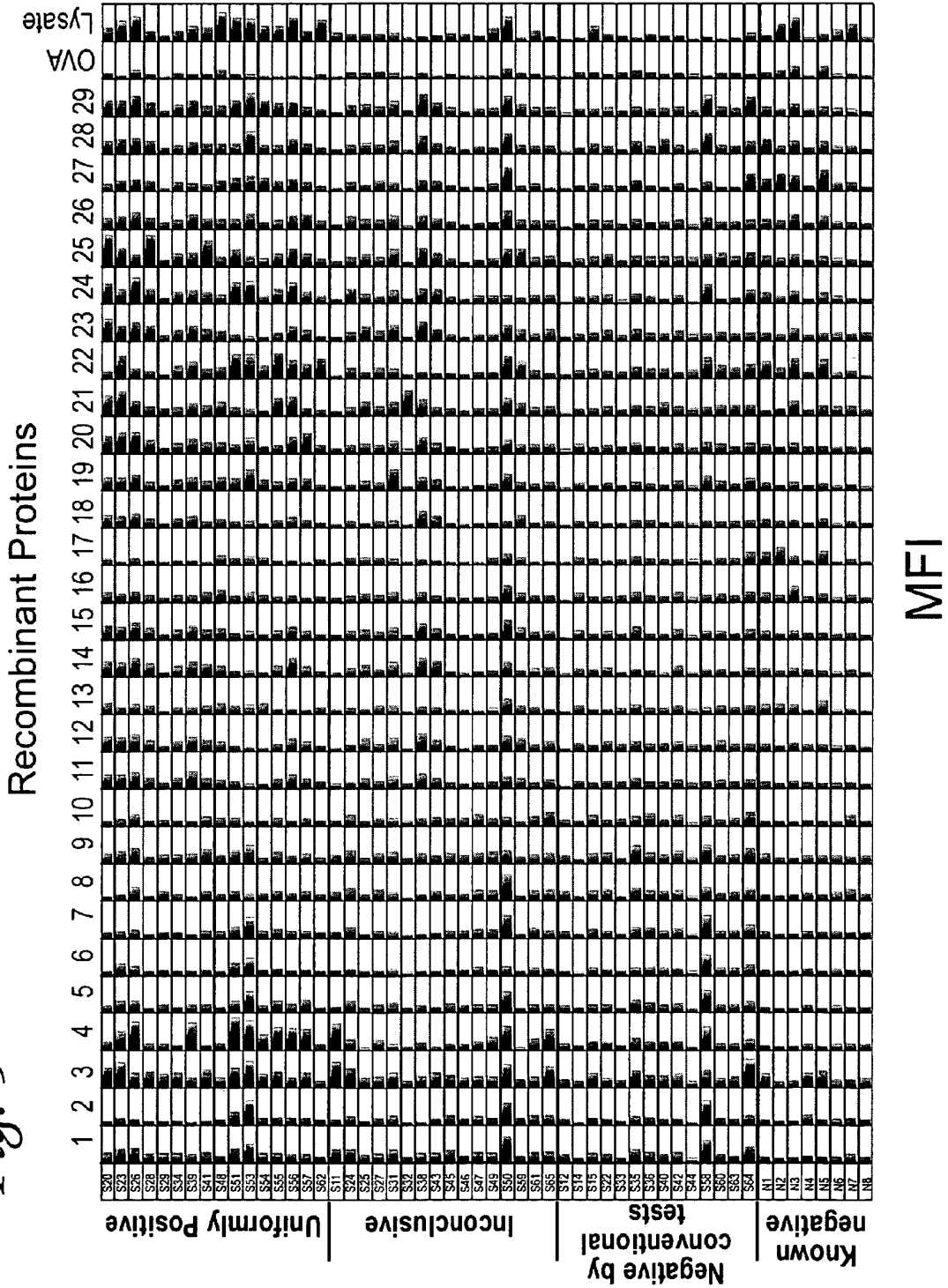
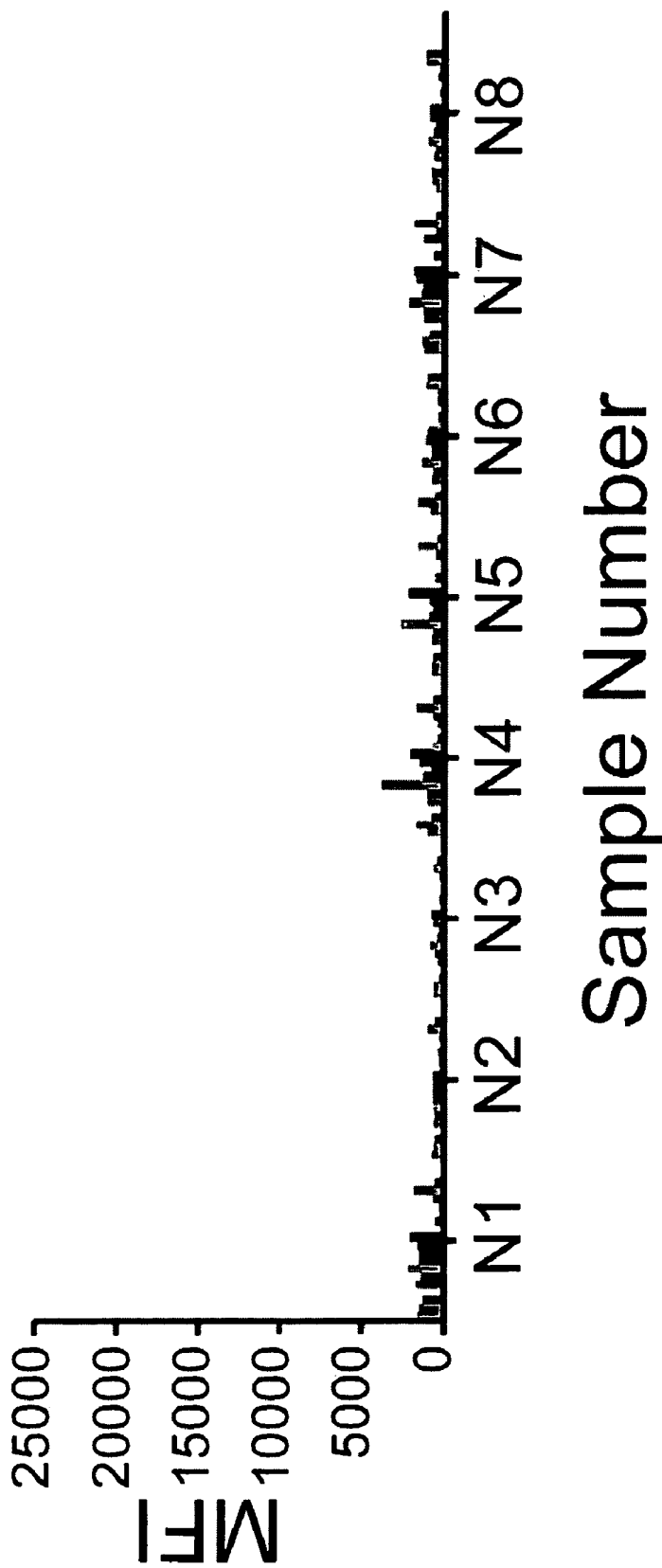


Fig. 10A



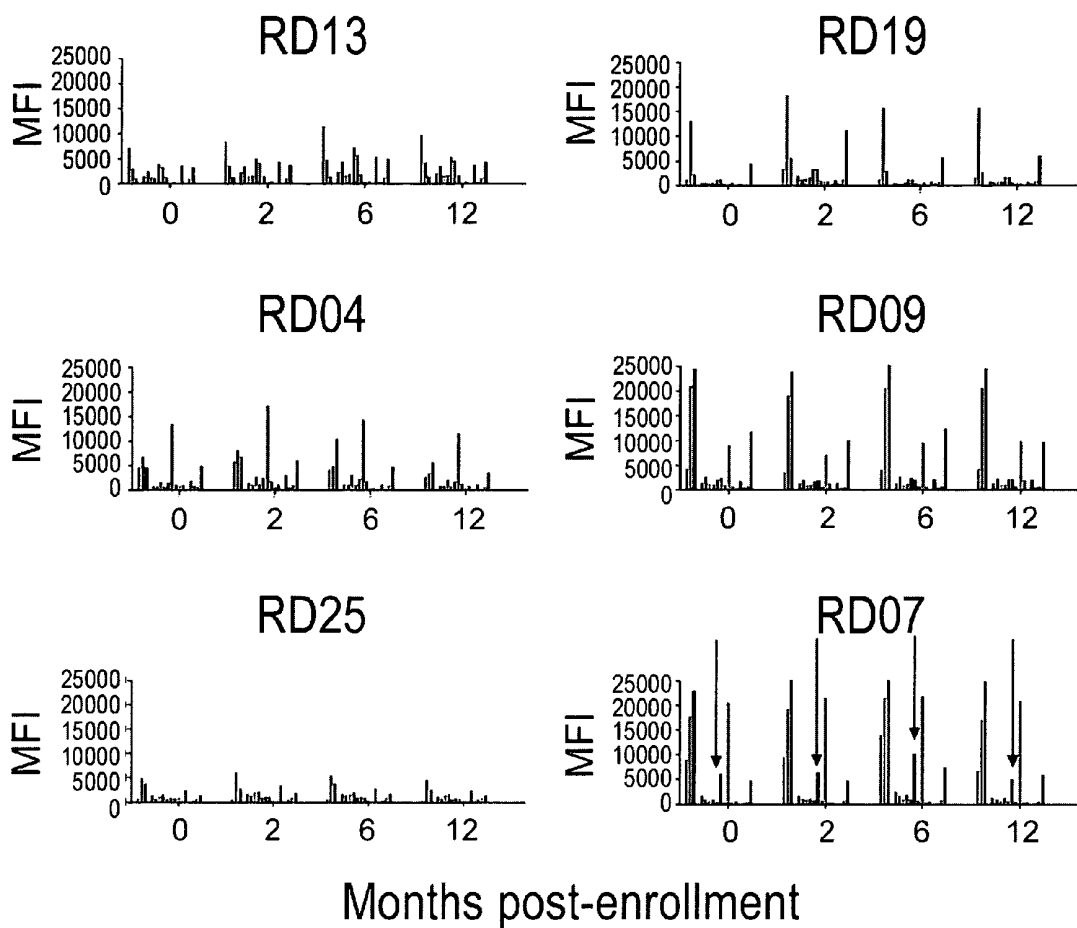
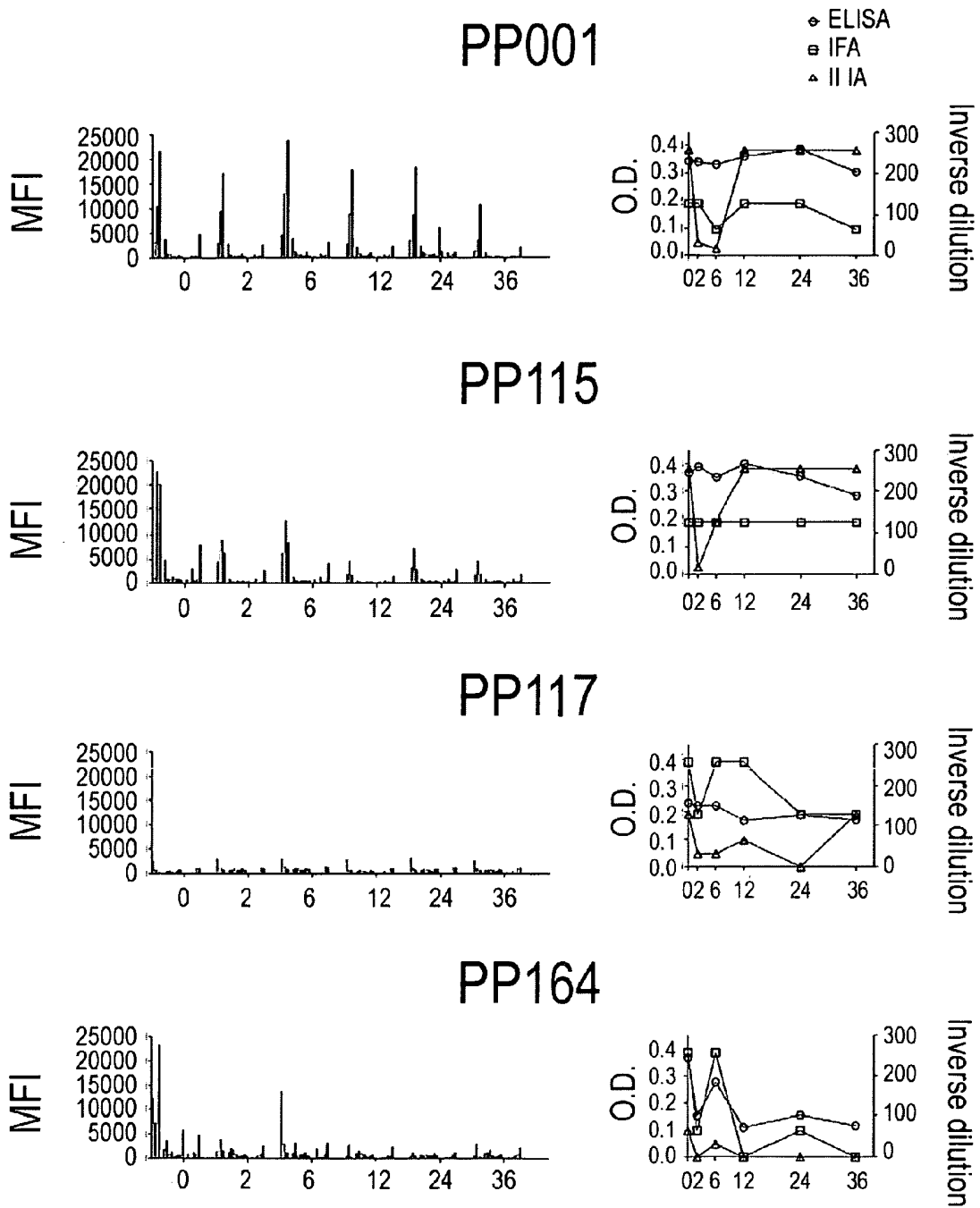


Fig. 10B

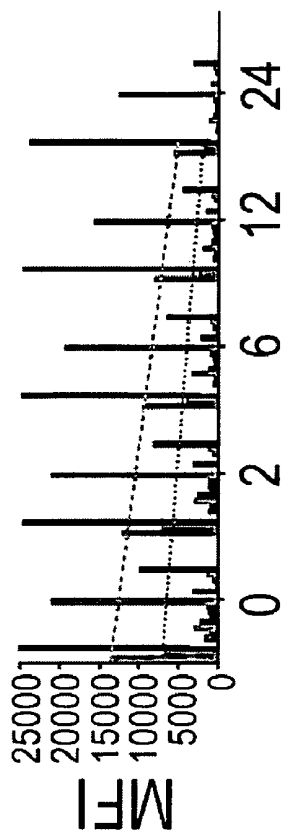


Months post-enrollment

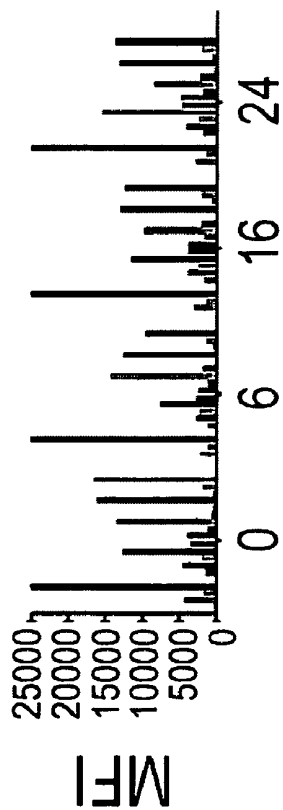
Fig. 11A

Fig. 11B

PP024



PP044



Months post-enrollment

Fig. 12A

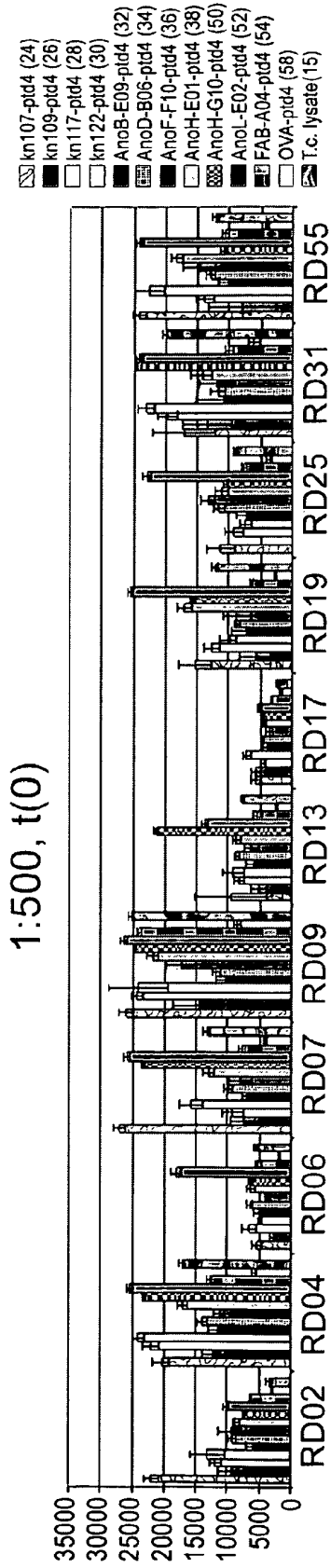


Fig. 12B

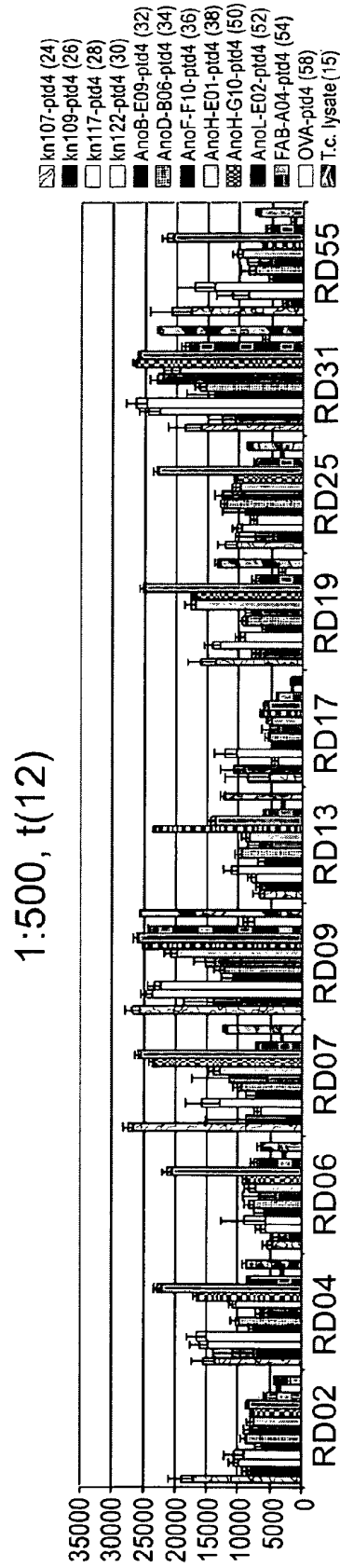
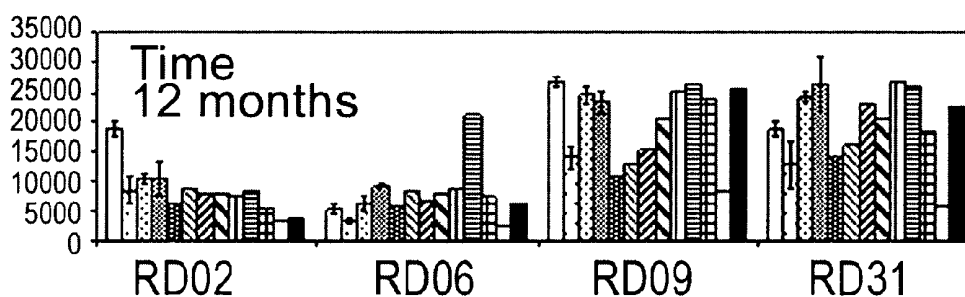
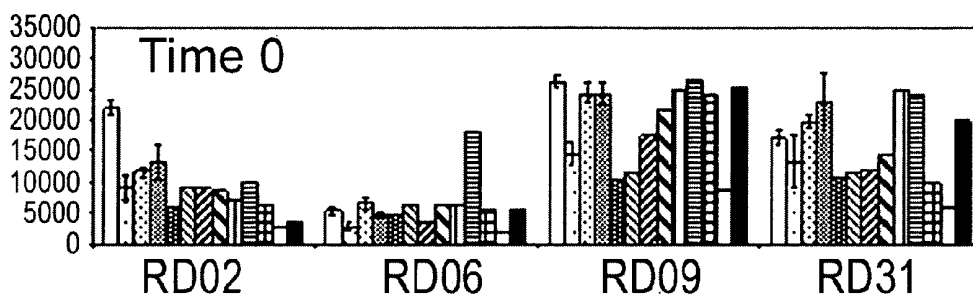


Fig. 13A

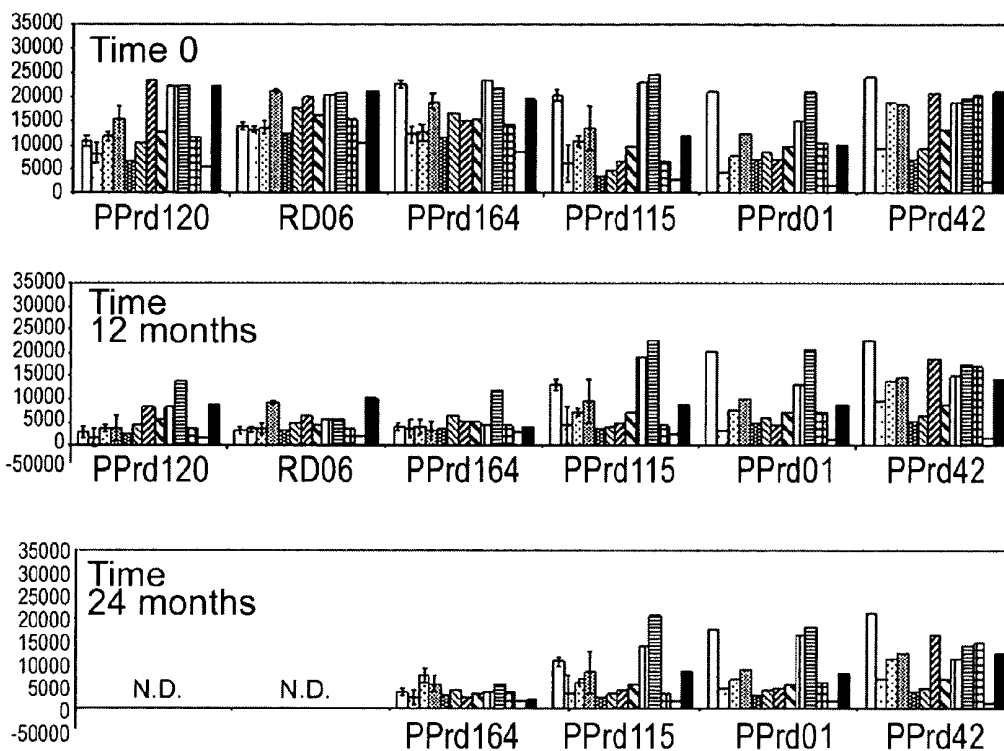
not treated



- kn107-ptd4 (24)
- kn109-ptd4 (26)
- kn117-ptd4 (28)
- kn122-ptd4 (30)
- AnoB-E09-ptd4 (32)
- AnoD-B06-ptd4 (34)
- AnoF-F10-ptd4 (36)
- AnoH-E01-ptd4 (38)
- AnoH-G10-ptd4 (50)
- AnoL-E02-ptd4 (52)
- FAB-A04-ptd4 (54)
- OVA-ptd4 (58)
- T.c. lysate (15)

Fig. 13B

treated



- kn107-ptd4 (24)
- kn109-ptd4 (26)
- kn117-ptd4 (28)
- ▨ kn122-ptd4 (30)
- AnoB-E09-ptd4 (32)
- ▨ AnoD-B06-ptd4 (34)
- ▨ AnoF-F10-ptd4 (36)
- ▨ AnoH-E01-ptd4 (38)
- ▨ AnoH-G10-ptd4 (50)
- ▨ AnoL-E02-ptd4 (52)
- ▨ FAB-A04-ptd4 (54)
- OVA-ptd4 (58)
- T.c. lysate (15)

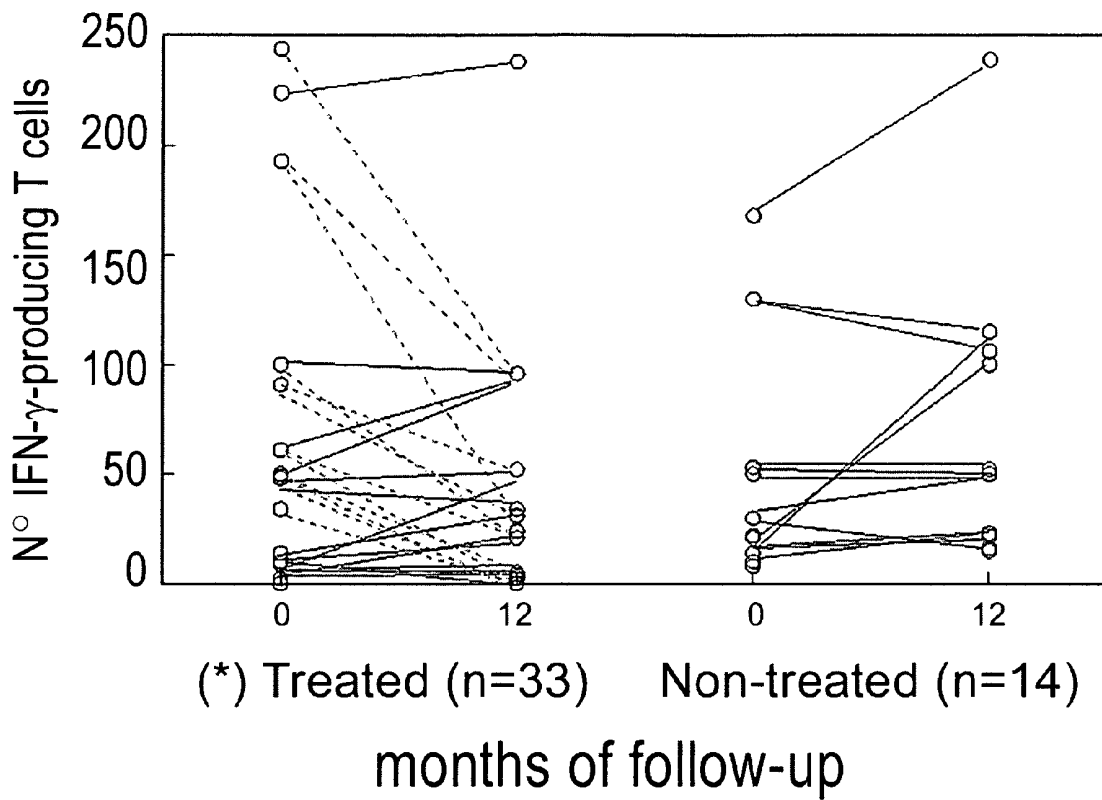


Fig. 14

Fig. 15A

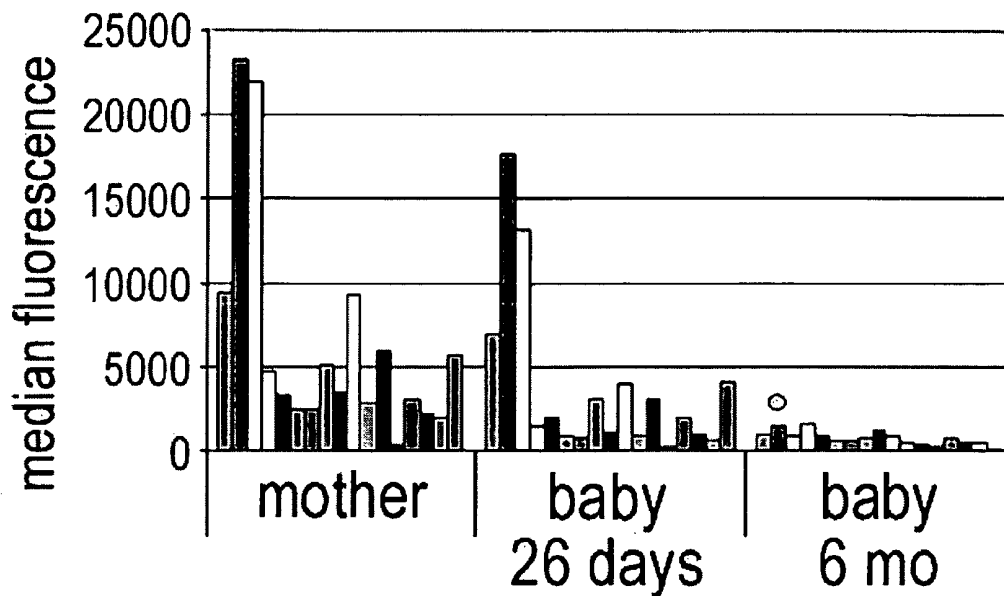
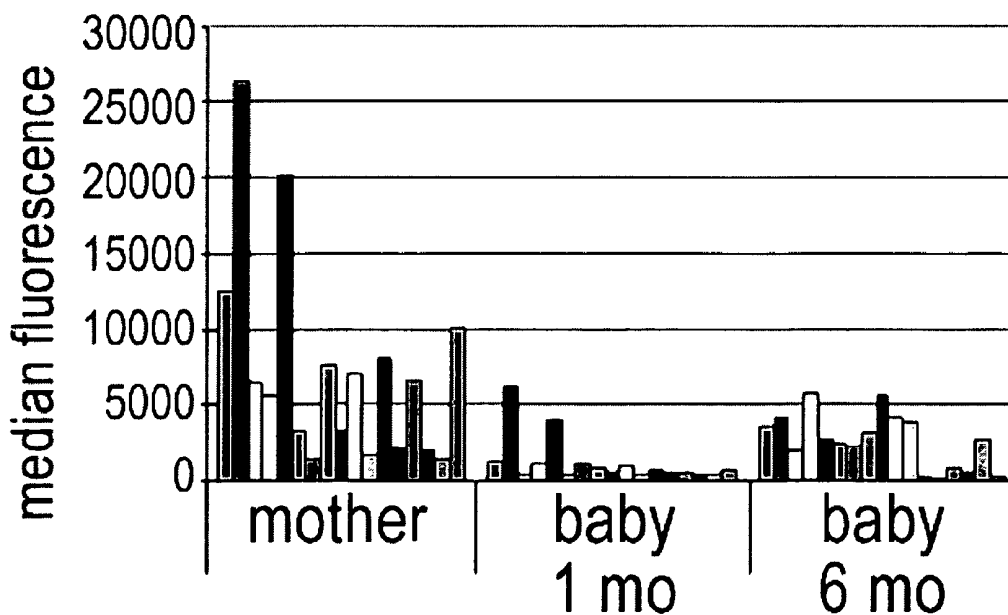


Fig. 15B



DIAGNOSTIC ASSAY FOR *TRYPANOSOMA CRUZI* INFECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 371 of PCT/US08/09174, filed on Jul. 30, 2008, which claims the benefit of U.S. Provisional Application Ser. No. 60/962,498, filed Jul. 30, 2007, and is a continuation-in-part application of U.S. Ser. No. 11/587,283, filed Aug. 2, 2007 (now issued as U.S. Pat. No. 7,888,135), which is a U.S. National Stage Application of international application PCT/US2005/013777, filed Apr. 22, 2005, which claims the benefit of U.S. Provisional Application Ser. No. 60/564,804, filed 23 Apr. 2004, and U.S. Provisional Application Ser. No. 60/623,299, filed 29 Oct. 2004, each of which is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT RIGHTS

This invention was made with government support under Program Project P01 AI0449790 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Trypanosoma cruzi is an obligate intracellular protozoan parasite. In mammalian hosts *T. cruzi* cycles between a trypomastigote stage which circulates in the blood and the amastigote stage which replicates in the cytoplasm of infected host cells (primarily muscle).

T. cruzi is the etiological agent of Chagas disease and is ranked as the most serious parasitic disease in the Americas, with an economic impact far outranking the combined effects of other parasitic diseases such as malaria, schistosomiasis, and *leishmania* (Dias et al., Mem. Inst. Oswaldo Cruz, 1999, 94:Suppl.1:103). Chagas Disease affects up to 20 million individuals primarily in the Americas where the insect vectors are present and where zoonotic transmission cycles guarantee a steady source of parasites. *T. cruzi* infection has its greatest human impact in areas of Latin America where housing conditions bring people, infected animals, and vector insects into close proximity. More than 90 million are at risk of infection in endemic areas, and roughly 50,000 children and adults die of chronic Chagas disease every year due to lack of effective treatments. Additionally, 2-5% of fetus carried by infected mothers in endemic areas are either aborted or born with congenital Chagas disease. Loss of revenue in terms of productivity lost due to sickness and medical costs have an overwhelming effect on economic growth of these countries.

Recently, increasing travel and immigration have brought *T. cruzi* infection into the spotlight globally, even in areas where transmission has previously been absent or very low. *T. cruzi* has spread beyond the borders of Latin America and has been detected in Europe, Asia, and the United States (Ferreira et al., J. Clin. Micro., 2001, 39:4390). In the U.S., 50-100 thousand serologically positive persons progressing to the chronic phase of Chagas disease are present, and the number of infected immigrants in developed countries is increasing. It is expected that, due to the exponential increase in emigration from Latin America, Chagas disease may become a serious health issue in North America and Europe in the next decade.

Congenital and transfusion/transplantation-related transmissions are thus becoming increasingly recognized as significant threats. As the number of infected individuals grows, transmission of *T. cruzi* to non-infected individuals through

blood transfusion and organ transplants from the infected immigrant donors is emerging as a route for *T. cruzi* transmission in more developed nations (Umezawa et. al. J. Clin. Micro., 1999, 37:1554; Silveira et. al. Trends Parasitol., 2001, 17; Chagas disease after organ transplantation—United States, 2001; MMWR Morb Mortal Wkly Rep. 2002 Mar. 15; 51(10):210-2). Each year, 15 million units of blood are transfused and approximately 23,000 organ transplants are performed in the United States alone, and presently almost none of the blood supply is tested for *T. cruzi*. A few cases of infection by *T. cruzi* through organ donation have already been reported to United States Centers for Disease Control since 2001. It has therefore become apparent that the screening of blood and organ donors is necessary not only in Latin America but also in developed countries that receive immigrants from endemic areas.

Diagnosis of *T. cruzi* infection is challenging for a number of reasons. The initial infection is seldom detected except in cases where infective doses are high and acute symptoms very severe, as in localized outbreaks resulting from oral transmissions. Classical signs of inflammation at proposed sites of parasite entry (e.g. “Romahia’s sign”) or clinical symptoms other than fever, are infrequently reported. As a result, diagnosis is very rarely sought early in the infection, when direct detection of parasites may be possible. In the vast majority of human cases, *T. cruzi* infection evolves undiagnosed into a well-controlled chronic infection wherein circulating parasites or their products are difficult to detect even with the use of amplification techniques. A “conclusive” diagnosis of *T. cruzi* infection is often reached only after multiple serological tests and in combination with epidemiological data and (occasionally) clinical symptoms. Further complicating matters, some researchers have reported positive PCR and clinical disease in patients with negative serology. Salomone et al. Emerg Infect Dis. 2003 December; 9(12):1558-62.

Unfortunately, multiple studies from geographically distinct areas and utilizing a wide range of tests and test formats have shown current diagnostics to be far from dependable (Pirard et al., 2005, Transfusion 45: 554-561; Salomone et al., 2003, Emerg Infect Dis 9: 1558-1562; Avila et al., 1993, J Clin Microbiol 31: 2421-2426; Castro et al., 2002, Parasitol Res 88: 894-900; Caballero et al., 2007, Clin Vaccine Immunol. 14:1045-1049; Silveira-Lacerda et al., 2004, Vox Sang 87: 204-207; Wincker et al., 1994, Am J Trop Med Hyg 51: 771-777; Gutierrez et al., 2004, Parasitology 129: 439-444; Marcon et al., 2002, Diagn Microbiol Infect Dis 43: 39-43; Picka et al., 2007, Braz J Infect Dis 11: 226-233; Zarate-Blades et al., 2007, Diagn Microbiol Infect Dis 57: 229-232). Many of the most widely employed serological tests, including one recently licensed by the United States Food and Drug Administration for use as a blood screening test in the U.S. (Tobler et al., 2007, Transfusion 47: 90-96), use crude or semi-purified parasite preparations, often derived from parasite stages present in insects but not in infected humans. The most widely accepted serological tests for *T. cruzi* infection utilize antigens from either whole to semi-purified parasite lysates from epimastigotes that react with anti-*T. cruzi* IgG antibodies. These tests show a degree of variability due to a lack of standardization of procedures and reagents between laboratories, and a number of inconclusive and false positive results occur due to cross-reactivity with antibodies developed against other parasites (Nakazawa et. al. Clin. Diag. Lab. Immunol., 2001, 8:1024).

Other tests have incorporated more defined parasite components, including multiple fusion proteins containing epitopes from various parasite proteins, which, individually

have shown some promise as diagnostics (Caballero et al., 2007, Clin Vaccine Immunol. 14:1045-1049; da Silveira JF et al., 2001, Trends Parasitol 17: 286-291; Chang et al., 2006, Transfusion 46: 1737-1744). Unfortunately, in the absence of a true gold standard, the sensitivity of new tests is generally determined using sera that have been shown to be unequivocally positive on multiple other serologic tests, but rarely with sera that are borderline or equivocal on one or more tests, an approach that assures only that the test being evaluated is no worse, but not necessarily any more sensitive, than the existing tests.

SUMMARY OF THE INVENTION

The present invention provides new tools for diagnosing and treating *T. cruzi* infections in people and animals. In one aspect, the invention provides a method of screening for antigenic *T. cruzi* polypeptides. First and second substrates are provided that each include a plurality of individually addressable candidate antigens derived from *T. cruzi*. The antigens present on the first and second substrate are substantially the same in order to facilitate comparison. The candidate antigens of the first substrate are contacted with a body fluid of a first mammal known to be positive for *T. cruzi* infection. The candidate antigens of the second substrate are contacted with a body fluid from a second mammal known or reasonably believed to be unexposed to *T. cruzi* infection. At least one antigenic *T. cruzi* polypeptide is then identified using a process in which the antigenic *T. cruzi* polypeptide binds to an antibody present in the body fluid of the first mammal but exhibits little or no binding to an antibody present in the body fluid of the second mammal. Optionally, the first and second mammals may be humans.

Positive evidence of *T. cruzi* infection in the first mammal may, for example, be based on a detection method such as a T cell assay, polymerase chain reaction (PCR), hemoculture or a xenodiagnostic technique. Evidence of negative serology in the second mammal is preferably shown by a negative result when the mammal is tested for *T. cruzi* infection utilizing a conventional serodiagnostic test that relies on antigens from whole or semi-purified parasite lysates from *T. cruzi*, such as, for example, from a *T. cruzi* epimastigote lysate.

More than two substrates that include a plurality of individually addressable candidate antigens may be used. Each substrate is contacted with the body fluid from a mammal which exhibits a different level of serological reaction to *T. cruzi* using a conventional serodiagnostic test that relies on antigens from whole or semi-purified parasite lysates from *T. cruzi*. The method optionally further includes the step of preparing the polypeptide antigens from an expression vector including a nucleotide sequence from *T. cruzi*.

Optionally, the screening method may further include a preliminary screening step. The preliminary screening step includes providing a first and a second substrate comprising a plurality of individually addressable antigen pools derived from *T. cruzi* in which the antigen pools present on the first and second substrate are substantially the same. The first substrate is contacted with a body fluid of a first mammal known to be positive for *T. cruzi* infection and the second substrate is contacted with a body fluid from a second mammal known or reasonably believed to be unexposed to *T. cruzi* infection. An antigen pool is then identified that binds to an antibody present in the body fluid of the first mammal but exhibits little or no binding to an antibody present in the body fluid of the second mammal.

In another aspect, the present invention provides an article that includes a substrate and a plurality of individually

addressable antigenic *T. cruzi* polypeptides. The antigenic polypeptides can be selected from the polypeptides identified in Table 1, 2 and/or 4, and include antigenic analogs or subunits thereof. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. The polypeptides are immobilized onto a surface of the substrate. Optionally, the article may include at least one antigenic *T. cruzi* polypeptide identified according to the screening method described above, or antigenic analogs or subunits thereof, immobilized onto the surface of the substrate. In an embodiment, the polypeptides are immobilized on the substrate surface to form a microarray. In another embodiment, the substrate includes at least one nanoparticle, with the polypeptides being immobilized on the surface of the nanoparticle.

The present invention also provides a kit for diagnosis of *T. cruzi* infection that includes an article that includes a substrate and a plurality of individually addressable antigenic *T. cruzi* polypeptides selected from the polypeptides identified in Table 1, 2 and/or 4, in which the polypeptides are immobilized onto a surface of the substrate. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. The kit also includes packaging materials and instructions for use. Optionally, the kit may include at least one antigenic *T. cruzi* polypeptide identified by the screening method described above and immobilized onto the surface of the substrate. The kit may be formulated for medical or veterinary use.

The present invention also provides a diagnostic method for obtaining information about a known or suspected *T. cruzi* infection in a mammal, or for determining whether a mammal is or has been infected by *T. cruzi*. Execution of the method involves obtaining a biological sample from the mammal, contacting the biological sample with a plurality of individually addressable antigenic *T. cruzi* polypeptides selected from the polypeptides identified in Table 1, 2, and/or 4, or antigenic analogs or subunits thereof, and evaluating the presence, absence, intensity or pattern of interaction of components of the biological sample with the antigenic *T. cruzi* polypeptides. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. Optionally, an antigenic *T. cruzi* polypeptide identified according to the screening method described herein, or antigenic analogs or subunits thereof, can be included in the plurality of antigenic *T. cruzi* polypeptides. In a preferred embodiment, the biological sample is contacted with an article that includes a substrate and a plurality of individually addressable antigenic *T. cruzi* polypeptides immobilized onto a surface of the substrate. Information that can be obtained according to the method includes, for example, the presence or absence of *T. cruzi* infection, the identity of the infective strain, the length of the infection, the stage of the infection, whether the infection is still present or the mammal has been cured, the vaccination status of the mammal, the success of treatment, or any combination thereof. The method can, for example, be a serodiagnostic method, wherein the biological sample component that interacts with an antigenic *T. cruzi* polypeptide is an antibody from the mammal. Alternatively, the method may be embodied by a cellular assay method where the biological sample component that interacts with an antigenic *T. cruzi* polypeptide is T

cell from the mammal. Like all diagnostic methods described herein, the method can be implemented as a multiplexed assay in which the biological sample is contacted simultaneously with the plurality of antigenic *T. cruzi* polypeptides. The biological sample can, for example, be obtained from a person suspected of having or being exposed to disease, or obtained from an actual or potential blood donor or transplant donor. Alternatively, the biological sample is obtained from a pooled blood product supply intended for use in transfusions or research.

Also provided by the invention is a method for detecting a *T. cruzi* infection, particularly a maternally transmitted *T. cruzi* infection, in an infant born to a mother who is known to have, or suspected of having, a *T. cruzi* infection. A biological sample is obtained from the infant and contacted with a plurality of individually addressable antigenic *T. cruzi* polypeptides, or antigenic analogs or subunits thereof. The biological sample is preferably a bodily fluid, more preferably blood, plasma or serum. The timing for obtaining the sample from the infant is important, as enough time should have elapsed after the birth such that antibodies produced by the infant can be detected. The sample can be obtained from the infant at about two or three months after birth but is preferably obtained about 4, 5 or 6 months after birth, or later. Preferably, at least one polypeptide is selected from the polypeptides listed in Table 1, Table 2 and/or Table 4. The presence, absence, intensity or pattern of interaction of components of the biological sample, particularly antibodies, with the antigenic *T. cruzi* polypeptides is evaluated to determine whether the infant exhibits an antibody response that exceeds background levels.

The method optionally further includes comparing the infant's antibody response to the plurality of antigenic *T. cruzi* polypeptides with the antibody response of the infant's mother to the same or similar panel of *T. cruzi* polypeptides. Comparison with the mother's antibody response is especially useful when the infant's antibody response is higher than background level. A biological sample is obtained from the infant's mother and contacted with the plurality of individually addressable antigenic *T. cruzi* polypeptides, or antigenic analogs or subunits thereof. The biological sample of the mother can be obtained prior to birth, during birth, or after birth. The presence, absence, intensity or pattern of interaction of components of the mother's biological sample with the antigenic *T. cruzi* polypeptides is compared to the presence, absence, intensity or pattern of interaction of components of the infant's biological sample with the antigenic *T. cruzi* polypeptides, to determine whether the infant's antibody response differs from the mother's antibody response. A difference in antibody responses, where the infant's response is above background levels, indicates that the infant may have a *T. cruzi* infection. Similar antibody responses for mother and infant indicate that maternal antibodies may still be present in the infant's bodily fluids. In that event, the comparison is optionally repeated using a biological sample can be obtained from the infant at a later date.

Additionally or alternatively, the method further optionally includes comparing the infant's antibody response to the plurality of antigenic *T. cruzi* polypeptides with the infant's antibody response to the same or similar panel of *T. cruzi* polypeptides as measured earlier, i.e., shortly after birth. Shortly after birth, the infant's antibody response is expected to mirror the mother's antibody response, reflecting the presence of maternal antibodies in the infant's bodily fluids. A biological sample is obtained from the infant shortly after birth, contacted with the plurality of individually addressable antigenic *T. cruzi* polypeptides, or antigenic analogs or sub-

units thereof, and the presence, absence, intensity or pattern of interaction of components of the infant's earlier biological sample with the antigenic *T. cruzi* polypeptides is compared to the presence, absence, intensity or pattern of interaction of components of the infant's later biological sample, or of the mother's biological sample, or both, with the antigenic *T. cruzi* polypeptides, to determine whether the infant's later antibody response differs from the mother's antibody response, wherein a difference in antibody responses indicates that the infant may have a *T. cruzi* infection.

In instances wherein the method identifies an infant having or suspected of having a *T. cruzi* infection, the method further optionally includes treating the infant for a *T. cruzi* infection, for example by administering a therapeutic agent to the infant.

In another aspect, the present invention provides a method for detecting contamination of a blood product supply with *T. cruzi*. The method of detecting contamination includes selecting a sample from the blood supply, contacting the sample with a plurality of individually addressable antigenic *T. cruzi* polypeptides selected from the polypeptides identified in Table 1, 2 and/or 4, or antigenic analogs or subunits thereof, and evaluating the presence, absence, intensity or pattern of interaction of components of the sample with the antigenic *T. cruzi* polypeptides to determine whether the blood supply is contaminated with *T. cruzi*. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. Optionally, an antigenic *T. cruzi* polypeptide identified according to the screening method described herein, or antigenic analogs or subunits thereof, can be included in the plurality of antigenic *T. cruzi* polypeptides. In a preferred embodiment, the blood supply sample is contacted with an article that includes a substrate and a plurality of individually addressable antigenic *T. cruzi* polypeptides immobilized onto a surface of the substrate.

Blood products that can be tested include whole blood, a blood product, or a blood fraction. For example, a cellular blood component, a liquid blood component, a blood protein, or mixtures thereof, or a red blood cell concentrate, a leukocyte concentrate, a platelet concentrate, plasma, serum, a clotting factor, an enzymes, albumin, plasminogen, or an immunoglobulin, or mixtures of thereof, can be tested for contamination according to the method.

The method of detecting contamination can be a serodiagnostic method, wherein the sample component that interacts with an antigenic *T. cruzi* polypeptide is an antibody. Alternatively, the method can take the form of a cellular assay method, wherein the sample component that interacts with an antigenic *T. cruzi* polypeptide is T cell.

In yet another aspect, the present invention provides a multicomponent vaccine. In one embodiment, the vaccine includes a plurality of immunogenic *T. cruzi* polypeptides selected from the *T. cruzi* polypeptides listed in Table 1, 2, and/or 4, or immunogenic subunits or analogs thereof. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. The multicomponent polypeptide vaccine optionally includes at least one immunogenic *T. cruzi* polypeptide identified according to the screening method described herein, or immunogenic subunit or analog thereof. In another embodiment, the multicomponent vaccine includes one or more polynucleotides operably encoding a

plurality of immunogenic *T. cruzi* polypeptides selected from the *T. cruzi* polypeptides listed in Table 1, 2, and/or 4 or immunogenic subunits or analogs thereof. In some embodiments, some or all of the polypeptides are selected from the polypeptides listed in Table 2 and/or Table 4, with the proviso that at least one of the polypeptides selected from Table 2 and/or Table 4 is a polypeptide that is not listed in Table 1. The multicomponent polynucleotide vaccine optionally includes a polynucleotide operably encoding a polypeptide identified according to the screening method, or immunogenic subunit or analog thereof. The multicomponent vaccine may be a therapeutic or prophylactic vaccine.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 provides a pictorial overview of the BIO-PLEX array analysis method; A) shows the protein-antibody-microsphere complex used by the BIO-PLEX method, B) shows multiple complexes in the well of a microplate substrate, and C) shows laser excitation of the complexes as they flow through a flow cytometer.

FIG. 2 provides a pictorial overview of the GATEWAY cloning method used to provide an expression vector used for the preparation of *T. cruzi* polypeptide antigens in one embodiment of the invention.

FIG. 3 shows assay development using varicella voster (VV)-ovalbumin sera; A, BIO-PLEX assay; B, ELISA assay.

FIG. 4 shows testing of protein pools for antigenic potential using A, negative control sera; B, very low positive sera; C, borderline positive sera; and D, strong positive sera. From left to right, in each panel at each of the sera dilutions, the tested samples are: lysate control, ovalbumin, pool 1C, pool 2A, pool 2I, pool 3A, pool 3K and pool 6. Pool 3K reacted with antibodies from infected individuals and was a candidate for further testing.

FIG. 5 shows testing of the component proteins of pool 3K for antigenic potential using A, negative control sera; B, very low positive sera; C, borderline positive sera; and D, strong positive sera. From left to right, in each panel at each of the sera dilutions, the tested samples are: lysate control, ovalbumin, protein 3K-1, 3K-2, 3K-3, 3K-4, 3K-5 and 3K-6, and pool 3K. Proteins 3K-1, 3K-2, 3K-3 and 3K-5 demonstrated varying degrees of reactivity to antibodies in sera from infected individuals.

FIG. 6 shows testing of four different serum samples using a panel of serodiagnostic proteins; A, strong seropositive serum; B, Subject 58: T cell reactive/seronegative serum; C, Subject 44: T cell non-reactive/seronegative serum; D, Subject 60: T cell non-reactive/seronegative. From left to right, in each panel at each of the sera dilutions, the test proteins are: lysate control, ovalbumin, protein 3K-1, 3K-3, 1A-1 and 4A-3, and pool 3K. Subject 58, declared seronegative by standard serological assay but exhibiting T cell reactivity to *T. cruzi* antigens, is of particular interest because antibodies are detected that recognize the recombinant *T. cruzi* antigens but not the parasite lysate.

FIG. 7 is a schematic showing a screening process for the high-throughput selection of diagnostic proteins for detection of *T. cruzi* infection.

FIG. 8 shows an SDS-PAGE gel of production of pooled protein. Sets of 6-8 genes were moved in pools from pDONR entry plasmids into Pdest[-PTD4] via a GATEWAY LR reaction INVITROGEN and the resulting plasmids transformed into BL21(DE3)pLysS cells for protein production. Recombinant HIS-tagged proteins were purified on Co+2 affinity resin and the bound proteins analyzed by SDS-PAGE. Protein pools depicted in lanes 1-5 were generated from the pooling

of 8 genes, while lanes 6, 7 and 8 were derived from 7, 6, and 6 genes respectively. Lane "S" contained molecular weight standards (BenchMark Prestained Protein Standard; INVITROGEN). Overall, approximately 80% of genes yielded proteins when expressed as pools.

FIG. 9 shows reactivity of a representative set of proteins tested with individual sera. A selection of 29 individual recombinant proteins was tested for the ability to bind IgGs present in the sera of 54 subjects. The sera are grouped as "uniformly positive" (reactive on all three conventional serological tests and a commercial assay kit), "inconclusive" (negative on at least one conventional serologic tests), "negative by conventional tests" (negative by all three conventional tests), and "known negative" (from residents of North America with very low chance of being infected based upon residency and travel history). Recombinant ovalbumin and *T. cruzi* lysate-coated beads were used as negative and positive controls, respectively. Horizontal bars in each box indicate mean fluorescence intensity (MFI) on a scale from 0 to 30,000 arbitrary light units. A number of the recombinant proteins either failed to discriminate between uniformly positive and known negative sera sets (e.g. 3, 16, 17, 22, 26, 27, 28) or showed no reaction with either set (e.g. 10). In contrast a number of proteins detected nearly the entire uniformly positive group, as well as some in the inconclusive and conventional negative groups but none in the known negative set (e.g. 4, 11, 19).

FIG. 10 shows stability of serological responses over time. The MFI of sera to a panel of 16 recombinant proteins (top 16 in Table 2), GFP negative control protein and *T. cruzi* lysate for a total of 18 measurements (bars) are shown for each serum. A) Reactivity of a set of 8 known negative sera. B) Stability of unique pattern of antigen activity for 6 seropositive subjects assayed at 4 time points over 12-21 months. Arrows in lower right panel (RD 07) indicate that detection of protein "8" (paraflagellar rod protein) which distinguishes the pattern of reactivity of serum RD07 from that of the similar RD09.

FIG. 11 shows the effect of benznidazole treatment on serological responses in chronically infected subjects. The MFI of sera to a panel of 16 recombinant proteins (top 16 in Table 2), GFP negative control protein and *T. cruzi* lysate for a total of 18 measurements (bars) are shown for each serum. A) Change in pattern of reactivity in 4 subjects over 36 months post-benznidazole treatment, measured using both the multiplex serologic assay (left) and conventional serology (right). B) Benznidazole-treated subjects exhibiting no evidence of change in multiplex assay for 24 months post-treatment (left; PP044) and or only changes in reactivity to selected recombinant proteins (right; PP024).

FIG. 12 shows the pattern of antigen-specific antibody responses in chronic Chagas disease over time (A, 0 months; B, 12 months) and among different individuals.

FIG. 13 shows the effects of benznidazole treatment over time on antibody titers to recombinant proteins; A, untreated; B, treated.

FIG. 14 shows the effect of treatment with benznidazole on *T. cruzi*-specific memory T cell responses in chronic Chagas disease subjects. The frequency of IFN-(gamma)-producing T cells specific for *T. cruzi* significantly decreased in the benznidazole-treated patient group vs non-treated, at 12 months after follow-up. ELISPOT responses became negative in 1/25 (44%), 6 patients at 12 months post-treatment and 5 patients at 24 months post treatment.

FIG. 15 shows the antibody profiles of four mothers (A, B, C, D) with chronic Chagas disease and their infants at a time point relatively soon after birth, and again later when the infant is 6-7 months old.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

In one aspect, the present invention is directed to the detection of *T. cruzi* infection in a mammal, particularly a human. A plurality of *T. cruzi* polypeptides, or subunits or analogs thereof, that are detectable by antibodies present in a bodily fluid, such as blood, plasma or serum, of at least some individuals that are infected with *T. cruzi* are included in a multicomponent panel for use in a diagnostic assay, which may be a serodiagnostic assay or a cellular assay. The term "serodiagnostic" is used because the assay is typically performed on a blood component such as whole blood, plasma or serum, but it should be understood that any bodily fluid that may provide evidence of an immune response to *T. cruzi* can be assayed using the serodiagnostic test of the invention.

The panel components are contacted with a bodily fluid of an individual such as blood, plasma, serum, urine, saliva or tears and the like, and the presence or absence of evidence of an immune response to *T. cruzi* in the individual is evaluated. The body fluid that is tested can be that of an individual patient to be screened, or it can be a body fluid that is part of a blood or plasma supply, for example, pooled or unpooled, that is available for transfusion and/or research.

An immune response indicative of *T. cruzi* infection may be evidenced by the binding of antibodies in the biological fluid to panel components. The panel components can likewise be used to assess the presence of a T cell response in the subject.

In a preferred embodiment, the diagnostic test is highly specific for *T. cruzi* infection and sufficiently sensitive to detect infection in subjects considered negative with conventional serological assays based on *T. cruzi* lysates due to a poor or inconsistent B cell response to infection. Optionally the test can include, as specificity controls, polypeptide antigens that are recognized when other infections are present.

The diagnostic test can detect the presence or absence of *T. cruzi* infection. In some embodiments, and depending on the antigenic polypeptides selected for including in the multicomponent panel, the pattern of antigen recognition may provide additional information such as the stage of infection or the severity of disease. The antigen recognition pattern may also be useful to discriminate among patients with active or latent infections, and those who have been cured or vaccinated.

The multicomponent diagnostic assay (also referred to herein as a multiplexed assay) has advantages over conventional serodiagnostic methods. For example, the multiplexed assay of the invention consistently detects infection, whereas conventional assays are plagued by high failure rates and inconsistent performance. The Examples below show that infected subjects produce individual patterns of antibody responses that differ from one another, rendering serodiagnostics based on a single antigen a less effective diagnostic than a multiplexed assay. There are numerous examples of the failure of conventional serology to detect infection, and parasitological tests are also unreliable. Individuals who are seropositive in the multiplex assay of the invention are likely to be infected with *T. cruzi*, particularly if they exhibit antibodies to at least 4, more preferably 6, and even more preferably 8 different recombinant *T. cruzi* proteins, and/or were born in endemic areas and/or have evidence of heart disease.

Such individuals are likely to be infected with *T. cruzi* even if they exhibit negative results with conventional serologic assays.

Additionally, the multiplexed serodiagnostic assay of the invention provides a better measure of the efficacy and effectiveness of therapeutic treatment than conventional serological or parasitological assays. Most subjects are negative by parasitological assays prior to treatment and remain positive by conventional serology for long periods of time after treatment. However a multiplexed assay using a selected set of recombinant proteins as described herein can detect changes in antibody levels upon completion of treatment. The use of multiple targets allows serologic changes to be detected following treatment when similar changes are not consistently observed using conventional serologic tests.

Also, the *T. cruzi* proteins identified herein for use in the multiplexed assays of the invention (both serodiagnostic and cellular) are expected to be effective diagnostics for different *T. cruzi* strains in different regions, since many of them are unique to *T. cruzi* and/or highly abundant proteins, such as "housekeeping" proteins, that are expected to show little variation among the different strains.

The invention is applicable to human disease but also has veterinary applications. For example, a diagnostic assay developed according to the invention can be used to diagnose *T. cruzi* infection in farm animals or pets, such as dogs.

Antigenic Polypeptides

A *T. cruzi* polypeptide, or subunit or analog thereof, that is suitable for inclusion in the panel is one that reacts to antibodies in the sera of individuals infected with *T. cruzi*. Such a polypeptide is referred to herein as an antigenic polypeptide or a polypeptide antigen.

A preferred antigenic polypeptide, or antigenic subunit or analog thereof, is one that detectably binds antibodies in a bodily fluid of a subject who is known to be infected or to have been infected by *T. cruzi*, but whose bodily fluid is seronegative when assayed by conventional means. A bodily fluid that is seronegative when assayed by conventional means is one that, for example, does not show a positive reaction (antibody binding) when exposed to antigens from either whole or semi-purified parasite lysates, for example those from epimastigotes, in conventional diagnostic tests. A subject who shows evidence of *T. cruzi* infection using, for example, a T cell assay, polymerase chain reaction (PCR), hemoculture, or xenodiagnostic techniques, is considered to be known to be infected or to have been infected by *T. cruzi*, even if the subject shows a negative response to a conventional serodiagnostic test.

Another preferred polypeptide, or subunit or analog thereof, is one that detectably binds antibodies in a bodily fluid of a subject who is seropositive when assayed by conventional means, regardless of whether the polypeptide also exhibits detectable binding to antibodies in a bodily fluid of a subject who is known to be infected or to have been infected by *T. cruzi*, but whose bodily fluid is seronegative when assayed by conventional means.

The antigenic *T. cruzi* polypeptides, and antigenic subunits and analogs thereof, bind antibodies in a bodily fluid of a subject, such as blood, plasma or sera, thereby providing evidence of exposure to *T. cruzi*. These antigenic polypeptides, and antigenic subunits and analogs thereof, may also be immunogenic; i.e., they may also, when delivered to a subject in an appropriate manner, cause an immune response (either humoral or cellular or both) in the subject. Immunogenic *T. cruzi* polypeptides, as well as immunogenic subunits and analogs thereof, are therefore expected to be useful in vaccines, as described below.

It should be understood that the term “polypeptide” as used herein refers to a polymer of amino acids and does not refer to a specific length of a polymer of amino acids. Thus, for example, the terms peptide, oligopeptide, and protein are included within the definition of polypeptide.

An antigenic *T. cruzi* polypeptide according to the invention is not limited to a naturally occurring antigenic *T. cruzi* polypeptide; it can include an antigenic subunit or antigenic analog of a *T. cruzi* polypeptide. Likewise the antigenic polypeptide can be a multivalent construct that includes epitopes obtained from different antigenic polypeptides of *T. cruzi*. An antigenic analog of an antigenic *T. cruzi* polypeptide is a polypeptide having one or more amino acid substitutions, insertions, or deletions relative to an antigenic *T. cruzi* polypeptide, such that antigenicity is not entirely eliminated. Substitutes for an amino acid are preferably conservative and are selected from other members of the class to which the amino acid belongs. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and tyrosine. Polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Examples of preferred conservative substitutions include Lys for Arg and vice versa to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a free —OH is maintained; and Gln for Asn to maintain a free NH₂. Antigenic subunits of an antigenic *T. cruzi* polypeptide are antigenic *T. cruzi* polypeptides that are truncated at either or both of the N-terminus or C-terminus, without eliminating their ability to detect serum antibodies against *T. cruzi*. Preferably, an antigenic subunit contains an epitope recognized by a host B cell or T cell. Fragments of an antigenic *T. cruzi* protein contain at least about eight amino acids, preferably at least about 12 amino acids, more preferably at least about 20 amino acids.

Examples of antigenic *T. cruzi* polypeptides suitable for inclusion in the multicomponent panel of the invention are listed in Tables 1, 2 and 4 in the Examples, below. The “Gene ID Numbers” represent gene numbers assigned by annotators of the *T. cruzi* genome and are accessed via the *T. cruzi* genome database on the worldwide web at “*TcruziDB.org*”.

Furthermore, as described below, the present invention also includes a method for identifying additional antigenic polypeptides indicative of *T. cruzi* infection. The use of the additional *T. cruzi* polypeptides thus identified, or antigenic subunit or analog thereof, alone or in combination with each other, with the antigenic *T. cruzi* polypeptides of Table 1, 2 and 4, and/or with other known antigens, in diagnostic and therapeutic applications relating to *T. cruzi* infection as described is also envisioned. It should be understood that the antigenic *T. cruzi* polypeptides described herein or identified using the screening method described herein are generally useful in any of diagnostic and/or therapeutic applications relating to *T. cruzi* infection.

Antigenic polypeptides used in the multicomponent panel of the invention preferably include polypeptides that are abundant during the two stages (amastigote and trypomastigote) that are prevalent in the life cycle of the parasite in mammals. In a mammalian host, *T. cruzi* cycles between a dividing intracellular stage (the amastigote) and a non-replicative extracellular trypomastigote form which circulates in the blood. The presence of two developmental stages of *T. cruzi* in mammalian hosts provides two anatomically and (to some degree) antigenically distinct targets of immune detection—the trypomastigotes in the bloodstream and the

amastigotes in the cytoplasm of infected cells. The intracellular location of amastigotes of *T. cruzi* has long been considered a “hiding place” for the parasite wherein it is not susceptible to immune recognition and control. Notably, most current serological tests for *T. cruzi* are based upon antigens from epimastigotes, the form of *T. cruzi* present in insects but not humans. Thus, in a preferred embodiment, an antigenic polypeptide for use in a *T. cruzi* diagnostic test or vaccine according to the invention can be one that is expressed by *T. cruzi* in the extracellular (trypomastigote) stage, in the intracellular (amastigote) stage, or during both stages of the life cycle.

Diagnostic Method

The diagnostic of the invention utilizes a multicomponent panel to assess the presence of an immune response (e.g., the presence of antibodies or reactive T cells) in the subject to multiple antigenic *T. cruzi* polypeptides, or antigenic subunits or analogs thereof. The panel may contain a number of antigenic *T. cruzi* polypeptides, or antigenic subunits or analogs thereof, wherein said number is between 5 and 50 or even more, depending on the embodiment and the intended application. For example, the panel may contain 5, 8, 10, 12, 15, 18, 20, 25, 30, 40 or more antigenic *T. cruzi* polypeptides. A typical multicomponent panel may contain 10 to 20 antigenic *T. cruzi* polypeptides. Preferably, some or all of the antigenic *T. cruzi* polypeptides used in the multicomponent panel are selected from those listed in one or more of Tables 1, 2 or 4. Conveniently, the *T. cruzi* polypeptides that are used in the multicomponent diagnostic test can be recombinant polypeptides; however they can be naturally occurring polypeptides or polypeptides that have been chemically or enzymatically synthesized, as well.

In one embodiment, the diagnostic test takes the form of a serodiagnostic assay, which detects a humoral (antibody) immune response in the subject. The binding of an antibody that is present in a biological fluid, such as a serum antibody, to any of the various components of the panel is determined. The threshold for a diagnosis of *T. cruzi* infection can be readily determined by the scientist, medical personnel, or clinician, for example based upon the response of known infected and control sera to the particular panel being used. For example, diagnosis criteria can be based on the number of “hits” (i.e., positive binding events) or they can represent a more quantitative determination based, for example, on the intensity of binding and optional subtraction of background. As an illustrative example, the multicomponent panel could contain 15 to 20 antigenic polypeptides, or antigenic analogs or subunits thereof, and a positive diagnosis could be interpreted as, say, 5 or more positive responses. Optionally, the serodiagnostic test could be further refined to set quantitative cutoffs for positive and negative based upon the background response to each individual panel component. So, for example, the response to each polypeptide could be set to be >2 standard deviations above the response of “pooled normal,” sera and an individual would have to have responses to a minimum of 5 out of 20 polypeptides.

The serodiagnostic assay of the invention can take any convenient form. For example, standard immunoassays such as indirect immunofluorescence assays (IFA), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescent bead technology and Western blots can be employed. Detection can be by way of an enzyme label, radiolabel, chemical label, fluorescent label, chemiluminescent label, a change in spectroscopic or electrical property, and the like.

In another embodiment, the diagnostic method can take the form of a cellular assay. In this embodiment, a multicompo-

nent panel of antigenic *T. cruzi* polypeptides as described herein is used to assess T cell responses in a mammalian subject, thereby providing another method for evaluating the presence or absence (or stage, etc.) of *T. cruzi* infection. Individuals are known who are serologically negative (based upon conventional tests) but who have T cells reactive with parasite antigens (usually a lysate of trypomastigotes and epimastigotes—but in some cases also against specific *T. cruzi* polypeptides). This suggests that T cell responses may be a sensitive way to assess infection, or to determine the stage of infection or exposure.

Recombinant antigenic *T. cruzi* polypeptides can be readily produced, for example, as histidine-tagged polypeptides. These His-tagged polypeptides can be purified onto a nickel-coated substrate, then added to a blood fraction comprising peripheral blood lymphocytes (e.g., a peripheral blood mononuclear cell, PBMC, fraction). The ability of the T cells to make IFN-gamma is then assessed, for example using an ELISPOT assay (e.g., Laucella et al., J Infect Dis. 2004 Mar. 1; 189(5):909-18). As another example, antigenic *T. cruzi* polypeptides, or antigenic analogs or subunits thereof, can be bound to major histocompatibility complex (MHC) tetramers and presented to T cells, for example in a composition of peripheral blood lymphocytes, in a microarray format. In this assay, smaller polypeptides, for example antigenic peptide subunits of antigenic *T. cruzi* polypeptides described herein, are preferred as they are more readily bound to the MHC tetramers and recognized by the T cells. Antigenic subunits of antigenic *T. cruzi* polypeptides can be predicted using various computer algorithms, and are amenable to chemical synthesis. Binding of T cells to the spots containing MHC-polypeptide complexes indicates recognition and hence *T. cruzi* infection. See, for example, Stone et al (Proc. Nat'l. Acad. Sci. USA, 2005, 102:3744) and Soen et al. (PLoS. Biol, 2003, 1:429) for a description of the general technique.

In a multiplexed assay, multiple analytes are simultaneously measured. Each polypeptide antigen is positioned such that it is individually addressable. For example, the polypeptide antigens can be immobilized in a substrate. In a preferred embodiment, the multiplexed serodiagnostic assay of the invention is performed using a bioassay such as the Luminex system (Luminex Corporation, Austin, Tex.). The Luminex system, which utilizes fluorescently labeled microspheres, allows up to 100 analytes to be simultaneously measured in a single microplate well, using very small sample volumes. However, other multiplex platforms such as protein microarrays can also be used, and the invention is not intended to be limited by the type of multiplex platform selected.

The panel components can be assembled on any convenient substrate, for example on a microtiter plate, on beads, or in a microarray on a microchip. A microarray format is advantageous because it is inexpensive and easy to read using a standard fluorescence microscope. In this format, one might just use the total number of spots (proteins) positive for each test patient to make a positive or negative diagnosis. In addition, the diagnostic test of the invention is well-suited to adaptation for use with commercially available high-throughput devices and immunoassay protocols, for example those available from Abbott Laboratories and Applied Biosystems, Inc. The serodiagnostic assay can also take the form of an immunochromatographic test, in the form of a test strip loaded with the panel components. The bodily fluid can be wicked up onto the test strip and the binding pattern of antibodies from the fluid can be evaluated.

Detection of *T. cruzi* Infection in Infants

In another aspect, the invention provides method for determining whether an infant has a *T. cruzi* infection. In situations where the infant's mother is infected with *T. cruzi*, the method facilitates early detection of a maternally transmitted infection. A maternally transmitted infection can be transmitted prior to or during birth (a congenital infection), or it may be transmitted after birth, as through breastfeeding.

At birth and for a period shortly thereafter, the antibody response of an infant born to an infected mother mirrors the antibody response of the infant's infected mother, reflecting the presence of maternal antibodies in the baby's fluids. With time, however, if the infant is infected, the infant will begin producing its own antibodies, and the pattern of response will begin to differentiate from that of the mother. Eventually, typically by about six months after birth, the antibody response of the infant will either diminish to near background levels (if the infant is not infected), or will appear distinct from that of the mother, indicating possible infection.

The method for detecting *T. cruzi* infection in an infant, particularly an infant born to a mother with a known or suspected *T. cruzi* infection, therefore includes analyzing at least one biological sample obtained from the infant. Preferably the biological sample is a body fluid such as blood, plasma or serum. The sample is obtained at a time after birth by which the infant's antibody response to the antigenic *T. cruzi* polypeptides, if the infant is infected, is detectably different from the mother's antibody response. Preferably the sample is obtained from the infant at about 6 months of age, but the sample can be obtained earlier, for example at about 5 months, 4 months, 3 months or 2 months. Likewise, the sample can be obtained later since after 6 months the baby is expected to be producing its own antibodies at a detectable level. Analysis is preferably performed using the multiplexed assay of the invention.

An infant that exhibits a background level antibody response to *T. cruzi* antigens in the multiplexed assay is unlikely to be infected with *T. cruzi*. However, an antibody response that exceeds background levels indicates possible infection. Optionally, the method therefore also includes administering a therapeutic agent an infant suspected of having a *T. cruzi* infection.

In a preferred embodiment of the method, the infant's antibody response to the *T. cruzi* antigen panel is first analyzed shortly after birth. When a neonate's antibody response is measured shortly after birth (preferably no later than one month after birth, more preferably no later than two months after birth), the neonate's antibody response will parallel that of its mother, due to the presence of maternal antibodies. Optionally, the mother's antibody response to the antigen panel is thus analyzed. The infant's antibody response at the later time point (when its own antibodies have begun to be produced) is compared to the antibody response of the mother, and/or to its own antibody response at a time shortly after birth. Comparison of the antibody response of the later infant sample with the antibody response of the earlier neonate sample and/or with the antibody response of the mother (preferably using a sample obtained from the mother at about the same time as the sample or samples are obtained from the infant, although the sample from the mother can be obtained at any convenient time as it is expected to be fairly stable) is preferred, as it facilitates the determination as to whether the infant's own antibody response is sufficiently different from the mother's to support the diagnosis of *T. cruzi* infection.

It should be understood that in this method, as in all methods involving the use of the multiplexed assay of the invention, the panel of serodiagnostic targets can include any anti-

genic *T. cruzi* polypeptide described herein, or subsequently discovered using the screening assay of the invention. Preferably, multiple *T. cruzi* polypeptide antigens are selected from those listed in Tables 1, 2 and/or 4 and used to assess the infant's antibody response in a multiplexed assay.

It should also be noted that the method of detecting *T. cruzi* infection in an infant according to the invention can take the form of either a serodiagnostic method, wherein the sample components that interact with an antigenic *T. cruzi* polypeptides are antibodies, or a cellular assay method, and wherein the sample components that interact with the antigenic *T. cruzi* polypeptides are T cells.

Blood Supply Screening

The diagnostic test of the invention can be used to detect the presence of *T. cruzi* infection in blood and blood products or fractions include whole blood as well as such as cellular blood components, including red blood cell concentrates, leukocyte concentrates, and platelet concentrates and extracts; liquid blood components such as plasma and serum; and blood proteins such as clotting factors, enzymes, albumin, plasminogen, and immunoglobulins, or mixtures of cellular, protein and/or liquid blood components. Details regarding the make-up of blood, the usefulness of blood transfusions, cell-types found in blood and proteins found in blood are set forth in U.S. Pat. No. 5,232,844. Techniques regarding blood plasma fractionation are generally well known to those of ordinary skill in the art and an excellent survey of blood fractionation also appears in Kirk-Othmer's Encyclopedia of Chemical Technology, Third Edition, Interscience Publishers, Volume 4.

A sample is contacted with a multicomponent panel of the invention, and a positive or negative response is detected as described above for clinical use of the assay in patients suspected of having *T. cruzi* infection. Advantageously, the diagnostic test is readily automated, for example using microchip technology, for the processing of large numbers of samples. Prophylactic and Therapeutic Immunization

In another aspect, the present invention is directed to both prophylactic and therapeutic immunization against *T. cruzi* infection and the chronic disease state, known as Chagas disease, that often eventually follows initial *T. cruzi* infection. Antigenic *T. cruzi* polypeptides described herein, or identified using a screening method described herein, may be immunogenic. That is, they may elicit a humoral (B cell) response and/or a cell-mediated immune response (i.e., a "T cell" response) in the subject. A cell-mediated response can involve the mobilization helper T cells, cytotoxic T-lymphocytes (CTLs), or both. Preferably, an immunogenic polypeptide elicits one or more of an antibody-mediated response, a CD4⁺ Th1-mediated response (Th1: type 1 helper T cell), and a CD8⁺ T cell response. Therapeutic administration of the polynucleotide or polypeptide vaccine to infected subjects is expected to be effective to delay or prevent the progression of the *T. cruzi* infection to a chronic disease state, and also to arrest or cure the chronic disease state that follows *T. cruzi* infection. Prophylactic administration of the polynucleotide or polypeptide vaccine to uninfected subjects is expected to be effective to reduce either or both if the morbidity and mortality associated with infection by *T. cruzi*. Further, if an uninfected, vaccinated subject is subsequently infected with *T. cruzi*, the vaccine is effective to prevent progression of the initial infection to a chronic disease state. As discussed in more detail hereinbelow, the vaccine can contain or encode a single immunogenic polypeptide or multiple immunogenic polypeptides. Methods for identifying nucleotide sequences encoding such polypeptides from a *T. cruzi* genomic library

using, for example, expression library immunization (ELI) or DNA microarray analysis are described below.

Advantages of a Genetic Vaccine

The choice of polynucleotide delivery as an immunization technique offers several advantages over other vaccine or antigen delivery systems. Vaccines containing genetic material are favored over traditional vaccines because of the ease of construction and production of the vectors, the potential for modification of the sequences by site-directed mutagenesis to enhance the antigenic potency of the individual epitopes or to abolish epitopes that may trigger unwanted response, in the case of DNA vaccines, the stability of DNA, the lack of the dangers associated with live and attenuated vaccines, their ability to induce both humoral and cell mediated immunity and, in particular, CD8⁺ T cell responses, and the persistence of the immune responses. Successful induction of humoral and/or cellular immune responses to plasmid-encoded antigens using various routes of gene delivery have been shown to provide partial or complete protection against numerous infectious agents including influenza virus, bovine herpes virus I, human hepatitis B virus, human immunodeficiency virus-1, as well as parasitic protozoans like *Plasmodium* and *Leishmania* (Donnelly et al., Ann. Rev. Immunol. 15:617-648, 1997). Representative papers describing the use of DNA vaccines in humans and primates include Endresz et al. (Vaccine 17:50-58, 1999), McCluskie et al. (Mol. Med. 5:287-300, 1999), Wang et al. (Infect. Immun. 66:4193-202, 1998), Le Borgne et al. (Virology 240:304-315, 1998), Tacket et al. (Vaccine 17:2826-9, 1999), Jones et al. (Vaccine 17:3065-71, 1999) and Wang et al. (Science 282(5388):476-80, 1998). The ability to enhance the immune response by the co-delivery of genes encoding cytokines is also well-established. Polynucleotide Vaccine

The polynucleotide vaccine of the invention includes at least one, preferably at least two, nucleotide coding regions, each coding region encoding an immunogenic polypeptide component from *T. cruzi* as identified herein and/or using the screening method described herein. When it contains two or more nucleotide coding regions, the polynucleotide vaccine is referred to herein as a "multicomponent" polynucleotide vaccine. It is desirable to minimize the number of different immunogenic polypeptides encoded by the nucleotide coding regions in the polynucleotide vaccine; however, it is nonetheless contemplated that a polynucleotide vaccine that generates the highest level of protection will encode 10 or more immunogenic polypeptides.

The polynucleotide vaccine can contain DNA, RNA, a modified nucleic acid, or any combination thereof. Preferably, the vaccine comprises one or more cloning or expression vectors; more preferably, the vaccine comprises a plurality of expression vectors each capable of autonomous expression of a nucleotide coding region in a mammalian cell to produce at least one immunogenic polypeptide or cytokine, as further described below. An expression vector preferably includes a eukaryotic promoter sequence, more preferably the nucleotide sequence of a strong eukaryotic promoter, operably linked to one or more coding regions. A promoter is a DNA fragment that acts as a regulatory signal and binds RNA polymerase in a cell to initiate transcription of a downstream (3' direction) coding sequence; transcription is the formation of an RNA chain in accordance with the genetic information contained in the DNA. A promoter is "operably linked" to a nucleic acid sequence if it is does, or can be used to, control or regulate transcription of that nucleic acid sequence. The invention is not limited by the use of any particular eukaryotic promoter, and a wide variety are known; preferably, however, the expression vector contains a CMV or RSV promoter. The

promoter can be, but need not be, heterologous with respect to the host cell. The promoter used is preferably a constitutive promoter.

A vector useful in the present invention can be circular or linear, single-stranded or double stranded and can be a plasmid, cosmid, or episome but is preferably a plasmid. In a preferred embodiment, each nucleotide coding region (whether it encodes an immunogenic polypeptide or a cytokine) is on a separate vector; however, it is to be understood that one or more coding regions can be present on a single vector, and these coding regions can be under the control of a single or multiple promoters.

There are numerous plasmids known to those of ordinary skill in the art useful for the production of polynucleotide vaccines. Preferred embodiments of the polynucleotide vaccine of the invention employ constructs using the plasmids VR1012 (Vical Inc., San Diego Calif.), pCMVI.UBF3/2 (S. Johnston, University of Texas) or pcDNA3.1 (INVITROGEN Corporation, Carlsbad, Calif.) as the vector. Plasmids VR1012 and pCMVI.UBF3/2 are particularly preferred. In addition, the vector construct can contain immunostimulatory sequences (ISS), such as unmethylated dCpG motifs, that stimulate the animal's immune system. Other possible additions to the polynucleotide vaccine constructs include nucleotide sequences encoding cytokines, such as granulocyte macrophage colony stimulating factor (GM-CSF), interleukin-12 (IL-12) and co-stimulatory molecules such B7-1, B7-2, CD40. The cytokines can be used in various combinations to fine-tune the response of the animal's immune system, including both antibody and cytotoxic T lymphocyte responses, to bring out the specific level of response needed to control or eliminate the *T. cruzi* infection.

The polynucleotide vaccine can also encode a fusion product containing the antigenic polypeptide and a molecule, such as CTLA-4, that directs the fusion product to antigen-presenting cells inside the host. Plasmid DNA can also be delivered using attenuated bacteria as delivery system, a method that is suitable for DNA vaccines that are administered orally. Bacteria are transformed with an independently replicating plasmid, which becomes released into the host cell cytoplasm following the death of the attenuated bacterium in the host cell.

An alternative approach to delivering the polynucleotide to an animal involves the use of a viral or bacterial vector. Examples of suitable viral vectors include adenovirus, polio virus, pox viruses such as vaccinia, canary pox, and fowl pox, herpes viruses, including catfish herpes virus, adenovirus-associated vector, and retroviruses. Exemplary bacterial vectors include attenuated forms of *Salmonella*, *Shigella*, *Edwardsiella ictaluri*, *Yersinia ruckerii*, and *Listeria monocytogenes*. Preferably, the polynucleotide is a vector, such as a plasmid, that is capable of autologous expression of the nucleotide sequence encoding the immunogenic polypeptide.

Preferably, the polynucleotide vaccine further includes at least one nucleotide coding region encoding a cytokine. Preferred cytokines include interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-18 (IL-18), γ -interferon, α , β (3-interferons, and chemokines. Especially preferred cytokines include IL-12 and GM-CSF.

Plasmids and other delivery systems are made using techniques well-known in the art of molecular biology. The invention should be understood as including methods of making and using the polynucleotide vaccine.

Polypeptide Vaccine

The polypeptide vaccine of the invention includes at least one, preferably at least two, immunogenic polypeptides from

T. cruzi as described herein and/or as identified using the screening method described herein. As with the polynucleotide vaccine, it is desirable to minimize the number of different immunogenic polypeptides supplied in the vaccine; however, it is nonetheless contemplated that a polypeptide vaccine that generates the highest level of protection will contain 10 or more immunogenic polypeptides.

Because a CD8⁺ T cell response cannot normally be directly triggered by the administration of a conventional protein subunit vaccine, the immunogenic polypeptides contained in the polypeptide vaccine preferably include one or more membrane transporting sequences (MTS) fused to their N-terminus or C-terminus or both. A membrane transporting sequence allows for transport of the immunogenic polypeptide across a lipid bilayer, allowing it to be delivered to the inside of a mammalian cell. In a particularly preferred embodiment, the immunogenic polypeptides are shocked with urea, as described further in Example VIII, prior to administration as a vaccine. From there, portions of the polypeptide can be degraded in the proteasome, and the resulting peptides can be displayed as class I MHC-peptide complexes on the cell surface. In this way, a polypeptide vaccine can stimulate a CD8⁺ T cell immune response. In another preferred embodiment, the immunogenic polypeptides are attached to nanoparticles and administered to a subject (e.g., Plebanski et al., *J. Immunol.* 2004, 173:3148; Plebanski et al., *Vaccine*, 2004, 23:258). A polypeptide vaccine of the invention is optionally adjuvanted using any convenient and effective adjuvant, as known to one of skill in the art.

The invention should be understood as including methods of making and using the polypeptide vaccine.

Pharmaceutical Compositions

The polynucleotide and polypeptide vaccines of the invention are readily formulated as pharmaceutical compositions for veterinary or human use. The pharmaceutical composition optionally includes excipients or diluents that are pharmaceutically acceptable as carriers and compatible with the genetic material. The term "pharmaceutically acceptable carrier" refers to a carrier(s) that is "acceptable" in the sense of being compatible with the other ingredients of a composition and not deleterious to the recipient thereof. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, salts, and/or adjuvants which enhance the effectiveness of the immune-stimulating composition. Methods of making and using such pharmaceutical compositions are also included in the invention.

Administration of the Polynucleotide Vaccine

The polynucleotide vaccine of the invention can be administered to the mammal using any convenient method, such as intramuscular injection, topical or transdermal application to the mammal's skin, or use of a gene gun, wherein particles coated with the polynucleotide vaccine are shot into the mammal's skin. The amount of polynucleotide administered to the mammal is affected by the nature, size and disease state of the mammal as well as the delivery method; for example, typically less DNA is required for gene gun administration than for intramuscular injection. Further, if a polynucleotide encoding a cytokine is co-delivered with nucleotide coding regions encoding the immunogenic polypeptide from *T. cruzi*, the amount of polynucleotide encoding the immunogenic polypeptide from *T. cruzi* in the vaccine is optionally reduced.

Hundreds of publications have now reported the efficacy of DNA vaccines in small and large animal models of infectious

diseases, cancer and autoimmune diseases (Donnelly et al., Rev. Immunol. 15:617, 1997). Vaccine dosages for humans can be readily extended from the murine models by one skilled in the art of genetic immunization, and a substantial literature on genetic immunization of humans is now available to the skilled practitioner. For example, Wang et al. (Science 282:476-480, 1998) vaccinated humans with plasmid DNA encoding a malaria protein, and the same group has developed a plan for manufacturing and testing the efficacy of a multigene *Plasmodium falciparum* liver-stage DNA vaccine in humans (Hoffman et al., Immunol. Cell Biol. 75:376, 1997). In general, the polynucleotide vaccine of the invention is administered in dosages that contain the smallest amount of polynucleotide necessary for effective immunization. It is typically administered to human subjects in dosages containing about 20 µg to about 2500 µg plasmid DNA; in some instances 500 µg or more of plasmid DNA may be indicated. Typically the vaccine is administered in two or more injections at time intervals, for example at four week intervals. Administration of the Polypeptide Vaccine.

Like the polynucleotide vaccine, the polypeptide vaccine can be administered to the mammal using any convenient method, such as intramuscular or intraperitoneal injection, topical administration, oral or intranasal administration, inhalation, perfusion and the like. The amount of polypeptide administered to the mammal is affected by the nature, size and disease state of the mammal, as well as by the delivery method. Intraperitoneal injection of 25 to 50 µg of polypeptide containing a membrane transducing sequence has been shown to result in import of the protein into nearly 100% of murine blood and spleen cells within 20 minutes (Schwarze et al., Science 285:1569-1572, 1999) and the sensitization of cytotoxic T cells (Schutze-Redelmeier et al., J. Immunol. 157:650-655, 1996). Useful dosages of the polypeptide vaccine for humans can be readily determined by evaluating its activity in vivo activity in mice.

Administration of a Combination of Polynucleotide Vaccine and Polypeptide Vaccine.

The invention contemplates administration of both a polynucleotide vaccine and a polypeptide vaccine to a mammal in a serial protocol. For example, a plasmid-based DNA vaccine may be administered to a mammal to "prime" the immune system, followed by the one or more administrations of a polypeptide vaccine or a viral vaccine (e.g., vaccinia vector carrying the genes that encode the immunogenic polypeptides and, optionally, cytokines) to further stimulate the mammal's immune system. The order of administration of the different types of vaccines, and the nature of the vaccines administered in any given dose (e.g., polypeptide vaccine, plasmid vaccine, viral vector vaccine) can be readily determined by one of skill in the art to invoke the most effective immune response in the mammal.

Screening Method for Identification of Antigenic *T. cruzi* Polypeptides

In another aspect, the invention provides high-throughput method to screen putative *T. cruzi* polypeptides for diagnostic potential. The antigenic polypeptides thus identified can be incorporated into a diagnostic test for *T. cruzi* as described herein.

T. cruzi polypeptides that are preferred candidates for screening, either individually or as part of a pool, have one or more of the following characteristics or features. The *T. cruzi* polypeptides may be abundant in the trypomastigote and/or amastigote stages of the *T. cruzi* life cycle in mammals, as described in more detail above. Additionally or alternatively, the *T. cruzi* polypeptides may be, or may be likely to be, surface-associated or secreted. Surface associated-antigenic

polypeptides include, for example, *T. cruzi* proteins that are anchored to the plasma membrane by glycosylphosphatidylinositols, or GPIs, and those that have transmembrane domains or are otherwise embedded in the plasma membrane. This property can be evaluated, for example, by analyzing the polypeptide sequence for the presence of an N-terminal leader sequence which directs the polypeptide to the cell membrane; by analyzing the polypeptide sequence for the presence of a known GPI sequence that facilitates attachment of the polypeptide to the cell surface; and/or by analyzing the polypeptide sequence for the presence of a transmembrane domain. Another preferred feature is that the polypeptide is unique to *T. cruzi* and not expressed in other organisms, including other kinetoplastids. This can be determined by performing BLAST searches of GenBank entries for other organisms and/or comparative genomics with *T. brucei* and *Leishmania major*. This feature enhances the specificity of the diagnostic test.

Another preferred feature is that the *T. cruzi* polypeptide be one that is less likely than others to be highly variant. For example, members of large gene families that appear to undergo rearrangements that create new variants are generally not preferred. However, pools of large gene family members (such as the trans-sialidase family, the Mucin-associated surface protein (MASP) family, and other smaller families of genes can be cloned and tested using degenerate primers. In that case, rather than a bead or a spot in the diagnostic test containing only one gene family member, it may have ten or hundreds, thereby circumventing the problem of recombination and variation in these families, and providing a better representation of the family than a single (possibly variant) protein.

The screening method involves providing two substrates that include a plurality of individually addressable candidate antigens derived from *T. cruzi*, in which the antigens present on both substrates are substantially the same. A substrate, as defined herein, is a surface of unreactive material that can be used to contain the individually addressable candidate antigens in isolation from one another. For example, a multi-welled array system such as a 96 well microplate is a substrate useful in the method of screening for serodiagnostic *T. cruzi* antigens. Individually addressed candidate antigens refers to potentially serodiagnostic *T. cruzi* antigens that have been positioned and/or labeled in such a way that differing antigens can be discretely identified using methods known to those skilled in the art. For example, antigens obtained directly or indirectly from *T. cruzi*, labeled with a fluorescent label with a different wavelength sensitivity from other fluorescent labels used with other antigens and positioned within a specific well or set of wells on a multi-welled array system, are individually addressed candidate antigens.

Candidate antigens immobilized on the first substrate are contacted with a body fluid from an organism known to be positive for *T. cruzi* infection based on a detection method such as a T cell assay, polymerase chain reaction (PCR), hemoculture or xenodiagnostic techniques. The organism is preferably a mammal, more preferably a dog or a human. Preferably, the organism exhibits negative serology when tested for *T. cruzi* infection utilize conventional serodiagnostic tests that rely on antigens from either whole to semi-purified parasite lysates, for example from epimastigotes, that react with anti-*T. cruzi* IgG antibodies.

Candidate antigens immobilized on the first substrate are contacted with the second substrate with a body fluid from an organism known or reasonably believed to be unexposed to *T. cruzi* infection. The second substrate serves as a control. The organism does not exhibit a strong positive serological signal

indicating infection by *T. cruzi*. Preferably, the organism shows no evidence of *T. cruzi* infection by any other diagnostic test as well. Optionally, the screening method includes testing of additional substrates using body fluids that are strongly, weakly and/or borderline seropositive using conventional tests for *T. cruzi*, as described in more detail below.

The body fluid may be any fluid found within the body of an organism that is capable of containing components of *T. cruzi* or immune system components prepared in response to exposure to *T. cruzi*. For example, an immune system component may be an antibody that specifically binds to a *T. cruzi* antigen. Such body fluids include, for example, blood, plasma, serum, urine, saliva, tears, lymphatic fluid, and the like.

The organism itself may be any organism that can be infected by *T. cruzi*, including vector organisms. For example, organisms may include insect vectors of Chagas disease belonging to the Hemiptera order, Reduviidae family, and Triatominae subfamily. The organism can also be a vertebrate reservoir of *T. cruzi* infection. Mammals are most susceptible to infection with *T. cruzi*, with approximately 150 species known to serve as reservoirs. Birds, amphibians, and reptiles are naturally resistant to infection. In the domestic cycle, frequently infected mammals, besides humans, are dogs, cats, mice, rats, guinea pigs, and rabbits. Pigs, goats, cattle, and horses can be infected by *T. cruzi*, but generally only manifest transitory parasitemia. Humans are a preferred organism due to the importance of diagnosing *T. cruzi* infection in humans.

Antigens that exhibit binding to antibodies present in the bodily fluid contacted with the first substrate but little or no binding to antibodies present in the control bodily fluid contacted with the second substrate are identified as antigenic *T. cruzi* polypeptides for use in the multicomponent diagnostic assay. The binding of an antigen to an antibody can be detected by various means known to those skilled in the art. For example, the association may be detected using flow cytometry, or by enzyme immunoassay (EIA) or enzyme-linked immunoassay (ELISA). Preferably, the association of one or more antibodies with multiple antigens is detected using a multiplex analysis system such as the BIO-PLEX multiplex analysis system commercially available from, for example, Bio-Rad® Laboratories (Hercules, Calif.).

The BIO-PLEX suspension array system is a biomarker assay system that includes a flow-based 96-well fluorescent microplate assay reader integrated with specialized software, automated validation and calibration protocols, and assay kits. The multiplex analysis system utilizes up to 100 fluorescent color-coded bead sets, each of which can be conjugated with a different specific antigen. The term "multiplexing" refers to the ability to analyze many different antigens essentially simultaneously. To perform a multiplexed assay, sample and reporter antibodies are allowed to react with the conjugated bead mixture in microplate wells. The constituents of each well are drawn up into the flow-based BIO-PLEX array reader, which identifies each specific reaction based on bead color and quantitates it. The magnitude of the reaction is measured using fluorescently labeled reporter antibodies specific for each antibody that may associate with the antigen being tested.

The BIO-PLEX suspension array system uses a liquid suspension array of about 100 sets of micrometer-sized beads, each internally dyed with different ratios of two spectrally distinct fluorophores to assign it a unique spectral address. The overall operation of the BIO-PLEX array system is illustrated in FIGS. 1A through 1C. As shown in FIG. 1A, polypeptide antigen 10 is bound to a microsphere bead 20 by, for example, a histidine tag. The polypeptide antigen 10 is

then contacted with a sample of sera containing an antibody; for example, an anti-*T. cruzi* antibody 30. This antibody, in turn, is contacted with a fluorescently labeled reporter antibody 40 to form a microsphere-antigen-antibody complex 50. As shown in FIG. 1B, since the microsphere beads 20 provide a large variety of different colors, and the microsphere beads 20 were earlier attached only to specific polypeptide antigens 10, a number of microsphere-antigen-antibody complexes 50 may be present in a microplate well 60. The complexes 50 are then run through a flow cytometry apparatus 70 that includes a classifying laser 70 and a reporting laser 80. The reporting laser 80 determines the amount of a particular antigen present, based on the amount of fluorescently labeled reporter antibody 40. The classifying laser 90, on the other hand, determines the frequency of fluorescence provided by the microsphere bead 20, and based on this frequency, the identity of the polypeptide antigen 10 can be determined.

In the embodiment used in this invention, the BIO-PLEX assay utilizes dyed beads containing nickel to capture the His-tagged *T. cruzi* polypeptides produced in the host bacterial cells. Each spectrally addressed bead captures a different protein. The protein-conjugated beads are allowed to react with a sample, and biomolecules in the sample (typically antibodies) bind to the bound protein antigens as further described in the Examples.

It should be understood that the invention is not limited to multiplexing as employed in the BIO-PLEX assay; other multiplexed approaches can readily be used. For example, protein arrays can be placed on a matrix, and the response to the individual proteins on the solid-phase array can be assayed.

An important advantage of using a multiplexed method is that a plurality of serodiagnostic antigens may be identified during a single run of the analysis. For example, serological responses to as many as a 100 individual proteins can be screened at one time, and the pattern of responsiveness to all 100, or any subset thereof, can be used to make or assist in making a diagnosis. Tests in current use employ crude antigen preparations from *T. cruzi* itself (an undefined composition thus not very reproducible), or utilize an individual recombinant protein (i.e., a single target thus not very robust) or a string of three portions of three different proteins combined in one synthetic protein. In the latter case, there is more than one target, but still the response to only a single entity is measured, thus the assay lacks robustness.

Optionally, the method of the invention can utilize more than two substrates that include a plurality of individually addressed candidate antigens. These additional substrates can be used, for example, to evaluate the body fluids from organisms classified as providing a very high positive sera response, intermediate positive sera response, very low positive sera response or a borderline positive sera response. When used to supplement data obtained on sera from control (uninfected) organisms and organisms that are or have been infected but show little or no positive serological response, these substrates can provide additional information on candidate *T. cruzi* antigens that may be used as serodiagnostic antigens. Measuring the pattern of responses to many antigens is also useful for evaluating other aspects of the *T. cruzi* infection such as the stage of the disease, its severity, or the particular strain of *T. cruzi* involved.

The method of screening for serodiagnostic *T. cruzi* antigens can also be done at the less specific level of an antigen pool, rather than specific antigens. An antigen pool, as defined herein, is a plurality of antigens in a mixture. Antigen pools may be used either as a means of evaluating a wide number of antigens more rapidly, as a means of evaluating mixtures of

antigens for possible interactions, or simply out of necessity when the identify of specific antigens is not known. The method of screening antigen pools can be done either as an independent analysis method, or it may be an optional preliminary step to the screening of individual *T. cruzi* antigens for potential as serodiagnostic antigens. In either case, the method of analyzing antigen pools includes providing two substrates that include a plurality of individually addressable antigen pools derived from *T. cruzi*, where the antigen pools present on the two substrates are substantially the same. The first substrate is then contacted with a body fluid from an organism known to be serologically positive for *T. cruzi* infection, while the second substrate is then contacted with a body fluid from an organism known to be not serologically positive for *T. cruzi* infection. Antigen pools that associate with an antibody present in the body fluid from an organism known to be serologically positive but that are absent or present to a lesser degree in the body fluid of an organism known to be not serologically positive for *T. cruzi* infection are thereby identified as serodiagnostic antigen pools that may be used by themselves or further evaluated to determine that specific antigens involved.

The candidate antigens derived from *T. cruzi* that are screened by the method of the invention can include antigens formed from polypeptides, polysaccharides, polynucleotides, or other substances present in *T. cruzi* that are capable of being specifically bound by antibodies. As polypeptides are known by those skilled in the art to be the most common and diverse antigens, the method of the invention preferably identifies polypeptide antigens. Polypeptide antigens can be obtained directly from *T. cruzi* using biochemical separation technology, particularly protein purification methods. More preferably, the polypeptide antigens are obtained using recombinant DNA technology. Using recombinant DNA technology, nucleotide sequences from *T. cruzi* are inserted into a host organism where they are used to direct product of a polypeptide that may contain one or more antigens that may be detected by the screening method.

The smallest useful peptide sequence contemplated to provide an antigen is generally on the order of about 6 amino acids in length. Thus, this size will generally correspond to the smallest polypeptide antigens that are screened for by the method of the invention. It is proposed that short peptides that incorporate a species-specific amino acid sequence will provide advantages in certain circumstances, for example, in the preparation of vaccines or for use in methods of detecting *T. cruzi*. Exemplary advantages of shorter peptides include the ease of preparation and purification, and the relatively low cost and improved reproducibility of production. However, the size of polypeptide antigens may be significantly larger. Longer polypeptide antigens identified by the method may be on the order of 15 to 50 amino acids in length, or may represent an entire protein, including modified proteins such as fusion proteins.

Preparation of the polypeptide antigens will generally include the use of an expression vector. An expression vector is a cloning vector that contains the necessary regulatory sequences to allow transcription and translation of a cloned gene or genes. An expression vector preferably includes a promoter sequence operably linked to one or more coding regions. A promoter is a DNA fragment that acts as a regulatory signal and binds RNA polymerase in a cell to initiate transcription of a downstream (3' direction) coding sequence; transcription is the formation of an RNA chain in accordance with the genetic information contained in the DNA. A promoter is "operably linked" to a nucleic acid sequence if it is does, or can be used to, control or regulate transcription of

that nucleic acid sequence. The invention is not limited by the use of any particular promoter. A wide variety of promoters are known by those skilled in the art.

A vector useful in the present invention can be circular or linear, single-stranded or double stranded and can be a plasmid, cosmid, or episome but is preferably a plasmid. In a preferred embodiment, each nucleotide coding region encoding an antigenic polypeptide is on a separate vector; however, it is to be understood that one or more coding regions can be present on a single vector, and these coding regions can be under the control of a single or multiple promoters.

There are numerous expression vectors known to those of ordinary skill in the art useful for the production of polypeptide antigens. A preferred expression vector is an expression vector formed using the GATEWAY cloning method. The GATEWAY cloning method is a universal cloning technique that allows transfer of DNA fragments between different cloning vectors while maintaining the reading frame, and has effectively replaced the use of restriction endonucleases and ligases. The steps involved in the GATEWAY cloning method are shown in FIG. 2. First, a gene is selected for cloning. For the present invention, this would be a gene selected from *T. cruzi*. The gene is then provided with primers and amplified using PCR technology with the help of an attB tagged primer pair, as readily practiced by those skilled in the art. The PCR fragment then combined with a donor vector (pDONR™) that includes attP sites to provide an entry clone, using the BP reaction. An integration reaction between the attB and the attP sites combines the PCR fragment with the donor vector. The resulting entry clone contains the gene of interest flanked by attL sites. The LR reaction is then used to combine the entry clone with a destination vector to produce an expression vector. In the LR reaction, a recombination reaction is used to link the entry clone with the destination vector (pDEST™) using the attL and attR sites and a CLO-NASE enzyme. The attL sites are already found in the entry clone, while the destination vector includes the attR sites. The LR reaction is carried out to transfer the sequence of interest into one or more destination vectors in simultaneous reactions, making the technology high throughput. For example, as this method allows multiple genes to be transferred to one or more vectors in one experiment, this method readily allows multiple antigenic polypeptides to be prepared that can then be screened by the method of the invention, potentially revealing a plurality of serodiagnostic antigens.

The *T. cruzi* genes are cloned into expression vectors, as described, which are then expressed in a host cell, such as a bacterial cell, yeast cell, insect cell, protozoan cell, or mammalian cell. A preferred host cell is a bacterial cell, for example an *E. coli* cell. Another preferred cell is a protozoan cell, more preferably a kinetoplastid cell, most preferably a *Crithidia* cell (U.S. Pat. No. 6,368,827; Apr. 9, 2002). Proteins are isolated from the host cell, purified, and analyzed. Preferably, the proteins are purified onto beads that are then used in a Bio-Plex assay; as described. Various serum samples (e.g., negative, mildly positive, strongly positive) can be efficiently screened for reactivity with a large number of gene products to identify those gene products that are associated with *T. cruzi* infection, for example those indicative of the existence of and, optionally, the extent and/or stage of *T. cruzi* infection. Typically, the identified antigens elicit an antibody response *T. cruzi* in a mammal. The genes and gene products thus identified are useful in diagnostic assays for *T. cruzi*.

The Examples that follow provide representative data from a screening of more than 350 *T. cruzi* gene products. Following FIGS. 4-6 in the Examples, a procedure including a preliminary screening of antigen pools, followed by screening

for specific antigens, is described. Four different substrates were used to provide data for the reactivity of the antigens in sera with various levels of reactivity to *T. cruzi* antigens. One pool, labeled "3K" on FIG. 4D, was selected for further analysis because it showed good reactivity with the positive sera. The "3K" pool was broken down into individual constituent gene products, and four of the gene products (antigens) were identified as potential candidates for diagnostic use. The "top" antigens identified this way can be combined into a single, robust diagnostic assay for *T. cruzi*; see, for instance, Example 9. Examples of gene products identified in accordance with the invention are also described.

The method of screening is also capable of identifying antigens that do not consistently elicit a strong B cell response. The majority of conventional and commercially available serological methods for diagnosis and blood screening of *T. cruzi* infection utilize either crude or semi-purified parasite lysates typically from epimastigotes. However the complex nature of molecules in these lysates creates a test that routinely gives false positive diagnosis. Research to improve serological diagnosis techniques has focused on the identification, characterization and cloning of particular *T. cruzi* antigens that elicit a strong B cell response. Experiments have demonstrated that some individuals declared negative by current serological tests in fact respond to parasite lysate by producing IFN-gamma in ELISPOT assays. These individuals therefore have T cells that have been exposed to parasite antigen but have a poor B cell antibody response to the antigens in the serological tests that use parasite lysate. The present invention is capable of detecting components present in the sera of such individuals, as shown in FIG. 6.

It is to be understood that other screening methods are applicable to the identification of antigenic *T. cruzi* polypeptides to be included in the multicomponent panel for the diagnostic test. For example, U.S. Pat. No. 6,875,584, issued Apr. 5, 2005, describes screening methods that can be used to identify additional antigenic *T. cruzi* polypeptides for use in a diagnostic test and/or as vaccine components. As another example, a nickel-coated substrate, such as a nanoparticle array, can be used to immobilize His-tagged candidate antigens which can then be contacted with serum or other blood product (in the case of the serodiagnostic test) or MHC-peptide complexes (in the case of the cellular test) to detect evidence of an immune response in the serum.

It is to be understood that any of the diagnostic, therapeutic or laboratory methods described herein can be performed with one or more protein antigens as set forth in Table 1, Table 3 and/or Table 4 herein; or as elsewhere described in the specification.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

EXAMPLES

The majority of current serological tests for *T. cruzi* infection utilize whole to semi-purified parasite lysates and are often inconclusive or result in false positives. Recent studies have identified individuals who are seronegative for *T. cruzi* infection by standard tests but are positive by PCR (Salomone et. al. *Emerg. Infect. Disease*, 2003, 9:1558) or have demonstrable cellular immune responses to *T. cruzi*. With respect to the latter, our lab has recently demonstrated that some individuals declared negative by current serological tests in fact have demonstrable T cell responses to parasite lysate as seen

in ELISPOT assays. These individuals therefore have T cells which have been exposed to parasite antigen but have a poor B cell antibody response to the mix of antigens in the serological test. It is apparent that the use of lysates is a poor test for *T. cruzi* infection and we expect that screening with multiple recombinant proteins will be able to reduce the number of false positives, and more importantly false negatives.

We have therefore developed a high-throughput method to screen large numbers of recombinantly expressed *T. cruzi* proteins for their serodiagnosis potential. Specifically, we combined a set of putative *T. cruzi* genes cloned into the GATEWAY with the BIO-PLEX LIQUICHIP bead technology to screen large numbers of recombinantly expressed proteins for their antigenicity using only a small volume of sample (<100 μ l). So far, we have produced 34 pools of approximately 10 proteins each and screened them for antigenicity. From the preliminary testing, 11 pools were found to bind readily detectable amounts of antibodies in the sera of *T. cruzi*-infected subjects. These pools were then broken down and each gene was expressed individually and tested. From these 81 genes we have been able to define more than 15 proteins with serodiagnostic potential.

Our method utilizes a blind screening process that has identified several known antigens as well as previously unidentified antigenic proteins from within pools containing multiple non-antigenic proteins. The use of the BIO-PLEX technology is not limited to antigen screening but its full potential may be realized as a novel method of blood donor screening. The highly antigenic proteins we discovered, and expect to continue to discover, with this method can be used to create a highly sensitive and specific test for *T. cruzi* infection.

Example 1

Buffer and Medium Preparation

A variety of buffers were used in the BIO-PLEX multiplex analysis. The buffers were prepared as follows. To prepare 1 liter of PBS/BSA (10 mM NaH_2PO_4 , 150 mM NaCl, and 0.1% (w/v) BSA), 8.77 g NaCl (MW 58.44 g/mol) and 1.4 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (MW 137.99 g/mol) were dissolved in 900 ml H_2O and the pH was adjusted to 7.4 using NaOH. Then, dissolve 1 gram of BSA and adjust the volume to 1 liter. Before use, filter the buffer using a 0.45 μ m filter. Sodium Azide should be added to 0.5% when storing the PBS/BSA buffer for long term. Azide should not be used with Carboxy Beads.

To prepare 1 liter of coupling buffer (50 mM MES), 11.67 g MES (MW 233.2 g/mol) was dissolved in 900 ml H_2O and the pH was adjusted to 5.0 using NaOH. The volume was then adjusted to 1 liter using additional H_2O . Before use, the buffer should be filtered using a 0.45 μ m filter.

To prepare 1 liter of activation buffer (100 mM NaH_2PO_4), 13.80 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (MW 137.99 g/mol) was dissolved in 900 ml H_2O and the pH was adjusted to 6.3 using NaOH. The volume was then adjusted to 1 liter using additional H_2O . Before use, the buffer should be filtered using a 0.45 μ m filter.

To prepare Buffer Z, 8 M urea, 20 mM Hepes, and 100 mM NaCl are combined and dissolved in deionized water to form a solution. The pH of the solution is adjusted to 8.0, and the solution is filtered through a 0.45 μ m filter and stored at room temperature. Imidazole (the side chain molecule in histidine) is added to Buffer Z at varying concentrations to either prevent the cobalt resin from binding non-specifically to some-

thing other than the histidine tag, or to out-competing the binding of the histidine tag and thus causing the protein to elute off the resin.

To prepare LB (*Luria-Bertani*) Medium, 10 g tryptone, 5 g yeast extract, and 10 g. NaCl were dissolved in 1 L deionized water and autoclaved for 25 minutes. For plates, 15 grams of agarose were also dissolved into the water prior to autoclaving.

Example 2

Production of Protein Pools or Individual Proteins

To provide a large set of *T. cruzi* proteins, over 350 proteins in pools of approximately 10 proteins each were prepared. The proteins were prepared using the GATEWAY universal cloning technique developed by INVITROGEN. The procedure can be carried out by cloning a pool of several genes together, which results in a pool of proteins, or by cloning an individual gene, resulting in the preparation of an individual protein. For preparation of an individual protein, a gene that codes for a desired *T. cruzi* protein is first selected for cloning. This gene is amplified from *T. cruzi* genomic DNA using gene specific primers flanked by lambda phage recombination sites, attB1 (5') and attB2 (3') and polymerase chain reaction. Gel purification of the att-flanked PCR product was carried out by separating the PCR reaction product on a 1% agarose gel using electrophoresis. The particular gene is identified by comparison with a DNA standard containing bands of known size. The band of the gene of interest is cut out of the gel and purified using Sigma-Aldrich's GenElute Minus EtBr Spin Columns (Catalog No. 5-6501).

The GATEWAY BP reaction is then used to insert the att-flanked *T. cruzi* gene fragment with a pDONRTM201 vector (Catalog No. 11798-014, INVITROGEN Corp., Carlsbad, Calif.). The BP reaction is conducted by adding the 5 µl of gel-purified attB-flanked PCR product (40-100 fmoles), 1 µl of the pDONRTM201 vector (supercoiled, 150 ng/µl), and 2 µl 5xBP CLONASE Reaction Buffer (Catalog No. 11789-013) to obtain a final volume of 8 µl. The BP CLONASE enzyme mix (Catalog No. 11789-013, INVITROGEN Corp., Carlsbad, Calif.) is mixed gently, and then 2 µl of the enzyme mix was added to the BP reaction mixture and mixed well. The reaction was then incubated at (room temperature) 25° C. overnight. Next, 1 µl of Proteinase K solution (Catalog No. 11789-013, INVITROGEN Corp., Carlsbad, Calif. 2 µg/µl) was added, and the mixture was allowed to incubate for 10 minutes at 37° C. Five microliters of the BP reaction are transformed by heat shock into chemical competent DH5α cells and grown up overnight at 37° C. shaking at 280 RPM in 5 mL of LB with 50 mg/L kanamycin to select for pDONR201-transformed cells. The plasmid is then purified from the culture using a QIAprep Spin Miniprep Kit (Catalog No. 27106, Qiagen Inc., Valencia, Calif.).

For the next step of protein production, the GATEWAY recombination reaction was used to insert the gene of interest in pDONR201 into a destination vector to provide the final expression clone. The destination vector in this case is a modified version of INVITROGEN's pRSET (Catalog No. V351-20), called pDEST-PTD4. First, the pDEST-PTD4 was linearized by restriction digest of a novel site (PvuII) within the cell death cassette. The linearized plasmid was purified using QIAquick Gel Extraction Kit (Catalog No. 28207, Qiagen Inc., Valencia, Calif.). The LR reaction between the gene of interest in the pDONRTM201 vector and the desired pDEST-PTD4 expression vector was then set up. First, 300 ng of the pDONR entry clone (prepared above), 300 ng of lin-

earized pDEST-PTD4 (INVITROGEN Corp., Carlsbad, Calif.), and 2 µl LR CLONASE Reaction Buffer (Catalog No. 11791-019, INVITROGEN Corp., Carlsbad, Calif.), 2 µl LR CLONASE Enzyme Mix, and deionized water are combined to obtain a final volume of 10 µl and mixed thoroughly by flicking the tube. The reaction was then incubated overnight at 25° C. Next, 2 µl proteinase K solution (2 µg/µl) was added and the mix was allowed to incubate for 10 minutes at 37° C. DH5α cells were then transformed by heat shock with 6 µl of LR reaction products, and plated onto LB agar plates containing 150 mg/L ampicillin and incubated overnight at 37° C. to select for ampicillin-resistant expression clones.

Next, all of the colonies were scraped clean with a clean sterile spatula, and used to inoculate a tube of 5 mL LB containing 150 mg/L ampicillin, and grown overnight at 37° C., 280 RPM. The pDEST-PTD4 containing the gene of interest is purified from the culture using a QIAprep Spin Miniprep Kit (Catalog No. 27106, Qiagen Inc., Valencia, Calif.). The miniprep preparation should contain copies of each gene of the pool from the desired pDEST vector. Three microliters of purified pDEST-PTD4 containing the gene of interest was then transformed into BL21(DE3)pLysS chemical competent cells. The culture was then directly inoculated into 10 mL LB/ampicillin (Amp)/chloramphenicol (CAM) (100 mg/L)/(34 mg/L) and grown overnight, shaking at 37° C. at 280 RPM.

On the fifth day, a 10 mL starter culture was inoculated into 500 mL LB/Amp/CAM and grown to an OD₆₀₀ of 0.4. Protein expression was then induced with 0.3 mM concentration of IPTG (isopropyl-β-D-thiogalactopyranoside), using 150 µl of 1M IPTG in 500 mL culture. The cells were spun down at 5,000 rpm for 8 minutes and 10 mL Buffer Z (8M urea, 20 mM Hepes, 100 mM NaCl) containing 15 mM imidazole was added. The cells were then sonicated three times for 25 seconds at an amplitude of 40. The samples were spun down at 13,000 rpm for 10 minutes and the supernatant is combined with 1 mL settled BD TALONTM Metal Affinity Resin (BD Biosciences Clontech, Catalog No. 635502) and rocked overnight at 4° C.

The resin/cell lysate slurry is then placed into an empty 0.8x4 cm chromatography column and the resin bed is allowed to settle. The liquid was allowed to run through and the resin bed was washed with 10 bed volumes (10 mL) of Buffer Z containing 15 mM imidazole. Once the 10 mL wash has run through, the His-tag protein was eluted with 3 bed volumes (3 mL) of Buffer Z containing 250 mM imidazole. The resulting sample contained the purified protein of interest. The sample was then desalted into Buffer Z (without imidazole) using a PD-10 desalting column (Amersham Biosciences, Catalog No. 17-0851-01). The resulting imidazole-free sample is quantified and diluted to a concentration of 10 µg/mL which is ready to be used to bind to BIO-PLEX beads for testing.

Example 3

Preparation of BIO-PLEX Beads

LIQUICHIP Ni-NTA beads (Qiagen Inc., Valencia, Calif.) were used to bind His-tagged purified proteins in the BIO-PLEX assay, but had to be prepared before use. First, the protein samples were desalted into Buffer Z that does not contain imidazole using Amersham PD-10 desalting columns (Amersham Biosciences Corp, Piscataway, N.J.). The protein was then quantified using a BCA assay and diluted to a concentration of 10 µg/ml with Buffer Z. The LIQUICHIP Ni-NTA Bead stock was then vortexed for 30 seconds at full

speed. Next, 50 μ l of bead suspension was pipetted out and placed into a 1.5 ml microcentrifuge tube. His-tagged protein dilution (50 μ l) was then added to the 50 μ l LIQUICHIP Bead suspension. The beads were then incubated at 4 $^{\circ}$ C. in the dark from at least 4 hours to overnight. Buffer (900 μ l PBS/BSA (10 mM NaH₂PO₄, 150 mM NaCl, 0.1% BSA pH 7.4)) was then added to the protein-coupled LIQUICHIP Bead suspension, adding 0.5% azide as a preservative.

Example 4

Preparation of Positive Controls

Positive and negative controls were used in the BIO-PLEX analysis of *T. cruzi* antigens. The positive control consists of proteins from a *T. cruzi* lysate coupled to LIQUICHIP Carboxy Beads. The beads thus contain a mix of *T. cruzi* proteins bound to their surface, and function as a general antigen mix. The LIQUICHIP Carboxy Beads bind to the proteins in a random manner, forming covalent bonds to amine groups in lysine side chains. The first step in the preparation of positive controls was the activation of Carboxy Beads using EDC/NHS. First, approximately 10 mg each of EDC (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (Fluka catalog No. 03449)) and NHS (N-hydroxysulfosuccinimide (Fluka catalog No. 56485)) were weighed into two microcentrifuge tubes. The LIQUICHIP CarboxyBead suspension (1 ml) was then centrifuged for 5 minutes at 10,000 rpm in a microcentrifuge. The supernatant was removed with a 200 μ l pipette and discarded. The beads were then washed twice by adding 80 μ l of activation buffer and centrifuged for 5 minutes at 10,000 rpm. The supernatant was then carefully removed. Activation buffer (80 μ l) was then added to the bead pellet at the bottom of the tube. The pellet should not be resuspended. The pellet in activation buffer was then vortexed for at least 2 minutes. De-ionized water was then added to the weighed EDC and NHS aliquots to provide solutions with a concentration of 50 mg/ml. NHS solution (10 μ l) and EDC solution (10 μ l) were then added to the bead suspension, which was then incubated for 20 minutes in the dark. Finally, the beads were centrifuged for 5 minutes at 10,000 rpm, after which the supernatant was removed and discarded.

The activated beads were then coupled to the *T. cruzi* lysate. First, the *T. cruzi* pellet was freeze/thawed about 5 times. Insoluble particles were removed by centrifugation. The protein stock was then diluted with coupling buffer to a concentration of 100 μ g/ml and a volume of 500 μ l. Any foreign protein, azide, glycine, Tris, or other reagent containing primary amine groups present in the protein preparation should be removed by dialysis or gel filtration. Coupling buffer (500 μ l) was then added to the beads, which were then resuspended by vortexing. The beads were then washed twice by adding 500 μ l of coupling buffer, centrifuging for 5 minutes at 10,000 rpm, removing the supernatant, and then repeating the process. Diluted protein solution (500 μ l), prepared earlier, was then added. Next, the tube containing the activated beads and the protein solution was gently agitated on a shaker for 2 hours in the dark at room temperature. The beads were then washed twice with PBS/BSA buffer. The beads were then resuspended in 500 μ l PBS/BSA, and 0.5% azide was added as a preservative. The bead number was then adjusted to provide the desired concentration per microliter.

Example 5

BIO-PLEX Analysis of Proteins

At the start of the analysis, a dilution series of the serum to be tested was prepared on a MILLIPORE 96 well filtration

plate. The BIO-PLEX Bead/Protein preparation, prepared according to Example 3, was then added to the wells on a MILLIPORE 96 well filtration plate. When preparing beads according to the normal protocol, 10 μ l of bead suspension is sufficient to make a useful data point. However when testing beads in which multiple proteins are bound to an individual bead, it may be necessary to combine the beads into a single tube and distribute them to wells so that enough of each bead is present in a given well to give an accurate data point. Controls are preferably included for each sample (sera/protein) being analyzed. For example, the BIO-PLEX analysis for *T. cruzi* antigens included a bead coated with ovalbumin (OVA) as a negative control and with *T. cruzi* lysate as a positive control.

To prepare for the BIO-PLEX analysis, 30 μ l of PBS/BSA buffer and 10 μ l of an individual bead suspension (or a predetermined volume containing multiple beads each with different proteins bound) were added to the MILLIPORE 96 well filtration plate. The filtration plate was then placed on the vacuum manifold and the sample liquid was pulled through the plate. Next, 50 μ l of PBS/BSA and 50 μ l of serum dilution were added. The beads were then incubated for 1 hour at room temperature while being shaken on a plate shaker. Each well was then washed four times with 200 μ l PBS/BSA to remove any unbound IgG antibodies from the well. PBS/BSA buffer (90 μ l) was then added to each well and beads that had settled to the bottom of the filtration plate well due to washing were resuspended. An aliquot (10 μ l) of the secondary reporter molecule was then added. This provided a 1:30 dilution (0.5 mg/ml) of antibody. A higher dilution may be used, but a 1:30 dilution makes sure that secondary antibody is not limited by residual unbound IgG. The solution was then incubated for 1 hour at room temperature while being shaken.

The assay solution was then drawn into the BIO-PLEX array reader, which illuminates and reads the sample. When a red diode "classification" laser (635 nm) in the BIO-PLEX array reader illuminates a dyed bead, the bead's fluorescent signature identifies it as a member of one of the 100 possible sets. BIO-PLEX Manager software correlates each bead set to the assay reagent that has been coupled to it. In this way the BIO-PLEX system can distinguish between the different assays combined within a single microplate well. A green "reporter" laser (532 nm) in the array reader simultaneously excites a fluorescent reporter tag (phycoerythrin, or PE) bound to the detection antibody used in the assay. The amount of green fluorescence is proportional to the amount of analyte captured in the immunoassay. Extrapolating to a standard curve allowed quantitation of the analyte in each sample. The results for specific proteins are described in Example 8, and shown in FIGS. 4-6.

Example 6

BIO-PLEX Assay of VV-Ovalbumin Sera

Ovalbumin (OVA) chosen as the protein antigen to develop the BIO-PLEX method. Mice were infected with Vaccinia virus (VV) containing the OVA gene in order to raise serum antibodies to the protein. Sera was collected at 7 days post infection, followed by a boost and an additional sera collection 7 more days later. OVA protein was expressed in *E. coli* and purified using a His-tag and bound to BIO-PLEX beads via a Ni-NTA residue and adsorbed to an ELISA plate for analysis. The sera was diluted and tested using the BIO-PLEX Assay described in Example 5. The results are shown

in FIG. 3A. The results obtained were very comparable to those obtained using ELISA, as described in Example 7.

Example 7

ELISA Assay of VV-Ovalbumin Sera

A comparison assay on the ovalbumin of mice infected with Vaccinia virus was run using the ELISA (Enzyme-linked Immunosorbent Assay) method. First, a 96-well polystyrene Immunolon microtiter plate (Dynex Technologies, Chantilly, Va.) was coated with 100 μ l of 10 μ g/ml ovalbumin (OVA) in PBS overnight at 4° C. or 2 hours at 37° C. The wells were then washed three times with PBS-T (PBS-TWEEN 20 buffer) and then blocked with 1% BSA for 2 hours. Serum dilutions were then added to each well and the wells were incubated for 2 hours at room temperature or overnight at 4° C. After incubation, the wells were washed five times with PBST. Biotinylated secondary mouse antibody (1:100 dilution) was then added and the wells were allowed to set for 1 hour at room temperature. The wells were then washed again for five times with PBST. Horseradish peroxidase-conjugated streptavidin was then added for 30 minutes at room temperature at a 1:100 dilution. The wells were then washed again five times with PBST. Finally, a developing reagent (2,2'-azido-di-[3-ethylbenzthiazoline sulfonate], ABTS) was added. The results of the ELISA assay of ovalbumin sera are shown in FIG. 3B.

Example 8

BIO-PLEX Assay Results for Pooled and Specific Proteins

Using the method of protein production described in Example 2, over 350 proteins in pools of approximately 10 proteins each were prepared. Each of the pools were screened for antigenicity using the BIO-PLEX technology, as described in Example 5. From the preliminary testing, 11 pools were found to bind readily detectable amounts of antibodies in the sera of *T. cruzi*-infected subjects. These pools were then broken down and each gene was expressed individually and tested. From the over 80 genes expressed, 15 proteins have been confirmed as having serodiagnostic potential.

A Hemagen® Diagnostics Chagas Disease Test Kit (Hemagen Diagnostics, Inc., Columbia, Md.) was used to evaluate and confirm the presence of anti-*T. cruzi* antibodies in sera from areas of active transmission in Argentina. Sera from non-endemic uninfected in-house sera served as the negative controls. Sera from 4 individuals from areas of active trans-

mission, all of which have tested seronegative using standard assays but 1 of which tests positive for T cell reactivity to *T. cruzi*, were used for the very low positive control. Sera from 5 individuals that were borderline positive/negative using standard serological assays were used to make up the borderline positive control and sera from 7 individuals that were consistently seropositive using standard serological assays make up the strong positive control.

Genes of interest were first cloned into the GATEWAY holding vectors (pDONR™ vector) and archived as single vectors or are placed into pools. Pools of genes in pDONR™ vectors can be moved simultaneously into either DNA vaccination vectors or protein expression vectors without the loss of individual genes in the pool. The resulting pools were expressed in *E. coli* strain BL21(DE3)pLysS cells, minimizing the possible toxic effects of individual genes. The protein pools are purified and tested using the BIO-PLEX bead technology for antigenicity. The results from analysis of the protein pools using the BIO-PLEX analysis method are shown in FIGS. 4A-2D. The headings in the figures indicate the type of sera being tested, based on the four categories (negative, very low positive, borderline positive, and strong positive) resulting from the evaluation using the Hemagen® Test Kit. As indicated by the arrow, FIG. 4D demonstrates a pool that shows high fluorescence, and hence contains a high level of protein that binds to *T. cruzi*-specific antibodies.

Once a pool of proteins was identified using the BIO-PLEX screening method as having possible antigenic properties, the individual genes in the pool were examined and tested to find which ones provided reactive antigens. The genes were first moved individually from the pDONR holding vector into an expression vector, followed by expression, purification and testing. Those proteins that exhibit binding to antibodies in infected individuals were then retested for confirmation and identified. The results of screening the pools for individual proteins is shown in FIGS. 5A-5D. The arrow in FIG. 5D shows a particular protein that reacted strongly with anti-*T. cruzi* antibodies present in strong positive sera.

From the proteins that were screened, many that showed antigenic activity were proteins that had been previously characterized as *T. cruzi* antigens. This provides a level of proof to the capacity of this technique to discover single antigens in pools. Selected ribosomal proteins, ubiquitin, calcium binding proteins, and paraflagellar rod proteins have all been described previously as being possible targets for serological diagnosis of *T. cruzi* infection. A list of the individual proteins identified as *T. cruzi* antigens using the BIO-PLEX screening method are shown below. The "Gene ID numbers" represent gene numbers assigned by annotators of the *T. cruzi* genome and are accessed via the *T. cruzi* genome database on the worldwide web at "TcruziDB.org."

TABLE 1

Assay Protein ID	Protein	<i>T. Cruzii</i> database accession numbers	Gene ID numbers	SEQ ID No:
1a-1	Tc beta-tubulin	Tc00.1047053506563.40	6998.t00004	1
1a-5	Tc alpha tubulin	Tc00.1047053411235.9	11788.t00001	2
1c-3	60S ribosomal protein L2, putative	Tc00.1047053508299.60	5568.t00006	3
2b-3	hypothetical protein, conserved	Tc00.1047053506529.460	6986.t00046	4
2c-1	cytochrome C oxidase subunit IV, putative	Tc00.1047053506529.360	6986.t00036	5
2c-9	hypothetical protein	Tc00.1047053506529.610	6986.t00061	6
2i-1	hypothetical protein, conserved	Tc00.1047053510887.50	6003.t00005	7
3d-3	iron superoxide dismutase, putative	Tc00.1047053509775.40	5781.t00004	8
3d-4	trans-splicing factor, putative	Tc00.1047053503583.40	4650.t00004	9

TABLE 1-continued

Assay Protein ID	Protein	<i>T. Cruzi</i> database accession numbers	Gene ID numbers	SEQ ID No:
3j-1	60S ribosomal protein L28, putative	Tc00.1047053506297.270	6890.t00027	10
3k-1	glycosomal phosphoenolpyruvate carboxykinase, putative (Phosphoenolpyruvate Carboxykinase (Pepck))	Tc00.1047053508441.20	7730.t00002	11
3k-2	ubiquitin-fusion protein, putative (polyubiquitin/ribosomal protein CEP52)	none	7355.t00001	12
3k-3	60S acidic ribosomal subunit protein, putative (Calmodulin-ubiquitin associated protein CUB2.8)	Tc00.1047053508355.250	7695.t00025	13
3k-5	ef-hand protein 5, putative	Tc00.1047053506391.30	6925.t00003	14
4a-3	paraflagellar rod protein 3	Tc00.1047053509617.20	8152.t00002	15
B1	axoneme central apparatus protein, putative	Tc00.1047053510955.40	8553.t00004	16
B2	serine carboxypeptidase (CBP1), putative	Tc00.1047053509695.220	8171.t00022	17
B5	aminopeptidase, putative	Tc00.1047053511289.30	8647.t00003	18
B7	elongation factor-1 gamma, putative	Tc00.1047053510163.20	8322.t00002	19
B8	hypothetical protein, conserved	Tc00.1047053506531.20	6987.t00002	20
D3	hypothetical protein, conserved	Tc00.1047053506489.30	6967.t00003	21

Research to improve serological diagnosis techniques has focused on the identification, characterization and cloning of particular *T. cruzi* antigens that elicit a strong B cell response. The use of *T. cruzi* specific antigens in a serological test gives a high level of specificity to a serological test, eliminating the problems that arise due to cross-reactivity to a parasite lysate. However using only a single antigen may not be sensitive enough to detect all individuals that are infected, and thus the use of multiple antigens is preferred. Recent evidence demonstrates that some individuals declared negative by current serological tests in fact respond to parasite lysate by producing IFN- γ in ELISPOT assays. These individuals therefore have T cells that have been exposed to parasite antigen, but have a poor B cell antibody response to the antigens in the serological tests that use parasite lysate. The ability to evaluate the T cell reactivity of individual proteins to sera from various subjects using the BIO-PLEX analysis is shown in FIG. 6A-6D.

Example 9

High Throughput Selection of Effective Serodiagnostics for *T. cruzi* Infection and Multiplex Diagnostic for Chagas Disease

As noted above, diagnosis of *T. cruzi* infection by direct pathogen detection is complicated by the low parasite burden in subjects persistently infected with this agent of human Chagas disease. In this study, we sought to improve upon current diagnostics for *T. cruzi* infection by screening for diagnostic candidates that displayed the ability to detect infection in subjects that went undetected or gave discordant results using other conventional serologic tests. We screened more than 400 recombinant proteins of *T. cruzi*, including randomly selected and those known to be highly expressed in the parasite stages present in mammalian hosts, for the ability to detect anti-parasite antibodies in the sera of subjects with confirmed or suspected *T. cruzi* infection. A set of 16 protein groups were then incorporated into a multiplex bead array format which detected 100% of more than 100 confirmed

positive sera and also documented consistent, strong and broad responses in samples undetected or discordant using conventional serologic tests. Each serum had a distinct but highly stable reaction pattern. The end result was thus the identification of a panel of recombinant proteins that more reliably detects *T. cruzi* infection than do a combination of existing conventional tests. Additionally, we show that a multiplex assay utilizing this diagnostic panel has utility in monitoring drug treatment efficacy in chronic Chagas disease. These results substantially extend the variety and quality of diagnostic targets for Chagas disease and offer a useful tool for determining treatment success or failure.

Methods

Parasites, gene selection and cloning. *T. cruzi* epimastigotes of the Sylvio, C L Brenner, Brazil, C L, Tulahuén, M83, M91 and Chapulin isolates were maintained in logarithmic phase growth (Kirchhoff et al., 1984, J Immunol 133: 2731-2735) and used as a source of genomic DNA. Primer sets incorporating lambda phage recombination sites flanking the 18-21 base gene-specific sequence (excluding both start and stop codons), were designed for each gene of interest, the genes cloned by PCR from the pooled DNA of the 8 *T. cruzi* isolates and GATEWAY adapted gene product inserted into the pDONR-201 plasmid (INVITROGEN, Carlsbad, Calif.). To speed the cloning process while improving our chances of cloning full-length non-mutated genes, at least 10 clones positive for the appropriate sized insert were pooled for each gene. *T. cruzi* lysate from Brazil strain amatigote and trypomastigotes was prepared as previously described (Lauella et al., 2004, J Infectious Diseases 189:909-918).

pDEST-PTD construction. The pDEST-PTD protein expression vector was created from pTAT_HA (Nagahara et al., 1998, Nat Med 4: 1449-1452) by replacing the BamHI-flanked TAT sequence with a BamHI-flanked PTD-4 encoding sequence (Ho et al., 2001, Cancer Res 61: 474-477), followed by GATEWAY (INVITROGEN)—adaptation of the plasmid utilizing the NcoI and XhoI cloning sites. The GATEWAY cloning cassette was PCR amplified from pDEST-YFP (gift from Dr. Boris Striepen, University of Georgia, Ga.).

Protein production and purification. Genes in pDONR plasmids were transferred to pDEST-PTD4 via a GATEWAY LR reaction and the proteins expressed in BL21(DE3)pLysS cells were extracted by sonication in 8M urea, 20 mM HEPES, 100 mM NaCl, pH 8.0 containing 15 mM imidazole. The lysate was then applied to TALON Metal Affinity Resin (BD Biosciences Clontech, Palo Alto, Calif.) and bound protein was eluted with 250 mM imidazole. Imidazole was removed on PD-10 desalting columns (GE Healthcare, Piscataway, N.J.) and protein concentration was estimated using a modified Bradford assay. Proteins were diluted to 10 µg/mL (in 8M urea) and stored in 1 mL aliquots at -20 C until use.

Human sera. Sera were obtained from individuals living in areas of Santiago del Estero, Argentina endemic for *Trypanosoma cruzi* and were analyzed using conventional serologic tests (e.g. immunofluorescence assay (IFI), hemagglutination (HAI), and ELISA) performed at the Diagnostic Department of the Instituto Nacional de Parasitología "Dr. Mario Fatala Chabén" and in our laboratory by a commercial ELISA serodiagnostic kit (Hemagen Diagnostics, Columbia, Md.). The latter assay was carried out as per the manufacturer's instructions with a positive response defined as 10% above the cutoff (0.250+mean of negative control absorbencies). Three serum pools were created: a "sero-negative" pool consists of 4 sera negative on all assays; a "borderline positive" pool made up of 5 sera with a response at or just above the equivocal zone of the Hemagen test (between cutoff and below cutoff+10%); a "strong positive" pool containing 7 sera that gave unequivocally positive responses on all tests. True negative controls were obtained from volunteer donors who were not from endemic areas. Sera used for subsequent analysis of individual proteins were obtained from *T. cruzi*-infected adult volunteers aged 29 to 61 recruited through the Chagas Disease Section of the Cardiology Department, Hospital Interzonal General de Agudos "Eva Perón", Buenos Aires, Argentina and infection status was determined serologically as described above. In some cases, subjects treated by a 30 day course of benznidazole as previously described (Viotti et al., 2006, Ann Intern Med 144: 724-734) donated serum samples prior to treatment and at regular intervals following treatment. The protocols were approved by the IRBs of the University of Georgia and the Hospital Interzonal General de Agudos "Eva Perón" and signed informed consent was obtained from all individuals prior to inclusion in the study.

Multiplex assay. Recombinant proteins were attached to LIQUICHP Ni-NTA beads (Qiagen) or Beadlyte Nickel Beads (Upstate Biotechnology) by overnight incubation at 4° C. in the dark. The sets of distinct addressable beads, each with a different protein attached, were pooled in equal volumes along with positive and negative control beads, consisting respectively of LIQUICHP Carboxy Beads (Qiagen) coupled to *T. cruzi* lysate and LIQUICHP Ni-NTA beads coated with recombinant HIS-tagged green fluorescent protein (GFP). Sera at 1:500 dilutions were added and the multiplex assays conducted using standard procedures (Waterboer et al., 2006, J Immunol Methods 309: 200-204). Antibody binding to individual beads was detected with goat anti-human IgG conjugated to phycoerythrin (Jackson ImmunoResearch, West Grove, Pa.) and quantified on a BIO-PLEX Suspension Array System (BioRad).

Statistical analysis. Serum samples were assayed in duplicate and the weighted mean fluorescence intensity (MFI) was calculated for a minimum of 30 beads per determination. The ratio of the specific MFI for each antigen to the MFI of the negative control (GFP- or OVA-coupled) protein was then calculated for each serum and antigen in the assay. Values

above the mean plus 4 standard deviations of a minimum of sixteen true negative sera run in the same assay, and individually determined for each antigen, were considered positive. Results

As part of a vaccine discovery effort, nearly 1500 genes from *T. cruzi* have been cloned into GATEWAY entry vector plasmids that allow them to be easily moved into a range of other plasmids. Genes were selected for cloning using a variety of criteria, initially including known expression in *T. cruzi* lifecycle stages that are present throughout infection in mammals (e.g. trypomastigotes and amastigotes), high likelihood of being surface expressed or secreted and expected presence in the genome at low copy number. With the completion of the *T. cruzi* genome sequencing project (El-Sayed et al., 2005, Science 309: 409-415) and whole organism proteome analysis (Atwood et al., 2005, Science 309: 473-476) the additional criterion of being relatively high in abundance in the proteomes of trypomastigotes and amastigotes was added as a basis for selection. Recombinant proteins produced in *E. coli* had N-terminal tags carrying the 6xHis-, PTD (Ho et al., 2001, Cancer Res 61: 474-477) and HA—tags for purification, protein translocation, and identification, respectively were captured by Ni-coupled Luminex beads for use in a multiplex bead array assay.

Selection of the diagnostic panel. The initial selection screen (FIG. 7) used approximately 420 proteins produced in pools of 8-10 proteins each. Production of pooled proteins was accomplished by moving sets of genes in batch into the PTD-4 expression plasmid and was confirmed by SDS-PAGE analysis (FIG. 8). In addition to the individual or pooled recombinant *T. cruzi* proteins, each screening experiment included negative control recombinant protein (ovalbumin or GFP) expressed from the PTD-4 plasmid as well as a lysate of trypomastigotes and amastigotes of *T. cruzi* that had been chemically coupled to BIO-PLEX beads.

To screen the pooled proteins we also took a pooling approach by assembling sera from subjects with and without documented infection with *T. cruzi*. Screening of 51 protein pools revealed 21 pools that were reactive with one or more of the serum pools 1-3 (FIG. 7). Reactive pools were then broken down into their individual constituent proteins; a total of 140 proteins were successfully expressed and individually rescreened with the serum pools, ultimately resulting in the selection of 55 proteins with serodiagnostic potential (FIG. 7 and Table 2). An additional 22 proteins that were either identified as high-abundance proteins using proteome analysis (Atwood et al., 2005, Science 309: 473-476) and/or as being unique to *T. cruzi* (and thus not encoded in the *T. brucei* or *Leishmania major* genomes) were then screened using the pooled sera, and 4 of these 22 were found to be reactive with one or more serum pools. Of the resulting 59 candidate proteins recognized by antibodies in the serum of *T. cruzi*-infected subjects, a substantial number were subsequently excluded from further testing either because they exhibited significant reactivity with sera from the true negative pool, or because they interfered with other beads in the multibead assays, perhaps because of protein-protein interactions. Preference was also given to *T. cruzi* proteins that detected antibodies in sera from the "borderline" pools. Ultimately 39 proteins (in bold and italics in Table 2) were selected for extensive further testing with a wider array of individual subject sera.

TABLE 2

The 59 candidate diagnostic proteins screened independently with individual (non-pooled) sera.

Gene Id	Gene name(s)	Notes	% reactive with 121 known positive sera	SEQ ID No:
Tc00.1047053506391.10, and	calmodulin and ATPase beta subunit	high abundance	32.23%	22
Tc00.1047053509233.180				23
Tc00.1047053507029.30	heat shock 70kDa protein, mitochondrial precursor, putative	high abundance	52.89%	24
Tc00.1047053510955.40	axonemal central apparatus protein, putative		42.15%	16
Tc00.1047053511215.119	69kDa paraflagellar rod protein, putative		23.97%	25
Tc00.1047053511271.10	dispersed gene family 1 fragment 4	unique to <i>T. cruzi</i>	5.08%	26
Tc00.1047053506529.610	hypothetical protein		17.27%	6
Tc00.1047053506391.30	EF-hand protein 5		2.48%	14
Tc00.1047053506635.130	hypothetical protein, conserved	high abundance	68.60%	27
Tc00.1047053511265.10	dispersed gene family 1 fragment 5	unique to <i>T. cruzi</i>	8.62%	28
Tc00.1047053511289.30	aminopeptidase, putative		11.57%	18
Tc00.1047053506195.110	malate dehydrogenase, putative	high abundance	24.79%	29
Tc00.1047053508461.140	poly(A)-binding protein	high abundance	34.17%	30
Tc00.1047053508441.20	glycosomal phosphoenolpyruvate carboxykinase, putative	high abundance	59.29%	11
Tc00.1047053508355.250	60S acidic ribosomal subunit protein, putative	high abundance	75.21%	13
Tc00.1047053511633.79	microtubule-associated protein homolog	high abundance	74.38%	31
Tc00.1047053510433.20, and	TolT proteins	unique to <i>T. cruzi</i>	74.38%	32
Tc00.1047053504277.11, and				33
Tc00.1047053504157.130				34
Tc00.1047053411235.9	alpha tubulin			2
Tc00.1047053510877.30	hypothetical protein, conserved			35
Tc00.1047053509695.220	serine carboxypeptidase (CBP1), putative			17
Tc00.1047053510887.50	hypothetical protein, conserved			7
Tc00.1047053509141.40	hypothetical protein, conserved			36
Tc00.1047053506247.220	histidine ammonia-lyase			37
Tc00.1047053509998.10	60S ribosomal protein L4, putative			38
Tc00.1047053504163.50	fructose-bisphosphate aldolase, glycosomal putative			39
Tc00.1047053507089.270	dihydropyridyl dehydrogenase, putative			40
Tc00.1047053511019.90	iron superoxide dismutase, putative			41
Tc00.1047053509017.20	hypothetical protein, conserved			42
Tc00.1047053506529.360	cytochrome C oxidase subunit IV, putative			5
Tc00.1047053510187.50	tyrosine aminotransferase, putative			43
Tc00.1047053505989.110	hypothetical protein, conserved			44
Tc00.1047053508209.140	protein disulfide isomerase, putative			45
Tc00.1047053506531.20	hypothetical protein, conserved			20
Tc00.1047053504153.280	hypothetical protein, conserved			46
Tc00.1047053509233.180	ATPase beta subunit, putative			47
Tc00.1047053506563.40	beta tubulin			1
Tc00.1047053506459.290	elongation factor-1-gamma, putative			48
Tc00.1047053508707.200	nucleoside diphosphate kinase, putative			49
Tc00.1047053506529.460	hypothetical protein, conserved			4
Tc00.1047053506297.270	60S ribosomal protein L28, putative			10
Tc00.1047053511527.34	60S ribosomal protein L2, putative			50
Tc00.1047053507483.4	polyubiquitin, putative			51
Tc00.1047053509053.70	p22 protein precursor, putative			52
Tc00.1047053506585.40	glucose-regulated protein 78, putative			53
Tc00.1047053511185	dispersed gene family 1 fragment 8			54
Tc00.1047053511589.130	14-3-3 protein, putative			55
Tc00.1047053511167.90	14-3-3 protein, putative			56
Tc00.1047053507241.30	arginine kinase, putative			57
Tc00.1047053510579.70	nascent polypeptide associated complex subunit, putative			58
Tc00.1047053506925.300	cyclophilin a			59
Tc00.1047053509775.40	iron superoxide dismutase, putative			8
Tc00.1047053503583.40	trans-splicing factor, putative			9
Tc00.1047053510099.120	d-isomer specific 2-hydroxyacid dehydrogenase-protein, putative			60
Tc00.1047053507093.300	hypothetical protein, conserved			61
Tc00.1047053508479.340	succinyl-CoA synthetase alpha subunit, putative			62
Tc00.1047053509815.120	dispersed gene family 1 fragment 9			63
Tc00.1047053511727.270	RNA-binding protein, putative			64
Tc00.1047053503781.80	universal minicircle sequence binding protein (UMSBP), putative			65
Tc00.1047053506201.39	translation elongation factor 1-beta, putative			66
Tc00.1047053506815.20	hypothetical protein			67

Note:

Tc00 numbers indicate closest homologue(s) present in the *T. cruzi* CL Brener sequence database (TeruziDb.org) based upon sequencing of the genes (for top 16) or predicted based upon primer sequences used in cloning. Because some primers for PCR cloning were designed prior to the release of the *T. cruzi* CL Brener sequence and the cloning involved the pooling of multiple clone derived from the PCR of a mixture of *T. cruzi* strains (see Material and Methods), some proteins were derived from mixtures of genes (e.g. numbers 1 and 16) and/or had a percent sequence identity <100% relative to the CL Brener strain (range 94.7 to 100%). In some cases (e.g. # 5 and 9) genes >2 kb in length were cloned in ~2 kb fragments in order to facilitate cloning and protein production. Items listed in bold type were selected for screening using >100 individual sera. Items underlined were selected to be part of the final 16 set bead array for screening of discordant sera or sera from subjects post-treatment with benznidazole.

Although the Luminex bead array technology theoretically accommodates up to 100 distinct, addressable beads in a single well—and thus the ability to assay up to 100 individual proteins—at the time of this work only 17 distinct beads were

available with the ability to capture his-tagged proteins. Thus our goal in the second part of the screen was to identify a set of the 16 best *T. cruzi* proteins (allowing a bead for a control non-*T. cruzi* protein). The 39 candidate diagnostic proteins

were tested in sets of 8-15, with each protein on a separate bead and with a negative control bead (HIS-tagged ovalbumin (OVA)) and a positive control bead (*T. cruzi* lysate) included in each assay sample. Between 38 and 48 individual sera from endemic subjects were used to test each protein. These sera are grouped as “uniformly positive” (reactive on all conventional serological tests), “inconclusive” (positive on at least one, but not all, conventional serologic tests), and “negative by conventional tests”, and “known negative” (from residents of North America). FIG. 9 shows a representative set of 29 proteins tested with 54 individual sera and indicates the range of reactivities of both sera and proteins. In addition to providing the basis on which to select the top proteins, this analysis also revealed that among the 30 sera that were inconclusive or negative on conventional tests, nearly half (14 of 30) had substantial reactivity to 3 or more recombinant *T. cruzi* proteins but not with the control OVA protein.

Following repeated screening, 16 proteins were selected to be part of the diagnostic panel (underlined in Table 2). DNA sequencing and mass spectrometric analysis confirmed the identity of each gene and protein and determined that one of the preparations contained two distinct proteins (Cahnodulin and an ATPase) and a second contained a mixture of related TolT proteins. This protein set was then used to screen a larger set of sera, most from chronically infected subjects living in Buenos Aires, and the percentage of these proteins reactive with 121 sera from well-characterized subjects was determined (Table 2). A serum was determined to be positive for any particular test antigen if the average luminescence (MFI) was >4 standard deviations above that of a set of true negative sera run in the same assay. Across all experiments, for the 19 true negative sera assayed multiple times (142 sample runs tested on 16 protein preparations for a total of 2272 determinations), none had S.D. >4 and only 17 of the 2272 determinations were >3 S.D. above the average negative serum values (and 9 of these 17 were from one serum sample reactive with the same antigen in multiple tests). Thus this was a highly stringent cutoff. Sera from all 121 of the confirmed chronically infected subjects reacted with at least 1 of the 16 recombinant protein preparations at the >4 S.D. cutoff and all but 7 reacted with >1 protein. As shown in Table 2, 6 of the 16 of the antigens each detected >50% of the sera and 3 antigens approached a 75% detection rate. Of the 121 sera tested, 118 would have been detected as positive using only 4 of the antigens and 100% would be detected using as few as 7 antigens.

Borderline samples. We then used our 16 bead multiplex test to attempt to resolve questionable infection status in subjects due to discordant results on conventional tests (Table 3). In this analysis, a cutoff for reactivity for each protein in the panel was set at the MFI plus 4 SD above the mean of a set of 16 negative sera. For comparison, the result of multiplex analysis of a pool of strongly positive sera assayed on different days is also shown. The strong positive serum pool showed excellent cross-assay consistency with 11 of the 16 protein preparations positive on each of 8 assays and consistent negative reactivity with 3 of 16. Antibodies to the remaining 2 proteins were also detected but at a lower level that sometimes fell below the strict cutoff of 4 S.D. above the mean. The sera classified as “conventional seronegative with no other evidence of infection” broke into 2 groups based upon the results of the multiplex test. Eight of the 16 failed to react with any of the 16 protein panel (although several reacted with the *T. cruzi* lysate) while the remaining 8 reacted with from 2-4 proteins. A similar nearly 50/50 split was observed in the group of 12 conventional seronegatives who were born in an endemic region, and in 5 individuals who had cardiopathologies consistent with Chagas disease. Lastly, testing in the multiplex assay of sera classified as “positive discordant” (based upon reactivity on 2 of the 3 conventional serologic tests but negative on the 3rd test) confirmed the positive diagnosis in all 7 cases with reactivity evident on 2-6 recombinant proteins by each serum. Without a clear gold standard diagnostic it is not possible certify on a case-by-case basis that the multiplex assay more accurately detects infection than does conventional serology—particularly in cases where there is reactivity to only 1 or 2 proteins and near the >4 S.D. cutoff. And while the birth place and presence of heart disease may support a positive diagnostic test, these criteria do not appear to distinguish between those likely to have reactivity with one or more recombinant proteins in the selected panel and those who do not react. However it is clear that conventional serological tests fail to detect a substantial number of individuals, many with antibodies to multiple *T. cruzi* antigens. It is noteworthy that screening of sera with a parasite lysate also routinely fails to detect sera that exhibit reactivity to multiple recombinant *T. cruzi* proteins. The set of 4 most frequently recognized proteins detected all 7 of the discordant positive samples as well as 13 of the 15 discordant negative or negative samples that reacted with at least 1 protein. Expanding the panel to the 7 proteins that detected all of the seropositive samples (see above) allowed us to detect all of these 15 questionable “negative” samples.

TABLE 3

Reactivity of negative, borderline or discordant sera in the 16 protein multiplex assay.

Sera	Serology	HAI	ELISA	IFI	Proteins																# >= 4 SD	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		lysate
Pooled sera from strongly reactive																						
					9.70	19.26	24.42	7.17	-0.12	nd	2.54	23.83	-0.37	5.48	5.68	17.55	8.79	15.94	23.91	42.37	12.58	12
					7.63	18.83	18.08	5.48	0.06	nd	2.19	31.80	-0.51	6.64	4.41	23.57	33.21	7.02	16.64	57.60	15.97	14
					8.47	16.74	16.65	5.46	0.04	nd	3.18	35.79	nd	4.00	3.63	20.01	nd	10.47	31.26	38.01	12.91	13
					7.33	17.46	15.47	3.63	-0.39	8.21	1.82	27.18	-0.49	4.30	4.35	15.60	20.38	13.70	17.22	44.23	11.83	14
					7.23	18.87	14.06	11.59	0.38	7.67	1.80	29.61	8.93	4.16	3.52	20.81	23.22	13.41	28.35	45.36	11.11	13
					17.64	46.65	30.56	7.84	nd	7.01	2.91	94.71	-0.05	12.30	9.57	45.06	10.81	22.94	22.01	95.05	20.28	13
					13.14	39.82	28.91	4.10	nd	6.35	2.12	72.56	-0.59	10.07	5.03	21.23	6.60	16.63	30.29	91.38	13.71	13
					9.03	31.25	20.79	3.92	nd	12.59	2.68	39.70	-0.08	4.31	3.51	27.05	6.67	10.11	70.18	27.91	19.22	11

Non-infected based upon conventional serology

no	Fe383	neg	nr	nr	0.41	-0.71	-0.16	-1.50	0.43	0.90	-1.22	0.47	-0.35	0.11	-0.45	0.73	0.65	1.61	0.78	-0.51	4.80	0
other	Fe323	neg	nr	nr	-1.07	-0.50	0.42	-1.94	-1.81	-1.37	-1.26	-0.49	0.16	-0.50	-0.84	-0.71	-1.32	-0.58	-0.36	2.89	-0.73	0
hard	Fe438	neg	nr	nr	-1.13	-0.32	0.41	0.52	-0.61	0.93	-0.12	-0.08	-1.15	0.15	-0.79	-0.88	-1.13	0.51	0.32	-0.77	-0.33	0
evi-	Fe410	neg	nr	nr	0.45	-0.11	0.66	-1.81	1.02	-0.68	-1.16	1.21	-0.25	-0.37	-0.97	-0.43	-0.86	0.97	0.99	1.08	-1.16	0
dence	Fe411	neg	nr	nr	0.03	0.40	0.42	-0.21	0.05	2.88	0.13	0.44	1.84	-0.29	1.01	-0.07	2.96	0.67	1.65	0.55	9.19	0
	Fe453	neg	nr	0.024	1.53	0.59	-0.68	1.51	1.24	-0.22	0.58	0.00	0.45	0.34	0.85	0.61	-0.24	-0.48	-0.06	2.09	0.10	0
	Fe408	discord	32	nr	0.55	-0.11	-0.18	0.39	0.94	-0.38	0.40	1.72	-0.04	0.02	-0.45	0.12	0.99	0.46	2.08	1.16	1.93	0
	Fe409	discord	32	nr	-0.39	1.42	1.61	0.65	-0.64	2.35	0.09	1.59	0.55	0.37	2.36	1.47	3.14	0.20	1.96	2.81	0.35	0
	Fe437	neg	nr	nr	-0.09	2.96	1.90	11.99	0.55	nd	0.07	1.73	-0.78	-1.02	1.13	1.11	4.05	2.29	2.73	0.91	-0.24	2
	Fe436	neg	nr	nr	1.26	5.11	1.41	-0.10	0.28	2.57	0.07	1.40	0.59	2.05	-0.81	4.38	3.05	1.30	1.40	3.10	0.58	2
	Fe415	neg	nr	nr	1.70	8.14	4.12	0.22	1.20	0.61	0.17	1.92	2.27	2.01	1.69	1.24	1.42	2.71	2.84	2.38	-0.27	2
	Fe324	neg	nr	nr	-0.50	0.03	9.62	0.18	-0.69	0.50	-0.11	-0.84	-1.66	2.55	-0.79	-0.35	-0.64	5.32	-0.76	-0.54	-0.24	2
	Fe426	discord	nr	0.088	-1.39	2.45	2.01	0.77	-2.06	-0.89	0.08	2.24	-0.81	-1.42	-1.15	-0.98	2.19	4.32	4.55	0.07	-0.06	2
	Fe320	neg	nr	nr	-0.79	0.76	5.19	-1.84	0.92	1.56	-1.67	-0.16	2.41	-0.49	3.86	1.28	4.75	0.63	5.05	17.82	3.63	4
	Fe434	neg	nr	0.038	8.46	1.78	6.73	-0.43	7.10	2.04	-0.73	2.82	3.18	4.75	1.82	1.58	2.69	0.48	1.91	3.56	0.82	4
	Fe439	neg	nr	nr	-1.31	-0.84	6.29	4.56	1.18	8.31	0.95	0.88	-1.09	0.17	-0.44	-0.97	0.80	4.76	1.23	1.18	0.43	4

TABLE 3-continued

Reactivity of negative, borderline or discordant sera in the 16 protein multiplex assay.																							
En-	Fc423	neg	nr	0.023	nr	-2.05	0.25	0.27	1.03	-1.72	-0.21	0.95	-1.14	-0.72	-0.86	2.02	-1.87	0.25	-1.16	-0.98	-0.91	0	
demic	Fc444	neg	nr	0.059	nr	-1.44	-0.91	-1.90	0.76	-1.24	-1.68	-0.14	-1.55	-0.96	-1.01	-0.75	-2.41	0.02	-1.67	-2.76	-0.69	0	
Born	PP048	neg	nr	0.096	nr	1.92	-0.56	-0.94	-1.18	-0.94	0.50	-1.24	-0.69	0.02	-0.56	0.40	1.84	-1.30	-0.62	0.13	7.94	0	
	PP035	neg	nr	nr	nr	-0.40	-1.86	-1.39	-1.65	-1.00	2.71	-1.68	-1.83	0.19	-1.11	0.18	-0.07	-1.80	1.11	-1.61	-1.43	0	
	RD46	neg	nr	0.041	nr	-0.01	0.81	-0.43	-0.44	-0.07	0.01	-0.17	0.53	-0.15	-0.36	0.05	2.63	-0.08	3.49	1.11	-0.09	-0.14	0
	Fc319	neg	nr	nr	nr	0.27	0.50	1.80	-0.45	nd	7.25	0.16	0.89	-0.98	-0.01	-0.47	-0.32	0.43	0.21	1.75	1.69	0.23	1
	PP050	neg	nr	0.04	nr	0.38	-2.44	-2.10	-1.23	-2.44	-0.67	-0.88	-2.25	-2.25	0.47	1.21	-1.06	40.29	-2.62	-4.10	-0.77	1	
	PP177	discord	16	0.15	128	1.70	-0.99	0.21	-0.85	1.79	-1.46	-1.00	-0.32	2.08	2.47	-0.99	7.25	-0.79	0.80	0.00	0.57	2.21	1
	Fc389	discord	nr	nr	32	-0.45	0.22	0.42	-0.61	-0.38	-0.86	-0.62	-0.48	0.19	0.10	-0.82	2.67	0.12	7.25	1.02	1.16	0.34	1
	Fc464	neg	nr	0.019	nr	2.55	0.10	1.03	-1.00	1.12	1.49	-1.01	1.99	1.98	4.07	4.17	0.29	4.92	2.59	1.19	3.35	7.94	3
	Fc448	neg	nr	0.018	nr	2.72	0.32	1.91	0.62	1.01	7.25	-0.54	0.58	1.02	2.59	1.20	3.54	79.63	61.34	1.00	2.24	2.10	3
	PP020	discord	nr	0.073	64	0.66	7.25	0.14	-0.61	-1.42	-0.05	-0.40	19.64	1.22	-1.19	1.76	0.40	5.64	1.52	7.25	-0.01	7.25	4
Heart	Fc560	neg	nr	0.041	nr	-0.27	-1.01	-1.18	-0.66	-0.73	-0.14	0.04	-0.09	-0.60	-1.03	-0.94	-0.45	-0.66	-1.02	0.83	-1.70	0.68	0
Dis-	Fc559	neg	nr	0.014	nr	0.06	-1.34	3.41	0.60	0.48	1.43	-0.46	-0.30	-0.82	-0.31	0.22	-0.34	0.27	0.25	0.69	0.22	0.81	0
case	Fc587	neg	nr	0.05	nr	2.03	4.23	6.11	-0.07	-2.03	7.06	-1.37	22.75	0.24	0.24	-0.57	11.29	2.83	3.48	37.83	28.10	4.41	8
	Fc565	neg	nr	0.056	nr	-0.24	2.40	-0.28	-0.28	-0.89	-0.74	-0.69	5.21	0.29	0.69	0.57	2.04	2.18	4.61	5.60	3.17	3.92	3
	Fc569	neg	nr	0.043	nr	2.42	4.23	6.11	-0.83	-0.62	-0.06	-1.21	12.25	-0.09	0.23	-0.25	4.86	12.80	4.44	11.70	11.58	1.66	8
Infected based upon conventional serology																							
posi-	Fc273	discord +	32	nr	128	-0.34	1.53	0.07	-0.35	-0.51	1.60	-0.67	0.77	1.12	-0.27	0.80	0.58	1.27	0.05	23.11	38.34	8.36	2
tive	Fc404	discord +	ND	1.15	32	1.60	-0.32	-0.37	1.54	1.46	-1.27	-0.28	-0.14	2.91	1.26	-0.09	0.87	1.06	14.22	6.40	3.15	-0.16	2
dis-	Fc331	discord +	nr	0.078	nr	-0.72	13.44	0.85	0.23	-0.75	-2.09	5.46	0.24	-1.35	0.61	-0.81	-0.52	-0.48	2.82	4.64	10.53	2.90	4
cor-	Fc428	discord +	nr	0.238	128	1.01	0.80	-0.16	-0.01	-0.07	0.15	-0.58	3.76	-0.57	-0.71	-1.32	0.07	52.71	9.30	4.03	12.36	2.40	4
dant	Fc440	discord +	nr	0.213	32	2.14	0.53	1.21	1.32	1.66	4.69	-0.66	0.51	1.85	1.86	0.43	0.07	9.06	15.31	7.30	116.71	3.35	5
	PP178	discord +	64	0.135	32	5.95	13.82	3.73	1.15	-0.83	17.71	2.23	2.59	-1.04	5.05	0.99	nd	3.83	51.95	0.61	4.45	3.61	6
	PP080	discord +	32	0.15	32	1.04	4.26	3.87	4.75	2.75	2.55	3.98	3.64	0.42	1.34	0.53	15.35	4.45	70.78	4.99	3.88	8.60	6

Sera judged cumulatively as "seronegative" based upon conventional serology were grouped into negative but "no other evidence" of exposure (16 sera), those "born in an endemic area" (12), those with evidence of "heart disease" consistent with Chagas disease (5) and compared to (top) pools of strongly positive sera (high reactivity in all serological tests) and to (bottom) sera from subjects who were negative on at least one of the three conventional serologic tests (discordant positive). Reactivity in the conventional serological tests (IFA, ELISA and IF) and the summary consensus of these tests (neg = below cut-off for all three tests; discord = positive on one of the three tests; discord + = positive on 2 of the 3 tests), as well as reactivity to the 16 recombinant protein sets and the *T. cruzi* lysate are shown. Cutoffs for a positive ELISA is an O.D. > 0.200 and for IFA and HAI is a dilution > 1/5 (a reaction at 1/5 is considered "reactive but negative" and < 1/5 non-reactive (nt)). The metric for reactivity of each serum for each protein is expressed as the number of standard deviations that the ratio of the MFI for *T. cruzi* protein to the MFI for GFP was above the average ratios of sixteen true negative sera run in the same assay. Values > 4 S.D. above this "background" reactivity are considered reactive and are highlighted. The total number of reactive recombinant proteins for each serum is indicated in the right-most column. nd = not determined (insufficient numbers of beads detected in this sample).

Monitoring treatment efficacy. There is a pressing need for a means to assess treatment efficacy in Chagas disease so we next used the multiplex assay to monitor changes in serology over time in subjects treated with benznidazole (BZ). Representative data from a set of 16 non-endemic normals (FIG. 10A) demonstrates the background level of detection of responses in uninfected individuals, displayed as the MFI for each protein. To establish the stability of serological responses over time in the absence of treatment, serial serum samples were obtained from chronically infected, seropositive subjects, all without clinical disease; a representative set of 6 subjects screened at 4 times points for up to 21 months is shown in FIG. 10B. Each subject exhibits a distinct pattern of serological responses and both the pattern and the potency of those responses are remarkably stable over time. In contrast, a representative set of 4 (from a total of 38) subjects followed for up to 36 months after treatment with BZ shows that some subjects exhibit a post-treatment decrease in the strength of responses to most *T. cruzi* antigens tested (FIG. 11A). In many cases this fall is evident by 2 months post-treatment (e.g. PP001, PP115, PP164) and is followed by a transient increase at 6 months. Interestingly, this early drop in antibody levels following treatment is also sometimes evident, although less consistently so, with conventional serological tests, particularly with indirect hemagglutination (FIG. 11A). Subject PP117 has borderline positive serology in both the multiplex and the conventional serologic assays, and is representative of a case in which documenting changes following treatment would be difficult. FIG. 11A). FIG. 11B presents 2 other patterns of responses following treatment. PP044 shows essentially no change in the pattern or potency of antibody responses up to 24 months post-treatment. Subject PP024 is similar in that responses to the several prominently detected proteins are relatively stable over time. However the MFI reading for numerous other antigens falls consistently over the 24 month monitoring period. Thus, although it might take more time and additional assays to determine treatment efficacy in these two subjects, a preliminary assessment would be that treatment failed in the case of PP044 but was successful for subject PP024.

Discussion

The poor quality of diagnostics for *T. cruzi* infection is a major impediment to coping with a disease that affects as many as 20 million people. Without quality diagnostics, the statistic of the disease burden is at best a guess, the ability to conclusively identify who should be treated, or should be allowed to donate blood or tissues is greatly compromised and the effectiveness of interventions to limit transmission or drugs to treat those infected is impossible to determine with any certainty.

In the early stages of *T. cruzi* infection, parasites can often be detected in blood. However, as immunity develops, even amplification techniques such as xenodiagnosis, hemaculture, and PCR, despite being repeated multiple times, routinely fail to detect infection (Castro et al., 2002, Parasitol Res 88: 894-900; Picka et al., 2007, Braz J Infect Dis 11: 226-233; Salomone et al., 2000, Am J Cardiol 85: 1274-1276; Duarte et al., 2006, Rev Soc Bras Med Trop 39: 385-387). Consequently, determination of infection status is largely dependent on the consensus results of multiple tests with different formats (e.g. ELISA, indirect fluorescent antibody, indirect hemagglutination, complement fixation). However the unreliability of these tests is well documented (Pirard et al., 2005, Transfusion 45: 554-561; Salomone et al., 2003, Emerg Infect Dis 9: 1558-1562; Avila et al., 1993, J Clin Microbiol 31: 2421-2426; Castro et al., 2002, Parasitol Res 88: 894-900; Caballero et al., 2007, Clin Vaccine Immunol. 14:1045-1049;

Silveira-Lacerda et al., 2004, Vox Sang 87: 204-207; Wincker et al., 1994, Am J Trop Med Hyg 51: 771-777; Gutierrez et al., 2004, Parasitology 129: 439-444; Marcon et al., 2002, Diagn Microbiol Infect Dis 43: 39-43; Picka et al., 2007, Braz J Infect Dis 11: 226-233; Zarate-Blades et al., 2007, Diagn Microbiol Infect Dis 57: 229-232). Many of these tests, including one recently licensed by the United States Food and Drug Administration for use as a blood screening test in the U.S. (Tobler et al., 2007, Transfusion 47: 90-96), use crude or semi-purified parasite preparations derived from parasite stages present in the insect vector but not in infected humans. Recently a number of recombinant parasite proteins or peptides have also come into limited use for diagnosis (da Silveira et al., 2001, Trends Parasitol 17: 286-291; Chang et al., 2006, Transfusion 46: 1737-1744; Kirchhoff et al., 1984, J Immunol 133: 2731-2735; Laucella et al., 2004, J Infectious Diseases 189:909-918).

A subject whose serum is consistently positive on multiple of the currently used tests is relatively easily determined to be infected. But the infection status of individuals positive on only one test (as in blood bank screening) is unclear and detection of parasites in subjects who are negative using multiple conventional serologic tests (Salomone et al., 2003, Emerg Infect Dis 9: 1558-1562; Gutierrez et al., 2004, Parasitology 129: 439-444; Marcon et al., 2002, Diagn Microbiol Infect Dis 43: 39-43; Picka, et al., 2007, Braz J Infect Dis 11: 226-233; Wincker et al., 1994, FEMS Microbiol Lett 124: 419-423) or who are positive by alternative but not widely available serological tests (Caballero et al., 2007, Clin Vaccine Immunol. 14:1045-1049; Zarate-Blades et al., 2007, Diagn Microbiol Infect Dis 57: 229-232) is not uncommon. Furthermore, currently available tests are inadequate for monitoring treatment efficacy (Sanchez Negrette et al., 2008, Clin Vaccine Immunol 15: 297-302; Bahia-Oliveira et al., 2000, J Infect Dis 182: 634-638; Solari et al., 2001, J Antimicrob Chemother 48: 515-519) and thus may also give inaccurate measurements of the effectiveness of other interventions.

With these deficits in mind, we set out to identify parasite proteins that would more effectively detect *T. cruzi* infection and provide a tool for monitoring changes in infection status over time. Development of a repository of nearly 1500 *T. cruzi* genes cloned into GATEWAY entry vectors provided a relatively straightforward approach to producing a large number and diversity of *T. cruzi* proteins appropriate for high-throughput screening of diagnostics. Adding the targeted approach of selecting proteins documented for high level expression in trypomastigote and amastigote stages of *T. cruzi* allowed us to also focus on the proteins that would be predicted to elicit the strongest antibody response in infected humans. The Luminex-based multiplex bead array system permitted us to screen many proteins simultaneously with very low requirements for serum. The production of histidine-tagged proteins also made it relatively uncomplicated to attach the recombinant proteins to Luminex beads. This latter point is not trivial as the proteins could be coupled to the assay beads directly from the denaturing urea-based lysis buffer without the requirement of movement to a non-denaturing buffer, wherein many of the proteins precipitated. The strong response detected using proteins prepared in this way suggests either that natively folded proteins are not required for the detection of these antibodies or that re-folding of the proteins attached to the Luminex beads during buffer exchange resulted in the formation of native conformational epitopes.

In addition to its utility for screening of a large number of proteins, the Luminex system also excels as a platform for

multiplex analysis of antibodies to a relatively large set of targets. We were restricted in this work by the number of Luminex bead sets manufactured with Ni+2 and thus sought to identify a maximum of 16 independent *T. cruzi* proteins that gave informative results from a large set of human sera. The ultimate panel selected by the screen included at least one protein previously identified as a potential diagnostic, the mitochondrial HSP-70 (Krautz et al., 1998, *Am J Trop Med Hyg* 58: 137-143). It is possible that other proteins revealed in our screen have been studied previously. However since the identity of some of these previously assayed proteins is somewhat cryptic (da Silveira et al., 2001, *Trends Parasitol* 17: 286-291) and few have been associated with annotated genes in the sequenced *T. cruzi* genome, this is difficult to determine. Also, over half of the antigens selected in our screen were among the 50 most abundant proteins in the trypomastigote and amastigote proteomes (Atwood et al., 2005, *Science* 309: 473-476). Two hypothetical proteins and 2 proteins unique to *T. cruzi* among the sequenced kinetoplastids, including 2 fragments from the very large and multicopy dispersed gene family protein, were among the proteins selected. Proteins that are unique to *T. cruzi* could be particularly useful in a serological screen as they are absent from *Leishmania*, one of the potentially confounding infections in terms of diagnosis of *T. cruzi*. However the dispersed gene family fragments were among the worst performers in the large scale screen—with only 5-9% of all confirmed positive sera having detectable antibodies to these. Similarly, other gene family proteins, including trans-sialidases, mucins and mucin-associated proteins (MASPS) were part of the screen but failed to make even the initial selection cuts in our assays, presumably because only a small fraction of their diversity would be represented in the recombinant proteins screened.

A multiplex approach like the Luminex also provided a more detailed examination of responses than is possible using a single target consisting of either an individual protein or a protein/peptide mixture. Each individual was seen clearly to have a distinct pattern of responses to the protein panel and that this pattern was impressively stable over time (several years). This is both interesting scientifically and serves as further validation of the quality and consistency of the data generated using this multiplex methodology. This heterogeneity of responses to pathogens among individuals appears to be more the norm than the exception, as similar results have been reported for individuals infected or immunized with viral (vaccinia), bacterial (*Francisella tularensis*) and protozoal (*Plasmodium falciparum*) pathogens (Davies et al., 2007, *Proteomics* 7: 1678-1686; Sundaresh et al., 2006, *Bioinformatics* 22: 1760-1766; Sundaresh et al., 2007, *Bioinformatics* 23: i508-518). Thus serodiagnostics in general are likely to need to move toward multiplex assays, as single antigens that are recognized by all individuals infected by any pathogen appear to be rare (Davies et al., 2007, *Proteomics* 7: 1678-1686).

The ability to simultaneously and independently assess antibody responses to multiple targets was instrumental to our success in addressing the issues of the detection of serological responses in subjects who are negative by conventional serology and the relatively rapid detection of changes in selected responses following drug treatment. The multiplex assay detected 100% of 121 samples consistently positive by conventional serology, and 100% of samples positive on 2 out of 3 conventional tests. In addition, however, we also detected antibodies specific for one or more recombinant proteins in 18 of 33 subjects judged as negative by conventional serology. Other investigators have documented cases of conventional seronegative subjects being seropositive on alternative

tests or even parasite positive (Salomone et al., 2003, *Emerg Infect Dis* 9: 1558-1562; Caballero et al., 2007, *Clin Vaccine Immunol.* 14:1045-1049; Gutierrez et al., 2004, *Parasitology* 129: 439-44; Marcon et al., 2002, *Diagn Microbiol Infect Dis* 43: 39-43; Picka et al., 2007, *Braz J Infect Dis* 11: 226-233; Zarate-Blades et al., 2007, *Diagn Microbiol Infect Dis* 57: 229-232; Wincker et al., 1994, *FEMS Microbiol Lett* 124: 419-423) although these previous reports of “infected seronegatives” have been somewhat anecdotal—presumably because investigators rarely screen for parasites in seronegative subjects. However in some studies parasite-positive conventional seronegatives are very well documented. For example Picka et al. (*Braz J Infect Dis* 11: 226-233, 2007) reported on one subject who was negative by up to 5 replicates of 4 different conventional serological tests yet was positive by a combined hemaculture-PCR approach. The multiple examples of failed conventional serology to detect infection in combination with the well-documented unreliability of parasitological tests, supports the conclusion that individuals who are seropositive in our multiplex assay are likely to be infected with *T. cruzi*. This conclusion is further supported by on-going studies demonstrating *T. cruzi*-specific T cell responses in subjects who are negative by conventional serology but positive in our multiplex assays. Without more sensitive parasitological tests we cannot conclusively determine if the subjects who are negative by conventional serology but positive in our multiplex assay are infected or possibly “exposed” but not still infected with *T. cruzi*. And without additional extensive validation, we cannot exclude the possibility that other infections or immunological conditions resulted in some of the multiplex positive responses, although standard clinical analysis failed to detect other complicating infections in these subjects. However, especially for subjects who have antibodies to up to 8 different recombinant *T. cruzi* proteins and were born in endemic areas and/or have evidence of heart disease, it is reasonable to conclude that they are indeed infected with *T. cruzi* despite their negative results with conventional serologic assays. Overall these studies support the already documented conclusion that current serological tests can misdiagnose infection—perhaps to a significant extent.

A second issue we addressed using the multiplex serological assay for *T. cruzi* infection was that of efficacy of therapeutic treatment. Because most subjects are negative by parasitological assays prior to treatment (making a negative result after treatment uninformative) and remain positive by conventional serology for extensive periods of time after treatment, assessing whether treatment actually achieved cure) has been problematic. When combined with other evidence of treatment failures and the adverse effects of the drugs, the absence of a method to detect treatment efficacy has resulted in a very low rate of treatment in chronic Chagas disease. This absence of a reliable and timely test for treatment efficacy is also a major impediment to the development and testing of new drugs—an area that has been at a virtual standstill for decades.

Herein we show that the multiplex assay using the selected set of recombinant proteins can detect significant changes in antibody levels, in some cases as early as the first post-treatment assay point (2 months post-treatment completion). These changes are not evident in all cases—an outcome that is not surprising given that treatment failure is common (Viotto et al., 1994, *Am Heart J* 127: 151-162). Our ability to assess responses to multiple targets on an individual basis appears to be crucial to the success of detection of serologic changes following treatment, as similar changes are not consistently observed using conventional serologic tests. Previ-

ous studies have suggested that various recombinant antigens may provide better assessment of treatment efficacy relative to conventional serology (Sanchez Negrette et al., 2008, Clin Vaccine Immunol 15: 297-302; Sosa Estani et al., 1998, Am J Trop Med Hyg 59: 526-529).

In conclusion, we define a set of diagnostic targets and an assay approach that we believe is a significant improvement upon current diagnostic tests for *T. cruzi* infection both for more consistently detecting infection and for assessing the effectiveness of treatment. Additional validation of these targets and the general methodology will require analysis of a larger set of subjects, a process that is currently on-going. Herein we have also not addressed the question of whether the antigens we identify would be useful throughout the wide endemic range for *T. cruzi*. Heterogeneity among different parasites strains in distinct regions could present a challenge. However here again this is a concern that a multiplex assay might rather easily address—it seems unlikely that all 16 proteins in our pool, most of which are abundant housekeeping proteins, would vary substantially among parasites in various regions. The problem of infection confirmation by detection of parasites or parasite products is likely to continue to be a roadblock to full acceptance of the results of this test, or any other, when they conflict with conventional serologic tests—despite the proven inadequacy of these “standard” tests. A downside of the Luminex system for multiplex analy-

sis is the reagent expense as well as the requirement for specialized equipment to “read” the results. However, other multiplex platforms such as protein microarrays could be more cost conservative and require less infrastructure (Davies et al., 2007, Proteomics 7: 1678-1686; Kartalov et al., 2006, Biotechniques 40: 85-90). Also, our results suggest that the number of proteins in the analysis could be reduced without substantial loss of sensitivity and the possibility exists for additional improvements in sensitivity by the inclusion of *T. cruzi* proteins previously validated by others, or that could be detected in additional screens like that described herein. At a minimum, these results begin to lay the groundwork for the removal of one of the major impediments to the development and effective implementation of treatments for *T. cruzi* infection.

Example 10

Preliminary Panel of Serodiagnostic Proteins

In a preliminary study that eventually resulted in the serodiagnostic proteins described in Example 9, 53 diagnostic proteins was selected (Table 4) from a group of 59 candidate proteins identified through screening with serum pools and individual sera. The preliminary serodiagnostic panel selected for further study consisted of the top 16 proteins in Table 4.

TABLE 4

Protein antigens				
Current panel constituents	Published as antigens?	Common Name	Annotated gene or Tc00 id of closest homolog	SEQ ID No:
1		hypothetical protein	Tc00.1047053508767.10	68
2		hypothetical protein, conserved	Tc00.1047053506635.130	69
3	1, 3 (ribosomal P protein, TcP0?)	60S acidic ribosomal subunit protein, putative	Tc00.1047053508355.250	13
4	1-2 (flagellar CaBP, 1F8)	flagellar calcium-binding protein, putative	Tc00.1047053507491.151	70
5	1, 3 (MAP)	microtubule-associated protein, putative	Tc00.1047053511633.79	31
6	1 (heat shock)	heat shock 70 kDa protein, mitochondrial precursor, putative	Tc00.1047053507029.30	24
7	1 (paraflagellar assoc. prot)	69 kDa paraflagellar rod protein, putative	Tc00.1047053511215.119	25
8		EF-hand protein 5	Tc00.1047053506391.30	14
9		aminopeptidase, putative	Tc00.1047053511289.30	18
10		axoneme central apparatus protein, putative	Tc00.1047053510955.40	16
11		hypothetical protein	Tc00.1047053506529.610	6
12		glycosomal phosphoenolpyruvate carboxykinase, putative	Tc00.1047053508441.20	11
13		dispersed gene family protein 1 fragment	Tc00.1047053511271.10	26
14		malate dehydrogenase, putative	Tc00.1047053506195.110	29
15		dispersed gene family protein 1 fragment	Tc00.1047053511265.10	28
16		poly(A)-binding protein	Tc00.1047053508461.140	30
Understudy - ranked by approximate potential				
17	1 (cytoskeleton assoc?)	beta tubulin	Tc00.1047053506563.40	1
18	1 (cytoskeleton assoc?)	alpha tubulin	Tc00.1047053411235.9	2
19	1 (ribosomal prot)	60S ribosomal protein L28, putative	Tc00.1047053506297.270	10
20	1 (ribosomal prot)	polyubiquitin (pseudogene), putative	Tc00.1047053507483.4	51
21		iron superoxide dismutase, putative	Tc00.1047053511019.90	41
22		iron superoxide dismutase, putative	Tc00.1047053509775.40	71
23		elongation factor-1 gamma, putative	Tc00.1047053506459.290	48
24		hypothetical protein, to be annotated	Tc00.1047053507515.4	72
25		dispersed gene family protein 1 fragment	Tc00.1047053509815.120	63
26		hypothetical protein, conserved	Tc00.1047053504153.280	46
27		p22 protein precursor, putative	Tc00.1047053509053.70	52
28		25 kDa translation elongation factor 1-beta, putative	Tc00.1047053506201.39	66
29		universal minicircle sequence binding protein (UMSBP), putative	Tc00.1047053503781.80	65
30		hypothetical protein, conserved	Tc00.1047053510877.30	35
31		d-isomer specific 2-hydroxyacid dehydrogenase-protein, putative	Tc00.1047053510099.120	60
32		RNA-binding protein, putative	Tc00.1047053511727.270	64
33		stress-induced protein sti1, putative	Tc00.1047053506321.290	73
34		glutamyl carboxypeptidase, putative	Tc00.1047053510837.20	74
35	1 (trans-sialidase)	chunk of conserved hypothetical protein	Tc00.1047053509099.160	75

TABLE 4-continued

Protein antigens				
Current panel constituents	Published as antigens?	Common Name	Annotated gene or Tc00 id of closest homolog	SEQ ID No:
36		centrin, putative	Tc00.1047053506559.380	76
37		possible salivary proline-rich protein rp15	Tc00.1047053506835.110	77
38		tuzin	Tc00.1047053507485.140	78
39	1 (trans-sialidase)	chunk of putative trans-sialidase	Tc00.1047053507997.14	79
40	4 (cruzipain)	cysteine peptidase, putative	Tc00.1047053507603.270	80
41	1 (heat shock)	Tc 85 kDa antigen with homology to heat shock proteins	Tc00.1047053509643.130	81
42	1 (heat shock)	Tc hsp 70	Tc00.1047053511211.170	82
43		serine carboxypeptidase (CBP1), putative	Tc00.1047053509695.220	17
44		hypothetical protein, conserved	Tc00.1047053510887.50	7
45		hypothetical protein, conserved	Tc00.1047053509141.40	36
46		dihydrolipoyl dehydrogenase, putative	Tc00.1047053507089.270	40
47		hypothetical protein, conserved	Tc00.1047053506529.460	4
48		trans-splicing factor, putative	Tc00.1047053503583.40	9
49		GTP-biNDing nuclear protein rtb2, putative	Tc00.1047053503539.30	83
50		14-3-3 protein, putative	Tc00.1047053511167.90	56
51		nascent polypeptide associated complex subunit, putative	Tc00.1047053510579.70	58
52		hypothetical protein, conserved	Tc00.1047053507093.300	61
53		succinyl-CoA synthetase alpha subunit, putative	Tc00.1047053508479.340	62

¹da Silveira, et al., TRENDS in Parasitology Vol. 17 No. 6 June 2001, p.286-291

² Umezawa, et al., TRANSFUSION Volume 43, January 2003, p. 91-97

³ Umezawa, et al., JOURNAL OF CLINICAL MICROBIOLOGY, January 2004, p. 449-452

⁴ Martinez, et al., INFECTION AND IMMUNITY, November 1991, p. 4275-4277

FIG. 12 shows the pattern of responses of 11 subjects to 11 antigens and 2 controls (ovalbumin as the negative control, and *T. cruzi* lysate as a positive control) at the time of the first bleed (FIG. 12A) and 12 months later (FIG. 12B). Three aspects are worthy of mention. First, the pattern of responses is unique in each individual; no one appears to respond similarly. Second, the pattern of responses is stable over the 12 month period. This is a strong indicator that the assay is indeed reproducibly detecting a persistent response. This is also important with respect to using the test to monitor changes after therapy. Third, no single recombinant protein (or even combination of 3 or 4 proteins) detects all responders. This result validates the need for a multicomponent test for *T. cruzi* infection.

Example 11

Monitoring Treatment for Chagas Disease

Benznidazole is the primary compound used for treatment of Chagas disease, although there is controversy about its efficacy in treating chronically infected subjects, such as those who have been infected >20 years). The left side of FIG. 13 below shows consistent serologic responses at 0 and 12 months in the absence of treatment in 4 subjects. The right side shows 6 treated subjects, treated at time 0 and reassayed at 12 and 24 months post-treatment. Changes in serology are obvious in 3 of the treated subjects at 12 months. The other 3 subjects have little to no change, even at 24 months. It is worth noting that conventional serology conducted on these same sera showed inconsistent or no changes. Furthermore, the percentage of individuals showing serological changes consistent with cure is similar to that reported in other like studies but using much longer follow-ups. E.g., a recent study by Fabbro et al. (Rev Soc Bras Med. Trop. 2007 January-February; 40(1):1-10), reported a treatment efficacy rate of 35-63% based upon conventional serology but required an average 16 year follow-up to see this change. Moreover, nearly 100% of those showing changes in serology also showed changes in T cell responses (FIG. 14; note decreasing to undetectable responses within 12-25 months). Similar changes were not seen in untreated subjects or in treated subjects who failed to

exhibit serological changes. Overall we conclude that this multiplex assay has the capability to relatively rapidly detect treatment success or failure—especially when coupled with assays of T cell reactivity.

Example 12

Maternal/Neonate Diagnostics

The unique pattern of responses in each individual may have utility with respect to monitoring congenital infection, which is currently very difficult. If the pattern of the serologic response in mother and newborn is similar, then we would suspect that the infant's serologic response is a result of maternally derived antibodies rather than to antibodies produced by the infant. However if the patterns are different, then we would suspect that the child is infected and therefore should be treated.

Four separate pairs of mother and newborn were evaluated. Serum antibody titers against individual recombinant *T. cruzi* proteins were determined for both the mother and the infant at a time point relatively soon after birth (within weeks), and again approximately 6 months later. For all pairs, the infant's pattern of response shortly after birth resembles the mother's pattern of response, indicating the presence of maternal antibodies (FIG. 15). At the later time point, two of the infants (FIGS. 15B, and D) show a pattern of response differs from the mother's, indicating that these infants may be infected and producing their own antibodies. Another infant (FIG. 15A) shows a response that is near background levels, suggesting that the infant is likely not infected. Circles over the individual measurements indicate statistically significant responses.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the

exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

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35          40          45
Val Tyr Phe Asp Glu Ala Thr Gly Gly Arg Tyr Val Pro Arg Ala Val
50          55          60
Leu Ile Asp Leu Glu Pro Gly Thr Met Asp Ser Val Arg Ala Gly Pro
65          70          75          80
Tyr Gly Gln Ile Phe Arg Pro Asp Asn Phe Ile Phe Gly Gln Ser Gly
85          90          95
Ala Gly Asn Asn Trp Ala Gln Gly His Tyr Thr Glu Gly Ala Glu Leu
100         105         110
Ile Asp Ser Val Leu Asp Val Cys Arg Lys Glu Ala Glu Ser Cys Asp
115         120         125
Cys Leu Gln Gly Phe Gln Ile Cys His Ser Leu Gly Gly Gly Thr Gly
130         135         140
Ser Gly Met Gly Thr Leu Leu Ile Ser Lys Leu Arg Glu Glu Tyr Pro
145         150         155         160
Asp Arg Ile Met Met Thr Phe Ser Ile Ile Pro Ser Pro Lys Val Ser
165         170         175
Asp Thr Val Val Glu Pro Tyr Asn Thr Thr Leu Ser Val His Gln Leu
180         185         190
Val Glu Asn Ser Asp Glu Ser Met Cys Ile Asp Asn Glu Ala Leu Tyr
195         200         205
Asp Ile Cys Phe Arg Thr Leu Lys Leu Thr Thr Pro Thr Phe Gly Asp
210         215         220
Leu Asn His Leu Val Ser Ala Val Val Ser Gly Val Thr Cys Cys Leu
225         230         235         240
Arg Phe Pro Gly Gln Leu Asn Ser Asp Leu Arg Lys Leu Ala Val Asn
245         250         255
Leu Val Pro Phe Pro Arg Leu His Phe Phe Met Met Gly Phe Ala Pro
260         265         270
Leu Thr Ser Arg Gly Ser Gln Gln Tyr Arg Gly Leu Ser Val Pro Glu
275         280         285
Leu Thr Gln Gln Met Phe Asp Ala Lys Asn Met Met Gln Ala Ala Asp
290         295         300
Pro Arg His Gly Arg Tyr Leu Thr Ala Ser Ala Leu Phe Arg Gly Arg
305         310         315         320
Met Ser Thr Lys Glu Val Asp Glu Gln Met Leu Asn Val Gln Asn Lys
325         330         335

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-continued

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 Phe Asn Thr Phe Phe Ser Glu Thr Gly Ala Gly Lys His Val Pro Arg
 50 55 60
 Ala Val Phe Leu Asp Leu Glu Pro Thr Val Val Asp Glu Ile Arg Thr
 65 70 75 80
 Gly Thr Tyr Arg Gln Leu Phe His Pro Glu Gln Leu Ile Ser Gly Lys
 85 90 95
 Glu Asp Ala Ala Asn Asn Tyr Ala Arg Gly His Tyr Thr Ile Gly Lys
 100 105 110
 Glu Ile Val Asp Leu Cys Leu Asp Arg Ile Arg Lys Leu Ala Asp Asn
 115 120 125
 Cys Thr Gly Leu Gln Gly Phe Leu Val Tyr His Ala Val Gly Gly Gly
 130 135 140
 Thr Gly Ser Gly Leu Gly Ala Leu Leu Leu Glu Arg Leu Ser Val Asp
 145 150 155 160
 Tyr Gly Lys Lys Ser Lys Leu Gly Tyr Thr Val Tyr Pro Ser Pro Gln
 165 170 175
 Val Ser Thr Ala Val Val Glu Pro Tyr Asn Ser Val Leu Ser Thr His
 180 185 190
 Ser Leu Leu Glu His Thr Asp Val Ala Ala Met Leu Asp Asn Glu Ala
 195 200 205
 Ile Tyr Asp Leu Thr Arg Arg Asn Leu Asp Ile Glu Arg Pro Thr Tyr
 210 215 220
 Thr Asn Leu Asn Arg Leu Ile Gly Gln Val Val Ser Ala Leu Thr Ala
 225 230 235 240
 Ser Leu Arg Phe Asp Gly Ala Leu Asn Val Asp Leu Thr Glu Phe Gln
 245 250 255
 Thr Asn Leu Val Pro Tyr Pro Arg Ile His Phe Val Leu Thr Ser Tyr
 260 265 270

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Ala Pro Val Ile Ser Ala Glu Lys Ala Tyr His Glu Gln Leu Ser Val
275 280 285

Ser Glu Ile Ser Asn Ala Val Phe Glu Pro Ala Ser Met Met Thr Lys
290 295 300

Cys Asp Pro Arg His Gly Lys Tyr Met Ala Cys Cys Leu Met Tyr Arg
305 310 315 320

Gly Asp Val Val Pro Lys Asp Val Asn Ala Ala Val Ala Thr Ile Lys
325 330 335

Thr Lys Arg Thr Ile Gln Phe Val Asp Trp Ser Pro Thr Gly Phe Lys
340 345 350

Cys Gly Ile Asn Tyr Gln Pro Pro Thr Val Val Pro Gly Gly Asp Leu
355 360 365

Ala Lys Val Gln Arg Ala Val Cys Met Ile Ala Asn Ser Thr Ala Ile
370 375 380

Ala Glu Val Phe Ala Arg Ile Asp His Lys Phe Asp Leu Met Tyr Ser
385 390 395 400

Lys Arg Ala Phe Val His Trp Tyr Val Gly Glu Gly Met Glu Glu Gly
405 410 415

Glu Phe Ser Glu Ala Arg Glu Asp Leu Ala Ala Leu Glu Lys Asp Tyr
420 425 430

Glu Glu Val Gly Ala Glu Ser Ala Asp Met Glu Gly Glu Glu Asp Val
435 440 445

Glu Glu Tyr
450

<210> SEQ ID NO 3
<211> LENGTH: 260
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 3

Met Gly Lys Thr Val Leu Thr Cys Arg Lys Gly Asn Gly Ser Val Tyr
1 5 10 15

Gln Leu His Gly His Lys Arg Leu Gly Pro Ala Lys Leu Arg Ile Leu
20 25 30

Asp Tyr Ala Glu Arg His Gly Phe Met Arg Gly Val Val Lys Thr Ile
35 40 45

Glu His Glu Pro Gly Arg Gly Ala Pro Leu Ala Arg Val Glu Phe Arg
50 55 60

His Pro Tyr Lys Tyr Arg Arg Val Lys Glu Leu Met Val Ala Pro Glu
65 70 75 80

Gly Met Phe Thr Gly Gln Ser Val Leu Cys Gly Val Lys Ala Pro Leu
85 90 95

Ala Ile Gly Asn Val Leu Pro Leu Gly Gln Ile Thr Glu Gly Cys Ile
100 105 110

Val Cys Asn Val Glu Ala Lys Val Gly Asp Arg Gly Thr Ile Ala Arg
115 120 125

Ala Ser Gly Asp Tyr Cys Ile Ile Ile Ser His Asn His Glu Thr Gly
130 135 140

Arg Thr Arg Leu Lys Leu Pro Ser Gly Gln Lys Lys Thr Val Pro Ser
145 150 155 160

Asn Cys Arg Ala Met Ile Gly Ile Ile Ala Gly Gly Gly Arg Ile Glu
165 170 175

Lys Pro Val Leu Lys Ala Gly Asn Ser Phe Tyr Arg Phe Arg Gly Lys
180 185 190

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Arg Asn Cys Trp Pro Lys Val Arg Gly Val Ala Arg Asn Pro Val Glu
195 200 205

His Pro His Gly Gly Gly Asn His Gln His Ile Gly His Pro Ser Thr
210 215 220

Val Ser Arg His Ala Pro Pro Gly Gln Lys Val Gly Leu Ile Ala Ala
225 230 235 240

Arg Arg Thr Gly Arg Ile Arg Gly Ser Arg Ala Val Lys Gly Ala Trp
245 250 255

His Pro Glu Glu
260

<210> SEQ ID NO 4
<211> LENGTH: 821
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 4

Met His Arg Gln Glu Ser Val Ser Ser Gly Gly Gly Asn Ala Thr Gly
1 5 10 15

Arg Gly Ser Leu Thr Thr Ala Glu Val Leu Asp Arg Ala Met Asn Gln
20 25 30

Cys Met Gln Arg Gly Leu Phe Asp Thr Ala Ser Trp Leu Gly Gln Leu
35 40 45

Ala Leu Asn Ala Thr Asp Ser Val Leu Arg Asp Ser Ile Ser Ala Thr
50 55 60

Ser Pro Ala Val Ala Ala Leu Gln Asp Pro Pro Leu Thr Gly Arg Ala
65 70 75 80

His Arg Tyr Leu Val Val Ala Leu Ser Leu Met Gln Lys Ser Glu Tyr
85 90 95

Ile Arg Cys His His Glu Leu Asn Ile Ala Leu Lys Glu Phe Ser Ala
100 105 110

Glu Ser Thr Pro Val Glu Ser Glu Lys Cys Ala Arg Asp His Pro Pro
115 120 125

Met Pro Arg Gly Ser Gly Arg Ser Thr Pro Leu Pro Ala Ala Ser Ser
130 135 140

Ser Pro Met Leu Pro Pro Pro Gln Leu Gln Phe Leu Cys Leu Tyr Ser
145 150 155 160

Leu Tyr Met Ala Gly Glu Cys Ile Lys Ser Thr Ser Ser Asn Pro Arg
165 170 175

Lys Ser Ser Asn Pro His Leu Arg Thr Leu Arg Gly Arg Leu Leu Thr
180 185 190

Leu Leu Glu Gln Gln Arg Arg Ser Leu Ser Ser Ser Pro Ala Ser Ile
195 200 205

Lys Ser Ser Met Lys Pro Thr Pro Leu Ser Ser Ala Ser Met Ala Val
210 215 220

Gly Ala Pro Ala Tyr Gly Asp Pro Phe Leu Cys Trp Leu His Gly Val
225 230 235 240

Val Leu Arg Glu Leu Gly Met Lys Gln Glu Ser Ala Thr Tyr Phe Leu
245 250 255

Ala Ala Leu Cys Asn His Pro Met Leu Trp Cys Ala Trp Glu Asp Leu
260 265 270

Cys Thr Leu Val Ser Arg Glu Asn Gln Ile Glu Glu Ile Glu Ala Ile
275 280 285

Ile Ala Ser Leu Glu Pro Arg Phe Met Ser Glu Ile Phe Leu Ala Ser
290 295 300

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Ala Lys Ala Ala Leu Asn Val Ala Pro Met Ser Leu Val Pro Pro Ser
 305 310 315 320

Leu Ser Thr Ala Ala Ala Ala Met Ala Gln Arg Ser Thr Ser Pro
 325 330 335

His Cys Gly Ser Leu Pro Arg Gln Thr Thr Ser Thr Leu Glu Thr Gln
 340 345 350

Glu Gln His Tyr Arg Pro Gln His His Gln Arg Arg Gly Glu Ser Gly
 355 360 365

Val Ser Pro Arg Leu Val Asn Ser Trp Glu Ala Leu Leu Glu Arg Phe
 370 375 380

Pro Asn Asn Leu Phe Leu Leu Ala Asn Leu Ala Gly Tyr Tyr Tyr Asn
 385 390 395 400

Val Lys Lys Asp Leu Glu Lys Ala Gln Ser Leu Tyr Lys Arg Leu His
 405 410 415

Glu Met Asn Pro Tyr Arg Leu Glu Ser Met Asp Asp Tyr Ser Ile Val
 420 425 430

Leu Phe Leu Arg Gly Asp Arg Ile Gly Leu Ser Ser Leu Ala Gln Gln
 435 440 445

Val Tyr Gln Ile Asp Pro Phe Arg Ala Glu Ser Asn Tyr Val Val Gly
 450 455 460

Asn Tyr Tyr Val Leu Met Gly Ala His Asp Arg Gly Val Leu His Phe
 465 470 475 480

Arg Arg Ala Val Ala Ala Asp Pro Thr Phe Leu Ala Ala Trp Thr Leu
 485 490 495

Leu Gly His Ala Tyr Leu Glu Thr Lys Asn Ser Ala Ala Ala Val Glu
 500 505 510

Ala Tyr Arg Ala Ala Val Asp Leu Asp Pro Arg Asp Tyr Arg Gly Trp
 515 520 525

Tyr Asn Leu Gly Gln Ile Tyr Glu Leu Leu Gln Phe Tyr His His Ala
 530 535 540

Leu Tyr Tyr Tyr Trp His Thr Thr Thr Leu Arg Pro Thr Asp Pro Arg
 545 550 555 560

Met Trp Ser Ala Val Ala Asn Cys Leu Asp Arg Glu Gly Arg Thr Gly
 565 570 575

Glu Ala Val Leu Cys Leu Glu Arg Ala Glu Ala His Glu Ser Ser Ser
 580 585 590

Ser Asp Tyr Tyr Pro Pro Leu Val His Arg Leu Gly Leu His Tyr Leu
 595 600 605

Gly Ile Arg Arg Leu Asp Arg Ala Val Ile Tyr Leu Glu Lys Leu Ala
 610 615 620

Leu Ser Glu Ala Arg Arg Arg Glu Asp Val Leu Phe Ala Ile Pro His
 625 630 635 640

Val Val Pro Tyr Tyr Leu Gln Gln Ala Arg Gln Leu Leu Asp Ile Pro
 645 650 655

Ser Arg Ser Pro Ser Tyr Glu Pro Gln Pro His His Ser Thr Thr Ala
 660 665 670

Gly Gly Gly Asp Gly Gln Leu Pro Gln Thr Met Ala Ser Ala Met Gly
 675 680 685

Ala Thr Asn Ala Ser Thr Gly Gly Asn Val Tyr Arg Ser Ser Leu Ala
 690 695 700

Asp Gln Trp Leu Thr Ala Asp Ala Ala Ala Arg Arg Asn Ile Glu Thr
 705 710 715 720

Arg Trp Glu Gln Ala Ala Leu Cys Leu Thr Ser Ser Glu Arg His Leu

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725				730				735							
Glu	Asn	Phe	Ala	Ser	Val	Leu	Gly	Ile	Pro	Val	Ala	Ser	Ala	Ala	Asp
			740								745				750
Asn	Gly	Ala	Arg	Lys	Ser	Thr	Glu	Tyr	Gly	Asp	Thr	Gly	Val	Ser	Gly
			755				760								765
Ser	Gly	Gly	Val	Ala	Gly	Val	Thr	Met	Asp	Glu	Gly	Arg	Ser	Gln	His
			770				775								780
Thr	Leu	Gln	Leu	Ala	Cys	Leu	Tyr	Arg	Glu	Leu	Asn	Lys	Ile	Arg	Gln
			785				790								800
Tyr	Leu	Thr	Ser	Gln	Gln	Glu	Gln	Val	Glu	Thr	Ala	Met	Arg	Met	Arg
			805												810
Gly	Gly	Gly	Asn	Ala											
			820												

<210> SEQ ID NO 5

<211> LENGTH: 334

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 5

Met	Leu	Ser	Arg	Arg	Ser	Leu	Thr	Thr	Ala	Phe	Ala	Ala	Met	Thr	Lys
1			5								10				15
Gln	Pro	Leu	Met	Gln	Gln	Arg	Arg	His	Asp	His	Asp	Arg	Trp	Tyr	Gly
			20												30
His	Ala	Leu	Glu	Leu	Asp	Ser	His	Asn	Tyr	Lys	Phe	Thr	Gly	Glu	Pro
			35				40								45
Pro	Ser	Trp	Met	Arg	Gln	Arg	Glu	Arg	Thr	Glu	Glu	Glu	Thr	Ala	Phe
			50				55								60
Ala	Lys	Ser	Val	Leu	Pro	His	Val	Asp	Phe	Ala	Ser	Ser	Tyr	Glu	Cys
			65				70								80
Leu	Leu	Phe	Asp	Ala	Asp	Arg	Leu	Asn	Gly	His	Leu	Asn	Arg	Lys	Glu
			85												95
Phe	Gly	Asn	Glu	Val	Thr	Phe	Arg	Leu	Glu	Lys	Gln	Ser	Asn	Thr	Val
			100												110
Ala	Arg	Ala	Gln	Gln	Met	Leu	Lys	Glu	Lys	Lys	Ser	Ser	Ser	Asp	Glu
			115				120								125
Arg	Leu	Glu	Asn	Thr	Met	Ile	Ala	Arg	Ile	Phe	Asp	Glu	Glu	His	Val
			130				135								140
Gln	Ala	Glu	Met	Lys	Tyr	Val	Lys	Cys	Ile	Arg	Ala	Asn	Glu	Leu	Ala
			145				150								160
Glu	Asp	Asn	Arg	Leu	Asp	Ile	Leu	Pro	Gly	Gly	Ser	Pro	Asn	Ser	Leu
			165												175
Arg	Glu	Lys	Thr	Arg	Trp	Asn	Val	Asn	Thr	Glu	Leu	His	Pro	Ala	Asp
			180												190
Arg	Ala	Glu	Ile	Gly	Ala	Arg	Leu	Thr	Ala	Trp	Leu	Pro	Glu	Lys	Tyr
			195				200								205
His	Ile	Val	Tyr	Phe	Asp	Asp	Phe	Gln	Thr	Val	Ala	Ala	Asn	Asp	Pro
			210				215								220
Ser	Ala	Arg	Arg	Glu	Met	Leu	Asn	Ile	Val	Gln	Asn	Val	Glu	Arg	Glu
			225				230								240
Tyr	Ala	Asp	Glu	Ala	Lys	Ser	Ser	Gly	Tyr	Glu	Lys	Asp	Leu	Lys	Glu
			245												255
Val	Val	Asn	Glu	Leu	Leu	Asp	Asp	Val	Asp	Pro	Ser	Arg	His	Ile	Thr
			260												270
Pro	Glu	Ala	Ile	Lys	Ala	Cys	Thr	Asp	Leu	Asn	Gln	Leu	Glu	Glu	Trp

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275	280	285
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Ser Arg Val Val His Glu Tyr Asn Gly Asp Asp Arg Ile Leu Asp Ile
 290 295 300

Tyr Ala Arg Ala Ala Glu Leu Thr Lys Asn Ala Asp His Gln Ala Leu
 305 310 315 320

Val Lys Asn Met Lys Glu Trp Arg Lys Leu Ala Asn Lys Ile
 325 330

<210> SEQ ID NO 6
 <211> LENGTH: 404
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 6

Met Met Gly Asp Val Asn Asn Val Glu Ala Lys Glu Lys Lys Met Gly
 1 5 10 15

Tyr Glu Ala Lys Lys Val Pro Val Ser Pro Val Lys Ser Ser Arg Pro
 20 25 30

Thr Ala Tyr Val Arg Lys Pro Ala Ser Ala Arg Asn Val Gly Ser Pro
 35 40 45

Ser Ala Lys His Asp Ala Leu Ala Ser Phe Thr Ser Pro Arg Asp Ser
 50 55 60

Lys Arg His Val Pro Asp Cys Gly Phe Ala Ser Pro His Ser Ser Arg
 65 70 75 80

Arg Pro Tyr Arg Thr Asp Pro Lys His Phe Glu Leu His Val Arg Ser
 85 90 95

Ser Val Glu Thr Ser Gly Ala Leu Lys Thr Pro Glu Ala Ala Lys Val
 100 105 110

Ser Ala Ser Gly Asn Gly Thr Asp Gly Pro Leu Phe Ser Ser Asp Val
 115 120 125

Glu Ser Ala Arg Leu Phe Pro Ser Ile Thr Ala Ala Glu Thr Arg Leu
 130 135 140

Pro Phe Leu Asp Gly Cys Phe Arg Pro Asn Thr Asp Gly Gly Ser Val
 145 150 155 160

Val Val Trp Ala Gly Gly Arg Arg Arg Gln Gln Gln Leu Gln Gln Gln
 165 170 175

Ser Leu Cys Ser Arg Gln Pro Ala Glu Arg Glu Glu Glu Ala Gly
 180 185 190

Ala Val Pro Gln Ala Glu Lys Ser Ala Val Phe Leu Pro Glu Ala Leu
 195 200 205

His Gln Glu Ala Lys Gly Phe Cys Leu Pro Leu Thr Ala Ser Leu Glu
 210 215 220

Asn Phe Thr Ala Ser Gly His Glu Arg Ser Leu His Pro Ser His Val
 225 230 235 240

Gly Ser Val Leu Pro Asn Asp Thr Thr Asp Leu Asn Glu Glu Arg Ser
 245 250 255

Phe Ala Gln Cys Met Pro Gly Met Asp Leu Ser Ala Ser Pro Leu Arg
 260 265 270

Met Asp Ala Arg Val Lys Glu Glu Leu Leu Leu His Phe Leu Asn Leu
 275 280 285

Ile Ser Ser Ser Pro Ser Ser Ser Ser Ser Glu Val Gly Ala Ser Phe
 290 295 300

Gln Ser Asp Arg Asp Ala Ala Thr Glu Thr Glu Leu Val Thr Val Phe
 305 310 315 320

Val Arg Gly Glu Asp Ala Gly Val Asp Ala Asp Thr Asn Thr Arg Arg

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Asp Gly Pro Arg Ala Glu Trp Arg His Leu Trp Thr Ser Asn Arg Pro
 305 310 315 320
 Lys Gly Glu Pro Leu Pro Tyr Gln Val Thr Phe Asn Asp Gly Ile Thr
 325 330 335
 Phe Glu Asp Asn Glu Gly Arg Pro Pro Val His Pro Glu Ser His Tyr
 340 345 350
 Glu Gln Thr Pro Lys Glu Ala Pro Tyr Lys Lys Tyr Glu Glu Gln Asp
 355 360 365
 Thr Lys Glu Glu Glu Glu Gln Lys Arg Arg Lys Ser Ala Trp Asp
 370 375 380
 Gln Ser Phe Lys Glu Ser Ile Ala Lys Tyr Glu Glu Arg Tyr Gly Val
 385 390 395 400
 Glu Ala Lys Lys Gly Asp Ser Asp Lys Lys Ser Ser Ser Asp Thr Gly
 405 410 415
 Lys Ser Ser Gly Gly Gly Asp Gly Ser Thr Pro Pro Pro Ser Ser Ser
 420 425 430
 Ser Ser His Glu Gly Gln Asp Gly Lys Lys
 435 440

<210> SEQ ID NO 8
 <211> LENGTH: 233
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <400> SEQUENCE: 8

Met Leu Arg Arg Ala Val Asn Ile Ser Ile Ala Arg Gly Arg Met Ala
 1 5 10 15
 Leu Met Ser Tyr Ala Thr Leu Pro Asp Leu Leu Lys Pro Ser Gly Ala
 20 25 30
 Pro Ala Glu Leu Pro Lys Leu Gly Phe Asn Trp Lys Asp Gly Cys Ala
 35 40 45
 Pro Val Phe Ser Pro Arg Gln Met Glu Leu His Tyr Thr Lys His His
 50 55 60
 Lys Ala Tyr Val Asp Lys Leu Asn Ala Leu Ala Gly Thr Thr Tyr Asp
 65 70 75 80
 Gly Lys Ser Ile Glu Glu Ile Ile Leu Ala Val Ala Asn Asp Ala Glu
 85 90 95
 Lys Lys Gly Leu Phe Asn Gln Ala Ala Gln His Phe Asn His Thr Phe
 100 105 110
 Tyr Phe Arg Cys Ile Thr Pro Asn Gly Lys Ala Met Pro Lys Ser Leu
 115 120 125
 Glu Ser Ala Val Thr Ala Gln Phe Gly Ser Val Glu Gln Phe Lys Asp
 130 135 140
 Ala Phe Val Gln Ala Gly Val Asn Asn Phe Gly Ser Gly Trp Thr Trp
 145 150 155 160
 Leu Cys Val Asp Pro Ser Asn Lys Asn Gln Leu Val Ile Asp Asn Thr
 165 170 175
 Ser Asn Ala Gly Cys Pro Leu Thr Lys Gly Leu Arg Pro Val Leu Ala
 180 185 190
 Val Asp Val Trp Glu His Ala Tyr Tyr Lys Asp Phe Glu Asn Arg Arg
 195 200 205
 Pro Asp Tyr Leu Lys Glu Ile Trp Ser Val Ile Asp Trp Glu Phe Val
 210 215 220
 Ala Lys Met His Ala Gln Ala Ile Lys
 225 230

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<210> SEQ ID NO 9
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 9
Met Asp Glu Asn Glu Gly Gly Trp Glu Glu Phe Ala Glu Glu Pro Gln
1      5      10     15
Gln Tyr Gly Glu Ala Glu Asp Ala Ala Asp Ile Tyr Ala Glu Glu Thr
20     25     30
Leu Ala Thr Ala Gln Lys Ile Ala Ser Asp Asp Asp Ala Leu Arg Phe
35     40     45
Asp Ser Val Lys Glu Val Thr Leu Leu Leu Arg Ser Ala Tyr Met Ser
50     55     60
Arg Met Leu Gln Lys Leu Gly Asp Tyr Ser Glu Gln Glu Val Val Lys
65     70     75     80
Lys Thr Ile Leu Pro Glu Asp Pro Glu Tyr Gln Phe Val Ile Asp Ser
85     90     95
Ser Thr Leu Val Leu Arg Ile Glu Val Glu Lys Ser Lys Ala Val Val
100    105    110
Tyr Leu Arg Ala His Tyr Gly Gln Arg Phe Pro Glu Leu Ala Met Phe
115    120    125
Phe Ser Asp Ser Val Leu Tyr Ala Arg Ile Val Arg Leu Ile Gln Asn
130    135    140
Asn Met Asp Leu Ser Val Val Ile Asp Gln Leu Asp Glu Leu Ile Pro
145    150    155    160
Ser Gln Leu Thr Ala Val Val Ile Ala Cys Ala Ser Thr Thr Ala Gly
165    170    175
Arg Glu Leu Ser Glu Glu Glu Leu His Arg Val Val Glu Ala Cys Gln
180    185    190
Glu Ile Asp Ile Leu Glu Ala Ala Lys Gln Thr Phe Leu Glu Tyr Ile
195    200    205
Gln Arg Ser Met Pro Leu Ile Cys Pro Asn Leu Cys Ala Phe Leu Gly
210    215    220
Thr Gly Ile Thr Ser Gln Leu Phe Ala Ile Ala Gly Gly Val Ser Ala
225    230    235    240
Leu Ser Thr Met Asp Ser Thr Glu Leu Ala Arg Leu Gly Ser Lys Arg
245    250    255
Ala Asp Ser Ser Gly Val Leu Ile Arg Thr Thr Gly Phe Leu Ser Asn
260    265    270
Ser Asp Leu Val Val Asn His Pro Pro Gln Met Arg Pro Lys Ala Leu
275    280    285
Arg Leu Val Ala Ser Thr Thr Ser Met Leu Ala Arg Ile Asp Ala Asn
290    295    300
Arg Arg Ala Ser Ser Gln His Glu Gly Tyr Arg Gln Arg Glu Met Val
305    310    315    320
Arg Leu Lys Met Leu Ser Trp Leu Asp Pro Pro Val Leu Arg Gly Ala
325    330    335
Ala Asn Asn Thr Tyr Ala Arg Arg Gly Arg Lys Arg Pro Arg Arg Gln
340    345    350

Thr Arg

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<210> SEQ ID NO 10
<211> LENGTH: 146

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<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 10

Met Thr His Ser Thr Asp Leu Gln Trp Leu Leu Val Arg Gln Asn Ser
1          5          10          15

Lys Phe Leu Gln Lys Arg Asn Gly Ile Arg Leu Ser Ser Asp Pro Phe
20          25          30

Asn Asn Asn Ala Asn Trp Thr Lys Arg His Ala Gly Phe Leu Asn Thr
35          40          45

Lys Ala Ala Val Val Lys Thr Lys Gly Asp Arg Ile Leu Val Thr Thr
50          55          60

Lys Asp Gly Lys Ala Gly Asn Lys Pro Lys Ser Met Tyr Lys Lys Ala
65          70          75          80

Val Met Asp Ala Gly Val Glu Ala Ser Val Val Ser Lys Ala Val Ala
85          90          95

Ala Val Arg Pro Asp Leu Ala Ser Ile Ala Ser Arg Arg Ala Arg Lys
100         105         110

Met Ala Ser Thr Leu Glu His Met Lys Lys Val Arg Ala Ala Arg Lys
115         120         125

Glu Arg Ser Ser Lys Ile Thr Phe Gln Arg Lys Ala Val Arg Pro Lys
130         135         140

Arg His
145

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<210> SEQ ID NO 11
<211> LENGTH: 525
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 11

Met Pro Pro Thr Ile His Arg Asn Leu Leu Ser Pro Glu Leu Val Gln
1          5          10          15

Trp Ala Leu Lys Ile Glu Lys Asp Ser Arg Leu Thr Ala Arg Gly Ala
20          25          30

Leu Ala Val Met Ser Tyr Ala Lys Thr Gly Arg Ser Pro Leu Asp Lys
35          40          45

Arg Ile Val Asp Thr Asp Asp Val Arg Glu Asn Val Asp Trp Gly Lys
50          55          60

Val Asn Met Lys Leu Ser Glu Glu Ser Phe Ala Arg Val Lys Lys Ile
65          70          75          80

Ala Lys Glu Phe Leu Asp Thr Arg Glu His Leu Phe Val Val Asp Cys
85          90          95

Phe Ala Gly His Asp Glu Arg Tyr Arg Leu Lys Val Arg Val Phe Thr
100         105         110

Thr Arg Pro Tyr His Ala Leu Phe Met Arg Asp Met Leu Ile Val Pro
115         120         125

Thr Pro Glu Glu Leu Ala Thr Phe Gly Glu Pro Asp Tyr Val Ile Tyr
130         135         140

Asn Ala Gly Glu Cys Lys Ala Asp Pro Ser Ile Pro Gly Leu Thr Ser
145         150         155         160

Thr Thr Cys Val Ala Leu Asn Phe Lys Thr Arg Glu Gln Val Ile Leu
165         170         175

Gly Thr Glu Tyr Ala Gly Glu Met Lys Lys Gly Ile Leu Thr Val Met
180         185         190

Phe Glu Leu Met Pro Arg Met Asn His Leu Cys Met His Ala Ser Ala

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195					200					205					
Asn	Val	Gly	Lys	Gln	Gly	Asp	Val	Thr	Val	Phe	Phe	Gly	Leu	Ser	Gly
210					215							220			
Thr	Gly	Lys	Thr	Thr	Leu	Ser	Ala	Asp	Pro	His	Arg	Asn	Leu	Ile	Gly
225					230					235					240
Asp	Asp	Glu	His	Val	Trp	Thr	Asp	Arg	Gly	Val	Phe	Asn	Ile	Glu	Gly
				245					250					255	
Gly	Cys	Tyr	Ala	Lys	Ala	Ile	Gly	Leu	Asn	Pro	Lys	Thr	Glu	Lys	Asp
			260					265						270	
Ile	Tyr	Asp	Ala	Val	Arg	Phe	Gly	Ala	Val	Ala	Glu	Asn	Cys	Val	Leu
		275					280							285	
Asp	Lys	Arg	Thr	Gly	Glu	Ile	Asp	Phe	Tyr	Asp	Glu	Ser	Ile	Cys	Lys
	290					295					300				
Asn	Thr	Arg	Val	Ala	Tyr	Pro	Leu	Ser	His	Ile	Glu	Gly	Ala	Leu	Ser
305					310					315					320
Lys	Ala	Ile	Ala	Gly	His	Pro	Lys	Asn	Val	Ile	Phe	Leu	Thr	Asn	Asp
				325					330						335
Ala	Phe	Gly	Val	Met	Pro	Pro	Val	Ala	Arg	Leu	Thr	Ser	Ala	Gln	Ala
			340						345					350	
Met	Phe	Trp	Phe	Val	Met	Gly	Tyr	Thr	Ala	Asn	Val	Pro	Gly	Val	Glu
		355					360						365		
Ala	Gly	Gly	Thr	Arg	Thr	Ala	Arg	Pro	Ile	Phe	Ser	Ser	Cys	Phe	Gly
	370					375					380				
Gly	Pro	Phe	Leu	Val	Arg	His	Ala	Thr	Phe	Tyr	Gly	Glu	Gln	Leu	Ala
385					390					395					400
Glu	Lys	Met	Gln	Lys	His	Asn	Ser	Arg	Val	Trp	Leu	Leu	Asn	Thr	Gly
			405						410					415	
Tyr	Ala	Gly	Gly	Arg	Ala	Asp	Arg	Gly	Ala	Lys	Arg	Met	Pro	Leu	Arg
			420					425						430	
Val	Thr	Arg	Ala	Ile	Ile	Asp	Ala	Ile	His	Asp	Gly	Thr	Leu	Asp	Arg
		435					440						445		
Thr	Glu	Tyr	Glu	Glu	Tyr	Pro	Gly	Trp	Gly	Leu	His	Ile	Pro	Lys	Tyr
	450					455					460				
Val	Ala	Lys	Val	Pro	Glu	His	Leu	Leu	Asn	Pro	Arg	Lys	Ala	Trp	Lys
465					470					475					480
Asp	Val	Arg	Gln	Phe	Asn	Glu	Thr	Ser	Lys	Glu	Leu	Val	Ala	Met	Phe
			485						490					495	
Gln	Glu	Ser	Phe	Ser	Ala	Arg	Phe	Ala	Ala	Lys	Ala	Ser	Gln	Glu	Met
			500					505					510		
Lys	Ser	Ala	Val	Pro	Arg	Tyr	Val	Glu	Phe	Ala	Arg	Leu			
		515					520					525			
<210> SEQ ID NO 12															
<211> LENGTH: 128															
<212> TYPE: PRT															
<213> ORGANISM: Trypanosoma cruzi															
<400> SEQUENCE: 12															
Met	Gln	Ile	Phe	Val	Lys	Thr	Leu	Thr	Gly	Lys	Thr	Ile	Ala	Leu	Glu
1				5					10					15	
Val	Glu	Ser	Ser	Asp	Thr	Ile	Glu	Asn	Val	Lys	Ala	Lys	Ile	Gln	Asp
			20					25					30		
Lys	Glu	Gly	Ile	Pro	Pro	Asp	Gln	Gln	Arg	Leu	Ile	Phe	Ala	Gly	Lys
		35					40					45			
Gln	Leu	Glu	Asp	Gly	Arg	Thr	Leu	Ala	Asp	Tyr	Asn	Ile	Gln	Lys	Glu

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 305 310 315 320

Ala Leu Phe

<210> SEQ ID NO 14
 <211> LENGTH: 208
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 14

Met Gln Ala Arg Gly Thr Val Lys Val Gln Gly Asp Ala Asn Val Asp
 1 5 10 15
 Gly Lys Met Ser Thr Gly Gln His Pro His His Gln His Leu Asn Ser
 20 25 30
 Thr Gln Ala Asn Ala Thr Thr Thr Ala Leu Glu Tyr Arg Ala Met Asn
 35 40 45
 Arg Pro Leu Tyr Arg Gly Pro Ile Ser His Asn Ile Ile Ser Glu Met
 50 55 60
 Ala Glu Gly Phe Tyr Val Leu Ser Gly Gly Tyr Lys Lys Leu Phe Ile
 65 70 75
 Pro Ser Lys Asp Val Tyr Ala Leu Met Gln Asn Val Gly Met His Leu
 85 90 95
 Thr Glu Glu Glu Phe His Asp Ala Leu Arg Val Ile Gly Gln Ser Glu
 100 105 110
 Pro Gln Asn Ala Asp Glu Leu Ser Phe Ser Asp Phe Leu Leu Leu Met
 115 120 125
 Thr Arg Glu Val Asp Asp Thr Met Ala Asp Glu Leu Arg Ser Ala Phe
 130 135 140
 Phe His Tyr Asp Lys His Lys Thr Gly Tyr Val Thr Arg Lys Gln Phe
 145 150 155 160
 Thr Glu Leu Phe Ala Thr Leu Gly Glu Arg Ser Thr Pro Glu Glu Leu
 165 170 175
 Glu Glu Leu Leu Ala Val Ala Glu Val Asp Glu Thr Asp Asp Lys Ile
 180 185 190
 Asp Tyr Asn Arg Phe Val Asn Glu Leu Thr Ser Arg Val Asn Cys Met
 195 200 205

<210> SEQ ID NO 15
 <211> LENGTH: 589
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 15

Met Ser Ala Glu Glu Ala Thr Gly Leu Glu Ala Ala Arg Lys Gln Lys
 1 5 10 15
 Ile His Asn Leu Lys Leu Lys Thr Ala Cys Leu Glu Asn Glu Glu Leu
 20 25 30
 Ile Gln Glu Leu His Val Ser Asp Trp Ser Glu Thr Gln Arg Gln Lys
 35 40 45
 Leu Arg Gly Ala His Leu Lys Ala Glu Glu Leu Val Ala Ser Val Asp
 50 55 60
 Val Gly Thr Lys Trp Asn Leu Thr Glu Ala Tyr Asp Leu Ala Lys Leu
 65 70 75 80
 Met Arg Val Cys Gly Leu Glu Met Ser Gln Arg Glu Leu Tyr Arg Pro
 85 90 95
 Glu Asp Lys Ala Gln Phe Met Asp Ile Ile Gly Val Lys Lys Val Leu
 100 105 110

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Gln	Asp	Leu	Lys	Gln	Asn	Arg	Asn	Lys	Thr	Arg	Val	Val	Ser	Phe	Thr
	115						120					125			
Gln	Met	Ile	Asp	Asn	Ala	Ile	Ala	Lys	Met	Glu	Lys	Val	Glu	Glu	Glu
	130				135						140				
Leu	Arg	Arg	Ser	Gln	Leu	Asp	Ala	Thr	Gln	Leu	Ala	Gln	Val	Pro	Thr
	145				150					155					160
Arg	Thr	Leu	Lys	Gln	Ile	Glu	Asp	Ile	Met	Asn	Ala	Thr	Gln	Ile	Gln
				165					170						175
Asn	Ala	Leu	Ala	Ser	Thr	Asp	Asp	Gln	Ile	Lys	Thr	Gln	Leu	Ala	Gln
		180						185					190		
Leu	Glu	Lys	Thr	Asn	Glu	Ile	Gln	Asn	Val	Ala	Met	His	Asp	Gly	Glu
		195					200					205			
Met	Gln	Val	Ala	Glu	Glu	Gln	Met	Trp	Thr	Lys	Val	Gln	Leu	Gln	Glu
	210					215					220				
Arg	Leu	Ile	Asp	Leu	Ile	Gln	Asp	Lys	Phe	Arg	Leu	Ile	Thr	Lys	Cys
	225				230					235					240
Glu	Glu	Glu	Asn	Gln	Pro	Phe	Lys	Lys	Ile	Tyr	Glu	Val	Gln	Lys	Gln
				245					250					255	
Ala	Asn	Gln	Glu	Thr	Ser	Gln	Met	Lys	Asp	Ala	Lys	Arg	Arg	Leu	Lys
			260					265					270		
Gln	Arg	Cys	Glu	Thr	Asp	Leu	Lys	His	Ile	His	Asp	Ala	Ile	Gln	Lys
		275					280					285			
Ala	Asp	Leu	Glu	Asp	Ala	Glu	Ala	Met	Lys	Arg	His	Ala	Ala	Asn	Arg
	290				295						300				
Glu	Lys	Ser	Asp	Gly	Phe	Val	Arg	Glu	Asn	Glu	Glu	Arg	Gln	Glu	Glu
	305				310					315					320
Ala	Trp	Asn	Lys	Ile	Gln	Asp	Leu	Glu	Arg	Gln	Leu	Gln	Lys	Leu	Gly
				325					330					335	
Thr	Glu	Arg	Phe	Glu	Glu	Val	Lys	Arg	Arg	Ile	Glu	Glu	Val	Asp	Arg
			340					345					350		
Glu	Glu	Lys	Arg	Arg	Val	Glu	Tyr	Ser	Gln	Phe	Leu	Glu	Val	Ala	Ser
		355					360					365			
Gln	His	Lys	Lys	Leu	Leu	Glu	Leu	Thr	Val	Tyr	Asn	Cys	Asp	Leu	Ala
	370				375						380				
Ile	Arg	Cys	Thr	Gly	Leu	Val	Glu	Glu	Leu	Val	Ser	Glu	Gly	Cys	Ala
	385				390					395					400
Ala	Val	Lys	Ala	Arg	His	Asp	Lys	Thr	Ser	Gln	Asp	Leu	Ala	Ala	Leu
				405					410					415	
Arg	Leu	Glu	Val	His	Lys	Glu	His	Leu	Glu	Tyr	Phe	Arg	Met	Leu	Tyr
			420					425					430		
Leu	Thr	Leu	Gly	Ser	Leu	Ile	Tyr	Lys	Lys	Glu	Lys	Arg	Met	Glu	Glu
		435					440					445			
Ile	Asp	Arg	Asn	Ile	Arg	Thr	Thr	His	Ile	Gln	Leu	Glu	Phe	Cys	Val
	450					455						460			
Glu	Thr	Phe	Asp	Pro	Asn	Ala	Lys	Arg	His	Ala	Asp	Met	Lys	Lys	Glu
	465				470					475					480
Leu	Tyr	Lys	Leu	Arg	Gln	Gly	Val	Glu	Glu	Glu	Leu	Ala	Met	Leu	Lys
				485					490					495	
Glu	Lys	Gln	Ala	Lys	Ala	Leu	Glu	Asp	Phe	Lys	Glu	Ser	Glu	Glu	Ala
			500					505					510		
Leu	Asp	Ala	Ala	Gly	Ile	Glu	Phe	Asn	His	Pro	Val	Asp	Glu	Asn	Asn
		515					520					525			
Glu	Glu	Val	Leu	Thr	Arg	Arg	Ser	Lys	Met	Val	Glu	Tyr	Arg	Ser	His
	530					535						540			

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Leu Ser Lys Gln Glu Glu Val Lys Ile Ala Ala Glu Arg Glu Glu Ile
545 550 555 560

Lys Arg Ala Arg Leu Leu Arg Thr Gly Gly Gly Gly Ser Gly Glu Gln
565 570 575

Pro Arg Ile Gly Asn Asn Thr Ala Pro Ala Arg Leu Glu
580 585

<210> SEQ ID NO 16

<211> LENGTH: 513

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 16

Met Pro Asn Arg Gln Ile Ile Gln Val Phe Glu Glu Tyr Gln Arg Ala
1 5 10 15

Arg Val Lys Phe Val Gln Thr Ile Ala Asp Leu Ala Ser Lys Pro Gln
20 25 30

His Ile Glu Ala Leu Gln Gln Ala Gly Val Met Gln Leu Leu Arg Pro
35 40 45

Leu Leu Leu Asp Ser Val Pro Ser Ile Gln Gln Ser Ala Ala Leu Ala
50 55 60

Leu Gly Arg Leu Ala Asn Tyr Ser Glu Glu Leu Ala Glu Asn Val Val
65 70 75 80

Ser Gly Asp Ile Leu Ala Gln Leu Val Tyr Ser Leu Ser Asp Gln Ser
85 90 95

Arg Phe Tyr Lys Lys Ser Ala Ala Phe Val Leu Arg Ser Val Ala Arg
100 105 110

His Ser Pro Gln Leu Ala Gln Ala Val Val Asp Ser Gln Ala Val Glu
115 120 125

Ala Leu Val Gly Cys Leu Glu Glu Phe Asp Pro Thr Val Lys Glu Ser
130 135 140

Ala Ala Trp Ala Leu Gly Tyr Val Ala Arg His Asn Ala Pro Leu Ala
145 150 155 160

Gln Glu Val Val Asp Lys Gly Ala Ile Pro Pro Leu Val Leu Cys Val
165 170 175

Gln Glu Pro Glu Leu Ser Leu Lys Arg Thr Ala Ala Ser Thr Leu Ser
180 185 190

Asp Ile Ala Lys His Leu Pro Glu Leu Ala Gln Ala Val Val Asp Gln
195 200 205

Asp Ala Val Thr His Leu Ala Pro Leu Ile Met Ser Asn Asp Ser Lys
210 215 220

Leu Arg Arg Gln Val Cys Gln Cys Leu Ala Gln Ile Ser Lys His Ser
225 230 235 240

Val Glu Leu Ala Glu Leu Val Val Glu Gly Glu Ile Phe Pro Lys Ile
245 250 255

Phe Thr Leu Leu Lys Asp Ser Asp Glu Val Val Arg Lys Asn Ala Ala
260 265 270

Thr Cys Ile Arg Glu Ile Ala Lys His Thr Pro Glu Leu Ala Gln Leu
275 280 285

Val Val Asn Ala Gly Gly Val Gly Ala Leu Val Asp Tyr Thr Ser Glu
290 295 300

Ser Arg Asp Ser Ala Arg Leu Pro Gly Ile Met Thr Leu Gly Phe Ile
305 310 315 320

Ser Ala Phe Ser Glu Thr Leu Ala Leu Ala Val Ile Val Ser His Gly
325 330 335

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Ile Val Pro Leu Ala Asp Ala Leu Glu Lys Glu Pro Glu Asp His Ile
 340 345 350

Lys Ala Ala Ala Ala Trp Ser Leu Gly Gln Ile Gly Arg His Ser Ala
 355 360 365

Asp His Ala Lys Ala Val Ala Asp Cys Asn Val Leu Pro Arg Leu Leu
 370 375 380

Asp Val Tyr Leu Asn Pro Lys Ser Ser Glu Asp Leu Arg Met Lys Ser
 385 390 395 400

Lys Arg Ala Leu Lys Asn Ile Ile Gln Arg Cys Leu Gln Leu Pro Ala
 405 410 415

Leu Glu Pro Leu Leu His Pro Asp Ala Pro Gln Lys Val Leu Lys Tyr
 420 425 430

Val Cys Gly Gln Phe Ala Lys Val Leu Pro Thr Asp Ile Ala Ala Lys
 435 440 445

Arg Glu Phe Val Ala Asn Arg Gly Leu Ala Thr Val Gln Arg Ile His
 450 455 460

Pro Glu Pro Gly Ser Lys Leu Ala Glu Tyr Ile Gln Ser Ile Asn Asn
 465 470 475 480

Cys Tyr Pro Pro Glu Ile Val Gln Tyr Tyr Ser Pro Gln Tyr Ala Gln
 485 490 495

Thr Phe Leu Glu Lys Ile Glu Asn Tyr His Val Gln Gln Val Gln Gln
 500 505 510

Ser

<210> SEQ ID NO 17
 <211> LENGTH: 466
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <400> SEQUENCE: 17

Met Ala Arg Pro Leu Ile Tyr Pro Ile Leu Ser Leu Val Ala Ala Ala
 1 5 10 15

Thr Leu Val Thr Thr Ala Val Glu Ala Leu Tyr Val Val Pro Gln Gly
 20 25 30

Arg Leu Arg Glu Thr Gly Ser Gly Trp His Pro Cys Asp Pro Asp Val
 35 40 45

Pro Gln Trp Ser Gly Tyr Phe Asp Ile Pro Gly Arg Glu Gly Asp Lys
 50 55 60

His Tyr Phe Tyr Trp Ala Phe Gly Pro Arg Asn Gly Asn Pro Glu Ala
 65 70 75 80

Pro Val Leu Leu Trp Met Thr Gly Gly Pro Gly Cys Ser Ser Met Phe
 85 90 95

Ala Leu Leu Ala Glu Asn Gly Pro Cys Leu Val Asn Glu Thr Thr Gly
 100 105 110

Asp Ile Tyr Lys Asn Asn Tyr Ser Trp Asn Asn Glu Ala Tyr Val Ile
 115 120 125

Tyr Val Asp Gln Pro Ala Gly Val Gly Phe Ser Tyr Ala Glu Val Glu
 130 135 140

Asp Tyr Asp Ser Asn Glu Glu Val Ser Glu Asp Met Tyr His Phe
 145 150 155 160

Leu Gln Ala Phe Phe Gly Ala His Gln Lys Leu Arg Lys Asn Lys Leu
 165 170 175

Phe Val Val Gly Glu Ser Tyr Gly Gly His Tyr Ala Pro Ala Thr Ala
 180 185 190

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His Tyr Ile Asn Lys Ala Asn Arg Glu His Val Gly Leu Pro Ile Arg
 195 200 205

Leu Ala Gly Leu Ala Val Gly Asn Gly Leu Thr Asp Pro His Thr Gln
 210 215 220

Tyr Ala Ala Tyr Pro Ser Leu Ala Trp Gly Trp Cys Arg Glu Lys Leu
 225 230 235 240

Gly Glu Pro Cys Val Ser Glu Glu Gly Tyr Gln Gln Met Ser Ser Met
 245 250 255

Val Thr Pro Cys Gln Lys Ala Ile Glu Ile Cys Asn Ser Asp Asn Asn
 260 265 270

Phe Ile Ala Lys Ala Ala Cys Val Thr Ala Arg Val Leu Cys Asn Pro
 275 280 285

Ile Ile Gly Val Tyr Ser Ala Thr Gly Leu Asn Asn Tyr Asp Ile Arg
 290 295 300

Lys Pro Cys Ile Gly Thr Leu Cys Tyr Asn Phe Asp Ala Leu Asn Ala
 305 310 315 320

Phe Met Asn Arg Glu Asp Val Gln Ser Ser Leu Gly Ala Lys Arg Gln
 325 330 335

Val Trp Gln Ser Cys Asn Met Glu Val Asn Leu Met Phe Leu Met Asp
 340 345 350

Trp Phe Lys Asn Phe Asn Tyr Thr Val Pro Thr Leu Leu Glu Asp Gly
 355 360 365

Val Ser Val Met Ile Tyr Ala Gly Glu Met Asp Phe Ile Cys Asn Trp
 370 375 380

Ile Gly Asn Lys Gln Trp Thr Thr Ala Leu Asn Trp Pro Gly Lys Ala
 385 390 395 400

Val Phe Asn Ala Ala Pro Asp Glu Pro Phe Arg Ala Pro Asp Gly Thr
 405 410 415

Val Ala Gly Leu Val Arg Thr Ala Ala Ala Ala Ser Thr Ser Asn Leu
 420 425 430

Thr Phe Val Gln Val Tyr Asn Ala Gly His Met Val Pro Met Asp Gln
 435 440 445

Pro Ala Ser Ala Phe Val Met Ile Ser Asn Phe Leu Gln Gly Arg Pro
 450 455 460

Phe Asn
 465

<210> SEQ ID NO 18
 <211> LENGTH: 381
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 18

Met Ser Arg Asn Val Ala Ala Glu Glu Glu Trp Glu Asp Val Asp Ala
 1 5 10 15

Pro Asn Glu Glu Asp Glu Glu Glu Glu Asp Thr Thr Ile Asn Asn Ser
 20 25 30

Asp Val Met Met Arg Tyr Lys Lys Ala Ala Leu Trp Cys Asn Glu Thr
 35 40 45

Leu Gln Leu Leu Leu Asp Ala Thr Lys Pro Gly Ala Lys Val His Glu
 50 55 60

Leu Cys Lys Leu Gly Asp Glu Thr Val Ala Lys Lys Leu Lys Thr Met
 65 70 75 80

Phe Lys Gly Thr Glu Lys Gly Leu Ala Phe Pro Thr Cys Ile Ser Val
 85 90 95

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Asn Ser Cys Val Ala His Asn Ser Pro Ser Ala Asp Asp Glu Val Ala
 100 105 110

Ser Gln Glu Ile Gln Leu Gly Asp Val Val His Ile Asp Leu Gly Ile
 115 120 125

His Val Asp Gly Tyr Cys Ala Gln Val Ala His Thr Val Gln Val Thr
 130 135 140

Glu Asn Asn Glu Leu Ala Ala Asp Asp Asp Ala Ser Lys Val Ile Ser
 145 150 155

Ala Thr Tyr Gly Ile Leu Asn Thr Ala Met Arg Lys Met Arg Pro Gly
 165 170 175

Val Ser Val Tyr Glu Val Thr Glu Val Ile Glu Lys Ala Ala Ala His
 180 185 190

Tyr Gly Val Thr Pro Val Asp Gly Val Leu Ser His Met Leu Lys Arg
 195 200 205

Tyr Ile Val Asp Ser Phe Arg Cys Val Pro Gln Arg Lys Val Ala Glu
 210 215 220

His Leu Val His Asp Tyr Thr Leu Glu Ala Gly Gln Val Trp Thr Leu
 225 230 240

Asp Ile Val Met Ser Ser Gly Lys Gly Lys Leu Lys Glu Arg Asp Val
 245 250 255

Arg Pro Thr Val Tyr Lys Val Ala Leu Asp Ser Asn Tyr Thr Met Lys
 260 265 270

Met Glu Ser Ala Arg Glu Leu Gln Arg Glu Ile Glu Ala Lys Tyr Gln
 275 280 285

Thr Phe Pro Phe Ala Leu Arg Asn Leu Glu Thr Lys Arg Ala Arg Leu
 290 295 300

Gly Leu Ser Glu Met Leu Lys His Gly Ala Val Val Pro Tyr Pro Val
 305 310 315 320

Leu Tyr Glu Arg Asp Gly Glu Val Val Gly His Phe Lys Ile Thr Leu
 325 330 335

Leu Ile Thr Ala Lys Lys Ile Glu Pro Val Thr Gly Leu Lys Pro Gln
 340 345 350

Lys Ala Pro Thr Leu Pro Ala Tyr Thr Asp Glu Leu Leu Leu Glu Ala
 355 360 365

Ser Lys Leu Pro Leu Thr Leu Glu Lys Lys Arg Lys Asn
 370 375 380

<210> SEQ ID NO 19
 <211> LENGTH: 411
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <400> SEQUENCE: 19

Met Ser Leu Thr Leu Trp Ser Gly Val Asn Pro Glu Asn Ala Arg Thr
 1 5 10 15

His Lys Leu Leu Ala Ala Ala Ala Leu Ala Asn Val Ala Val Thr Leu
 20 25 30

Lys Ala Cys Glu Tyr Gly Arg Glu Asn Glu Thr Ala Glu Tyr Cys Arg
 35 40 45

Asn Cys Ser Pro Cys Gly Arg Tyr Pro Val Leu Gln Thr Glu Glu Gly
 50 55 60

Cys Val Phe Glu Ser Asn Ala Ile Leu Arg His Ile Ala Arg Leu Asp
 65 70 75 80

Arg Ser Gly Gly Phe Leu Tyr Gly Arg Thr Pro Leu Glu Gly Ser Gln
 85 90 95

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Val Asp Met Trp Leu Asp Phe Ser Ala Thr Glu Leu Asp Ala Ala Ser
 100 105 110

Glu Pro Phe Val His His Ala Phe Arg Gly Glu Pro Leu Pro Ala Asn
 115 120 125

Ala Met Asp Arg Val His Glu Val Leu Arg Ala Leu Glu Ala Trp Leu
 130 135 140

Glu Thr Arg Thr Phe Leu Val Gly Glu Arg Met Thr Val Ala Asp Val
 145 150 155 160

Ala Val Ala Phe Ala Leu Gln Trp His Tyr Arg Leu Asn Gly Ala Glu
 165 170 175

Gly Glu Ala Leu Thr Lys Lys Tyr Arg Asn Ala Tyr Arg Leu Tyr Asn
 180 185 190

Thr Val Met Gln Gln Pro Lys Thr Val Glu Val Leu Arg Ser Gln Gly
 195 200 205

Ala Thr Phe Gly Pro Val Lys Ala Glu Arg Lys Gly Lys Asp Ala Ala
 210 215 220

Ala Pro Ala Arg Ala Glu Lys Lys Pro Lys Ala Ala Ala Ala Ala
 225 230 235 240

Asp Gly Ala Glu Glu Asp Glu Ala Pro Arg Glu Lys Lys Lys Pro
 245 250 255

Asn Pro Leu Asp Glu Leu Pro Pro Ser Pro Phe Val Leu Asp Ala Phe
 260 265 270

Lys Arg Glu Tyr Ser Asn Thr Asp Thr Arg Thr Val Ala Ala Pro Tyr
 275 280 285

Phe Phe Gln His Tyr Asp Ala Ala Gly Tyr Thr Thr Phe Trp Cys Arg
 290 295 300

Tyr Lys Tyr Asn Glu Asp Asn Lys Met Gln Phe Met Thr Ala Asn Leu
 305 310 315 320

Ile Arg Gly Trp Phe Gln Arg Met Glu His Val Arg Lys Tyr Ala Phe
 325 330 335

Gly Val Ala Leu Ile Ile Gly Glu Glu Arg Arg His Asp Ile Val Ala
 340 345 350

Leu Trp Val Phe Arg Gly Arg Gly Met Pro Ala Ile Val Glu Asp Val
 355 360 365

Glu Asp Thr Glu Leu Phe Asp Trp Glu Glu Val Ala Asp Val Ala Ala
 370 375 380

Gln Arg Glu Arg Ile Thr Asp Tyr Leu Cys Trp Glu Gly Pro Thr Ile
 385 390 395 400

Pro Arg Pro Val Leu Glu Gly Arg Val Phe Lys
 405 410

<210> SEQ ID NO 20
 <211> LENGTH: 982
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 20

Met Ser Gly Asp Gly Asp Ser Ser Leu Asp Pro Ser Ile Leu Val Val
 1 5 10 15

Glu Ala Arg Phe Asn Glu Ser Leu Gly Asn Gln Ser Val Ser Gly Gly
 20 25 30

Gly Gly Ser Glu Arg Trp Gln His His Glu Glu Lys Gln Gln Gln
 35 40 45

Gln Gln Pro Leu Ser Leu Pro Pro Arg Ser Arg Gly Asp Val Asn Trp
 50 55 60

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Asn Ala Ser Ser Ser Ser Ser Pro Ser Thr Ile Glu Glu Ala Glu Gly
 65 70 75 80

Gly Asp Gly Asp Arg Arg Thr Ala Asp Arg Arg Trp Ser Asp Asp Gly
 85 90 95

Ser Asn Ala Gly Asn Asp Arg Asp Gly Gly Ile Glu Thr Asn Glu Glu
 100 105 110

Asn Glu Asp Glu Ile Ala Glu Arg Val Leu Arg Ala Leu Arg Cys Lys
 115 120 125

Asp Met Leu Met Asp Glu Gln Ala Arg Lys Leu Gln Arg Arg Glu Met
 130 135 140

Glu Ala Arg Gln Leu Arg Arg Glu Leu Asp Glu Leu Arg Gly Glu Lys
 145 150 155 160

Gln Leu Leu Met Gln Gln Leu Arg Gly Phe Leu Asp Gly Ser Thr Pro
 165 170 175

Met Thr Thr Ala Ser Glu Thr Gly Pro Leu Lys Asp Ser Gly Gln Leu
 180 185 190

Tyr Pro Ser Met Leu Leu Gln Arg Ala Asp Ser Gln Leu Gln Asp Glu
 195 200 205

Arg Ala Glu Arg Gln Gln Asp Ala Arg His Phe Met Ala His Ile Glu
 210 215 220

Gln Leu Thr Ala Gln Leu Ala Glu Ala Gln His Glu Ala Arg Thr Arg
 225 230 235 240

Glu Ala Arg His Ala Gln Asp Leu Asp Thr Ile Gln Gln Glu Met Gln
 245 250 255

Glu Leu Ser Thr Val Val Asp Asp Met His Ala Thr Lys Ala Ala Leu
 260 265 270

Cys Arg Thr Gln Glu Gln Leu Ala Lys Ala Asn Glu Glu Lys Ala Gln
 275 280 285

Cys Gln Leu Glu Arg Asp Arg Leu Val Arg Ser Leu Gln Glu Ala Leu
 290 295 300

Arg Arg Glu Gly Ser Glu His Gln Arg Thr Leu Glu Arg Met Arg Ala
 305 310 315 320

Glu Ala Gly Ala Tyr Glu Arg Ala Lys Ala Ala Ala Glu Ala Lys Cys
 325 330 335

Arg Arg Ala Glu Ala Glu Gln Leu Lys Leu Ala Glu Glu Leu Arg Ala
 340 345 350

Leu Arg Ile Glu Met Gln Gln Leu Val Asp Glu Asn Ala Ala Leu Thr
 355 360 365

Leu Arg Met Glu Ser Ser Glu Gln Gln Leu Arg Arg Ala Gln Lys Gln
 370 375 380

His Val Glu Glu Arg Ala Ala Glu Ala Glu Ala Arg Arg Arg Leu Gln
 385 390 395 400

Glu Glu Leu Asp Ala Lys Val Arg Glu Met Ala Gln Leu Arg Ser Thr
 405 410 415

Arg Asp Ala Gln Ser Gln Leu Leu Val Glu Glu Glu Gly Arg His Ala
 420 425 430

Leu Phe Gln Ala Glu Val Glu Glu Cys Val Gln Ser Thr Arg Gln Leu
 435 440 445

Glu Glu Ala Leu Met Arg Cys Glu Arg Arg Arg Cys Glu Ala Glu Glu
 450 455 460

Arg Glu Thr Arg Val Ala Ala Glu Arg Asp Ala Leu Arg Val Gln Leu
 465 470 475 480

Gln Arg Val Thr Ala Ala Ser Arg Gln Glu Leu Leu Glu Gln Gln Gln
 485 490 495

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Leu Thr Glu Glu Met Arg Ser Phe His Gln Ala Lys Leu Gln Gln Met
 500 505 510
 Gln Gln Ala Ala Glu His Gln Arg Gln Arg Ala Glu Arg Leu Glu Glu
 515 520 525
 Lys Ser Glu Glu Ala Val Arg Glu Tyr Arg Thr Leu Gln Ala Leu Leu
 530 535 540
 Asp Ser Thr Gln Arg Gln Met Glu Glu Val Ala Gly Lys Leu His Glu
 545 550 555 560
 Leu Arg Gln Gln Arg Met Ser Leu Glu Ser Met Leu Ala Glu Thr Gln
 565 570 575
 Gln Glu Asn Asn Glu Cys Ala Ala Arg Glu Lys Asn Ala Ala Ala Gln
 580 585 590
 Leu Asp Ala Ile Arg Ser Arg Leu Lys Gln Arg Glu Cys Ala Trp Arg
 595 600 605
 Glu Leu Arg Ala Lys Met Gln Arg Leu Glu Glu Arg Glu Gln Arg Arg
 610 615 620
 Arg Leu Ala Glu Ala Ala Asp Ser Leu Leu Arg Met Arg Gln Asn His
 625 630 635 640
 His Ser Gln Gly Lys Cys Lys Thr Lys Leu Gln Thr Cys Ile Arg Asp
 645 650 655
 Lys Ile Ser Arg Ala Arg Leu Glu Glu Asn Leu Leu Asp Asn Ile Ala
 660 665 670
 Gly Val Asp Val Asn Thr Thr Leu Ser Thr Lys Glu Pro Ser Ser Met
 675 680 685
 Thr Ala Pro Pro Pro Pro Pro Glu Thr Lys Arg Thr Pro Leu Arg Gly
 690 695 700
 Pro Gln Leu Asp Ala Trp Gln Ala Lys Leu Gln Ala Leu Glu Ala Arg
 705 710 715 720
 Asn Ala Asn Leu Glu Arg Gln Leu Ala Ser Arg Gln Ile Gly His Arg
 725 730 735
 Ala Leu Val Glu Asp Arg Lys Ala Leu His Gln Gln Met His Thr Leu
 740 745 750
 Gln Glu Thr Ala Gln Gly Leu Met Ser Ala Leu Glu Arg Gln His Arg
 755 760 765
 Asp Ala Ile Lys His Leu Glu Glu Ala His Arg Arg His Thr Leu Val
 770 775 780
 Ala Cys Arg Glu Ala Ser Asp Ala Leu Ala Ser His Glu Ser Cys Val
 785 790 795 800
 Arg Ser Gly Val Val Arg Val Val Ser Glu Leu Met Ala Phe Ile Arg
 805 810 815
 Ala Leu Glu Ala Asn Ala Thr Leu Val Ala Arg His His Glu Arg Leu
 820 825 830
 Gln Glu Ala Pro Val Gln Met Ala Asp Asp Asp Asp Lys Glu Asn Met
 835 840 845
 Leu Arg Ala Ala Cys Asp Asp Ile Thr Arg Asn Phe Leu Gly Val Glu
 850 855 860
 Gly Gly Trp Glu Ala Leu Leu Gln His Ser Leu Arg Thr Thr Ser Gly
 865 870 875 880
 Arg Lys Ser Ser Arg Ala Asp Gly Gly Ser Leu Thr Ala Ala Met Arg
 885 890 895
 Arg Arg Ile Arg Cys Phe Leu Met Asp Tyr Leu Gln Ala Gln Leu Leu
 900 905 910
 Gly Lys Pro Ala Ala Ala Val Leu Pro Ala Leu Arg Arg Gln Ser Gly

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Leu Gln Asp Met Ile Asn Glu Val Asp Gln Asp Gly Ser Gly Thr Ile
 50 55 60
 Asp Phe Pro Glu Phe Leu Thr Leu Met Ala Arg Lys Met Gln Asp Ser
 65 70 75 80
 Asp Ser Glu Glu Glu Ile Lys Glu Ala Phe Arg Val Phe Asp Lys Asp
 85 90 95
 Gly Asn Gly Phe Ile Ser Ala Ala Glu Leu Arg His Val Met Thr Asn
 100 105 110
 Leu Gly Glu Lys Leu Thr Asp Glu Glu Val Asp Glu Met Ile Arg Glu
 115 120 125
 Ala Asp Val Asp Gly Asp Gly Gln Ile Asn Tyr Glu Glu Phe Val Lys
 130 135 140
 Met Met Met Ser Lys
 145

<210> SEQ ID NO 23
 <211> LENGTH: 518
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 23

Met Leu Thr Arg Leu Arg Ser Ala Ala Leu Arg Gly Ala Ala Gly Thr
 1 5 10 15
 Arg Ala Ala Ser Gly Met Pro Thr Ala Asp His Lys Gly Arg Val Gly
 20 25 30
 Tyr Val Ser Gln Val Ile Gly Ala Val Val Asp Val His Phe Ala Glu
 35 40 45
 Gly Val Pro Pro Val Leu Thr Ala Leu Asp Val Val Glu Lys Leu Gly
 50 55 60
 Arg Asp Glu Pro Leu Thr Leu Glu Ile Val Gln His Leu Asp Ala His
 65 70 75 80
 Thr Gly Arg Cys Ile Ala Met Gln Thr Thr Asp Leu Leu Lys Leu Lys
 85 90 95
 Ser Lys Val Val Ser Ser Gly Gly Asn Ile Ser Val Pro Val Gly Arg
 100 105 110
 Glu Thr Leu Gly Arg Ile Phe Asn Val Leu Gly Asp Ala Ile Asp Gln
 115 120 125
 Arg Gly Pro Val Gly Glu Lys Gln Arg Met Pro Ile His Ala Val Ala
 130 135 140
 Pro Lys Leu Ala Asp Gln Ala Ala Glu Asp Thr Ile Leu Thr Thr Gly
 145 150 155 160
 Ile Lys Val Ile Asp Leu Ile Leu Pro Tyr Cys Lys Gly Gly Lys Ile
 165 170 175
 Gly Leu Phe Gly Gly Ala Gly Val Gly Lys Thr Val Ile Ile Met Glu
 180 185 190
 Leu Ile Asn Asn Val Ala Lys Gly His Gly Gly Phe Ser Val Phe Ala
 195 200 205
 Gly Val Gly Glu Arg Thr Arg Glu Gly Thr Asp Leu Tyr Leu Glu Met
 210 215 220
 Met Gln Ser Lys Val Ile Asp Leu Lys Gly Asp Ser Lys Cys Val Leu
 225 230 235 240
 Val Tyr Gly Gln Met Asn Glu Pro Pro Gly Ala Arg Ala Arg Val Ala
 245 250 255
 Gln Ser Ala Leu Thr Met Ala Glu Tyr Phe Arg Asp Val Glu Gly Gln
 260 265 270

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Asp Val Leu Leu Phe Ile Asp Asn Ile Phe Arg Phe Thr Gln Ala Asn
 275 280 285
 Ser Glu Val Ser Ala Leu Leu Gly Arg Ile Pro Ala Ala Val Gly Tyr
 290 295 300
 Gln Pro Thr Leu Ala Glu Asp Leu Gly Gln Leu Gln Glu Arg Ile Thr
 305 310 315 320
 Ser Thr Thr Lys Gly Ser Ile Thr Ser Val Gln Ala Val Tyr Val Pro
 325 330 335
 Ala Asp Asp Ile Thr Asp Pro Ala Pro Ala Thr Thr Phe Ser His Leu
 340 345 350
 Asp Ala Thr Thr Val Leu Asp Arg Ala Val Ala Glu Ser Gly Ile Tyr
 355 360 365
 Pro Ala Val Asn Pro Leu Glu Cys Ala Ser Arg Ile Met Asp Pro Asp
 370 375 380
 Val Ile Ser Val Asp His Tyr Asn Val Ala Gln Asp Val Val Gln Met
 385 390 395 400
 Leu Thr Lys Tyr Lys Glu Leu Gln Asp Ile Ile Ala Val Leu Gly Ile
 405 410 415
 Asp Glu Leu Ser Glu Glu Asp Lys Leu Ile Val Asp Arg Ala Arg Lys
 420 425 430
 Val Thr Lys Phe Leu Ser Gln Pro Phe Gln Val Ala Glu Val Phe Thr
 435 440 445
 Gly Met Thr Gly His Tyr Val Gln Leu Glu Glu Thr Ile Glu Ser Phe
 450 455 460
 Ser Gly Leu Leu Met Gly Thr Tyr Asp Gln Val Pro Glu Met Ala Phe
 465 470 475 480
 Tyr Met Val Gly Gly Ile Thr Ser Val Leu Glu Lys Gly Lys Lys Met
 485 490 495
 Ala Glu Glu Ala Ala Glu Leu Glu Lys Leu Arg Arg Ala Arg Ala Ala
 500 505 510
 Gln Ala Gly Gln Lys Glu
 515

<210> SEQ ID NO 24

<211> LENGTH: 655

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 24

Met Phe Ala Arg Arg Leu Cys Gly Ala Gly Ser Leu Ala Ala Ala Ser
 1 5 10 15
 Leu Ala Arg Trp Gln Ser Ser Lys Val Thr Gly Asp Val Ile Gly Ile
 20 25 30
 Asp Leu Gly Thr Thr Tyr Ser Cys Val Ala Val Met Glu Gly Asp Lys
 35 40 45
 Pro Arg Val Leu Glu Asn Thr Glu Gly Phe Arg Thr Thr Pro Ser Val
 50 55 60
 Val Ala Phe Lys Gly Gln Glu Lys Leu Val Gly Leu Ala Ala Lys Arg
 65 70 75 80
 Gln Ala Ile Thr Asn Pro Gln Ser Thr Phe Phe Ala Val Lys Arg Leu
 85 90 95
 Ile Gly Arg Arg Phe Glu Asp Ser Asn Ile Gln His Asp Ile Lys Asn
 100 105 110
 Val Pro Tyr Lys Ile Val Arg Ser Ser Asn Gly Asp Ala Trp Val Gln
 115 120 125

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Asp Ala Asn Gly Lys Gln Tyr Ser Pro Ser Gln Val Gly Ala Phe Val
 130 135 140
 Leu Glu Lys Met Lys Glu Thr Ala Glu Asn Phe Leu Gly Arg Lys Val
 145 150 155 160
 Ser Asn Ala Val Val Thr Cys Pro Ala Tyr Phe Asn Asp Ala Gln Arg
 165 170 175
 Gln Ala Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Asn Val Ile Arg
 180 185 190
 Val Val Asn Glu Pro Thr Ala Ala Ala Leu Ala Tyr Gly Leu Asp Lys
 195 200 205
 Thr Lys Asp Ser Met Ile Ala Val Tyr Asp Leu Gly Gly Gly Thr Phe
 210 215 220
 Asp Ile Ser Val Leu Glu Ile Ala Gly Gly Val Phe Glu Val Lys Ala
 225 230 235 240
 Thr Asn Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Leu Cys Leu
 245 250 255
 Ser Asp Tyr Ile Leu Thr Glu Phe Lys Lys Ser Thr Gly Ile Asp Leu
 260 265 270
 Ser Asn Glu Arg Met Ala Leu Gln Arg Ile Arg Glu Ala Ala Glu Lys
 275 280 285
 Ala Lys Cys Glu Leu Ser Thr Thr Met Glu Thr Glu Val Asn Leu Pro
 290 295 300
 Phe Ile Thr Ala Asn Gln Asp Gly Ala Gln His Val Gln Met Thr Val
 305 310 315 320
 Ser Arg Ser Lys Phe Glu Ser Leu Ala Glu Lys Leu Val Gln Arg Ser
 325 330 335
 Leu Gly Pro Cys Lys Gln Cys Ile Lys Asp Ala Ala Val Asp Leu Lys
 340 345 350
 Glu Ile Ser Glu Val Val Leu Val Gly Gly Met Thr Arg Met Pro Lys
 355 360 365
 Val Ile Glu Ala Val Lys Gln Phe Phe Gly Arg Asp Pro Phe Arg Gly
 370 375 380
 Val Asn Pro Asp Glu Ala Val Ala Leu Gly Ala Ala Thr Leu Gly Gly
 385 390 395 400
 Val Leu Arg Gly Asp Val Lys Gly Leu Val Leu Leu Asp Val Thr Pro
 405 410 415
 Leu Ser Leu Gly Ile Glu Thr Leu Gly Gly Val Phe Thr Arg Met Ile
 420 425 430
 Pro Lys Asn Thr Thr Ile Pro Thr Lys Lys Ser Gln Thr Phe Ser Thr
 435 440 445
 Ala Ala Asp Asn Gln Thr Gln Val Gly Ile Lys Val Phe Gln Gly Glu
 450 455 460
 Arg Glu Met Ala Ala Asp Asn Gln Met Met Gly Gln Phe Asp Leu Val
 465 470 475 480
 Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe
 485 490 495
 Asp Ile Asp Ala Asn Gly Ile Cys His Val Thr Ala Lys Asp Lys Ala
 500 505 510
 Thr Gly Lys Thr Gln Asn Ile Thr Ile Thr Ala Ser Gly Gly Leu Ser
 515 520 525
 Lys Glu Gln Ile Glu Arg Met Ile Arg Asp Ser Glu Ser His Ala Glu
 530 535 540
 Ser Asp Arg Leu Lys Arg Glu Leu Val Glu Val Arg Asn Asn Ala Glu

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545		550		555		560									
Thr	Gln	Ala	Asn	Thr	Ala	Glu	Arg	Gln	Leu	Thr	Glu	Trp	Lys	Tyr	Val
			565						570						575
Ser	Asp	Ala	Glu	Lys	Glu	Asn	Val	Arg	Thr	Leu	Leu	Ala	Glu	Leu	Arg
			580						585					590	
Lys	Ser	Met	Glu	Asn	Pro	Asn	Val	Thr	Lys	Asp	Glu	Leu	Ser	Ala	Ala
		595					600					605			
Thr	Asp	Lys	Leu	Gln	Lys	Ala	Val	Met	Glu	Cys	Gly	Arg	Thr	Glu	Tyr
	610					615						620			
Gln	Gln	Ala	Ala	Ala	Ala	Asn	Ser	Ser	Ser	Ser	Ser	Gly	Asn	Thr	Asp
	625				630						635				640
Ser	Ser	Gln	Gly	Glu	Gln	Gln	Gln	Gln	Gly	Asp	Gln	Gln	Lys	Gln	
			645						650					655	

<210> SEQ ID NO 25
 <211> LENGTH: 600
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 25

Met	Ser	Tyr	Lys	Glu	Ala	Ser	Gly	Ala	Val	Gly	Pro	Ala	Asp	Gln	Gln
1				5					10					15	
Gln	Pro	Ala	Val	Pro	Glu	Val	Thr	Asp	Val	Thr	Leu	Glu	Ala	Ala	Arg
			20					25					30		
Lys	Gln	Lys	Ile	His	Asn	Leu	Lys	Leu	Lys	Thr	Ser	Cys	Leu	Ser	Asn
		35					40					45			
Glu	Glu	Phe	Ile	Gln	Asp	Leu	His	Val	Ser	Asp	Trp	Ser	Glu	Thr	Gln
	50					55					60				
Lys	Gln	Lys	Leu	Leu	Ala	Ala	His	Glu	Lys	Ala	Gln	Glu	Leu	Leu	Ser
	65				70					75					80
Ser	Val	Glu	Gly	Gly	Thr	Lys	Trp	Asn	Leu	Thr	Glu	Ala	Tyr	Asp	Ile
			85					90						95	
Lys	Lys	Leu	Met	Arg	Val	Cys	Gly	Leu	Gln	Leu	Ser	Val	Arg	Glu	Leu
			100					105						110	
Tyr	Lys	Pro	Glu	Asp	Lys	Pro	His	Phe	Met	Glu	Val	Val	Ala	Leu	Lys
		115					120						125		
Lys	Thr	Leu	Asn	Glu	Leu	Lys	Gln	His	His	Asn	Lys	Thr	Arg	Thr	Val
	130					135					140				
Ser	Phe	Thr	Gly	Thr	Ile	Asp	Asn	Ala	Ile	Ala	Lys	Leu	Glu	Lys	Ile
	145				150					155					160
Glu	Asp	Glu	Leu	Arg	Arg	Ser	Gln	Leu	Asp	Ala	Ser	Glu	Met	Ala	Gln
				165					170					175	
Val	Pro	Val	Ala	Val	Leu	Lys	Asn	Leu	Glu	Glu	Cys	Met	Asn	Val	Thr
			180					185						190	
Val	Val	Gln	Thr	Ala	Leu	Leu	Gly	Asn	Glu	Glu	Gln	Ile	Lys	Ala	Gln
			195				200						205		
Leu	Ala	Ala	Ile	Glu	Lys	Ala	Lys	Glu	Ile	Arg	Asn	Val	Ala	Ile	Ala
	210					215						220			
Asp	Gly	Glu	Met	Ala	Ile	Ala	Glu	Glu	Gln	Tyr	Tyr	Ile	Lys	Ala	Gln
	225				230					235					240
Leu	Leu	Glu	His	Leu	Val	Glu	Leu	Val	Ala	Asp	Lys	Phe	Arg	Ile	Ile
			245						250					255	
Gly	Gln	Thr	Glu	Asp	Glu	Asn	Lys	Pro	Phe	Gly	Arg	Ile	Gln	Asp	Val
			260					265						270	
Gln	Lys	Lys	Ser	Phe	Gln	Glu	Thr	Ser	Ala	Ile	Lys	Asp	Ala	Lys	Arg

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275					280					285					
Arg	Leu	Lys	Gln	Arg	Cys	Glu	Asp	Asp	Leu	Lys	Asn	Leu	His	Asp	Ala
290					295					300					
Ile	Gln	Lys	Ala	Asp	Met	Glu	Asp	Ala	Glu	Ala	Met	Lys	Arg	Phe	Ala
305					310					315					320
Thr	Gln	Lys	Glu	Lys	Ser	Glu	Lys	Phe	Ile	Gln	Glu	Asn	Leu	Asp	Arg
					325					330					335
Gln	Asp	Glu	Ala	Trp	Arg	Arg	Ile	Gln	Glu	Leu	Glu	Arg	Val	Leu	Gln
					340					345					350
Arg	Leu	Gly	Thr	Glu	Arg	Phe	Glu	Glu	Val	Lys	Arg	Arg	Ile	Glu	Glu
					355					360					365
Asn	Asp	Arg	Glu	Glu	Lys	Arg	Lys	Val	Glu	Tyr	Gln	Gln	Phe	Leu	Asp
					370					375					380
Val	Cys	Gly	Gln	His	Lys	Lys	Leu	Leu	Glu	Leu	Ser	Val	Tyr	Asn	Cys
					385					390					395
Asp	Leu	Ala	Met	Arg	Cys	Ile	Gly	Met	Met	Glu	Glu	Leu	Val	Ala	Glu
					405					410					415
Gly	Cys	Ser	Ala	Ile	Lys	Ser	Arg	His	Asp	Lys	Thr	Asn	Glu	Leu	Leu
					420					425					430
Ala	Asp	Leu	Arg	Leu	Gln	Val	His	Gln	Glu	Tyr	Leu	Glu	Ala	Phe	Arg
					435					440					445
Arg	Leu	Tyr	Lys	Thr	Leu	Gly	Gln	Leu	Val	Tyr	Lys	Lys	Glu	Lys	Arg
					450					455					460
Leu	Glu	Glu	Ile	Asp	Arg	Asn	Ile	Arg	Thr	Thr	His	Ile	Gln	Leu	Glu
					465					470					475
Phe	Ala	Ile	Glu	Thr	Phe	Asp	Pro	Asn	Ala	Lys	Lys	His	Ser	Asp	Ala
					485					490					495
Lys	Lys	Glu	Leu	Tyr	Lys	Leu	Arg	Ala	Gln	Val	Glu	Glu	Leu	Glu	
					500					505					510
Met	Leu	Lys	Asp	Lys	Met	Ala	Gln	Ala	Leu	Glu	Met	Phe	Gly	Pro	Thr
					515					520					525
Glu	Asp	Ala	Leu	Asn	Gln	Ala	Gly	Ile	Glu	Phe	Val	His	Pro	Ala	Glu
					530					535					540
Glu	Val	Glu	Asp	Gly	Asn	Leu	Thr	Arg	Arg	Ser	Lys	Met	Val	Glu	Tyr
					545					550					555
Arg	Ala	His	Leu	Ala	Lys	Gln	Glu	Glu	Val	Lys	Ile	Ala	Ala	Glu	Arg
					565					570					575
Glu	Glu	Leu	Lys	Arg	Ser	Lys	Thr	Leu	Gln	Ser	Gln	Gln	Tyr	Arg	Gly
					580					585					590
Lys	Thr	Val	Gln	Gln	Ile	Thr	Gln								
					595					600					

<210> SEQ ID NO 26

<211> LENGTH: 2655

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (2625)..(2625)

<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 26

Cys Phe Ala Ala Arg Thr Ser Ser Ile Ser Gly Cys Glu Cys Val Cys
 1 5 10 15

Ala Ala Gly Gly Tyr Gly Asp Thr Cys Val Pro Ala Ala Val Pro Asp
 20 25 30

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Gly	Leu	Gly	Pro	Leu	Pro	Leu	Pro	Asp	Ala	Asp	Asp	Thr	Glu	Val	Arg
	35						40					45			
Cys	Val	His	Gly	Gly	Ser	Ile	Ser	Pro	Val	Asp	Tyr	Pro	Asp	Pro	Gly
	50					55					60				
Val	Arg	Gly	Leu	Cys	Phe	Val	Asn	Val	Thr	Phe	Thr	Ala	Ala	Ile	Val
65					70					75					80
Leu	Asp	Leu	Ser	Arg	Phe	Asp	Ala	Gln	Gln	Lys	Thr	Leu	Ser	Ile	Thr
				85					90					95	
Leu	Leu	Gln	Cys	Val	Leu	Met	Gly	Leu	Ser	Val	Arg	Gly	Ser	Gly	Ala
			100					105						110	
Arg	Val	His	Val	Asn	Val	Thr	Ser	Ser	Met	Met	Asp	Ser	Gly	Ala	Leu
		115					120						125		
Lys	Phe	Arg	Gly	Asp	Phe	Gly	Ala	Ser	Ser	Gln	Ile	Leu	Val	Ala	Gly
	130					135					140				
Ser	Thr	Leu	Val	Thr	Lys	Ser	Arg	His	Ala	Thr	Ala	Phe	Trp	Glu	Phe
145					150					155					160
Thr	Leu	Gly	Thr	Asn	Ser	Thr	Leu	Leu	Leu	Leu	Asp	Asn	Tyr	Ile	Glu
				165					170					175	
Ala	Asn	Ile	Tyr	Ala	Leu	Ser	Phe	Ser	Asp	Ser	Val	Val	Asp	Gly	Gly
			180					185					190		
Gly	Ile	Ile	Met	Lys	Gly	Asn	Thr	Leu	Arg	Ala	Thr	Gly	Asn	Asp	Asp
		195					200					205			
Gly	Val	Glu	Ser	Ala	Val	Tyr	Ile	Glu	Thr	Phe	Asp	Val	Gly	Asn	Gly
	210					215					220				
Gly	Tyr	Phe	Asp	Val	Glu	Asn	Asn	Thr	Met	Ser	Gly	Ala	Asn	Gly	Ile
225					230					235					240
Tyr	Leu	Leu	Gly	Gly	Thr	Thr	Val	Asn	Ser	Ala	Gly	Leu	Leu	Arg	Val
			245						250					255	
Ala	Asp	Cys	Ser	Phe	Ala	Gly	Ser	Thr	Phe	Pro	Phe	Asp	Ser	Thr	Leu
			260					265					270		
Ile	Tyr	Leu	Asp	Ser	Ser	Val	Thr	Leu	Glu	Gly	Gly	Ala	Gln	Trp	Arg
		275					280					285			
Val	Glu	Gly	Asn	Glu	Val	Ser	Ala	Ala	Ser	Val	Leu	Ile	Met	Pro	Lys
	290					295					300				
Ser	Ala	Tyr	Ile	Ile	Gln	Val	Ser	Gly	Ser	Gly	Thr	Thr	Val	Ala	Leu
305					310					315					320
Ala	His	Asn	Arg	Gln	Val	Asp	Asn	Ser	Tyr	Pro	Phe	Ala	Asp	Leu	Pro
			325						330					335	
Leu	Ser	Lys	Thr	Val	Val	Glu	Ser	Pro	Ala	Arg	Phe	Val	Val	Gly	Cys
			340						345					350	
Asn	Leu	Gln	Gly	Gly	Glu	Glu	Val	Ser	Cys	Asp	Gly	Val	Phe	Pro	Glu
		355					360						365		
Asp	Val	Val	Leu	Phe	Ile	Cys	Gly	Thr	Cys	Asn	Asp	Asp	Ala	Ala	Cys
	370					375						380			
Tyr	Val	Pro	Gly	Thr	Glu	Ser	Val	Asp	Arg	Ser	Ser	Cys	Ser	Cys	Ser
385					390					395					400
Cys	Lys	Glu	Gly	Trp	His	Gly	Ala	Ser	Cys	Leu	Pro	Phe	Glu	Val	Pro
			405						410					415	
Asn	Ala	Val	Val	Pro	Pro	Val	Ala	Glu	Arg	Ala	Val	Asp	Gly	Asp	Thr
			420					425					430		
Ser	Cys	Val	Val	Asn	Gln	Thr	Leu	Lys	Asn	Leu	Thr	Leu	Asn	Met	Trp
		435					440						445		
Lys	Thr	His	His	Cys	Tyr	Val	Gly	Val	Thr	Phe	Ser	Gly	Val	Gly	Ala
	450					455						460			

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Val Leu Thr Phe Phe Leu Asp Arg Met Pro Leu His Leu Leu Ile Asn
 465 470 475 480
 Ile Thr Leu Thr Gly Cys Thr Phe Ser Gly Gly Ala Ala Leu Gln Phe
 485 490 495
 Val Gly Gly Ala Gly Ala Ala Glu Ser Ser Gly Val Leu Ile Arg Val
 500 505 510
 Ser Gln Thr Val Met Arg Ser Ser Val Val Ala Phe Ser Leu Ala Leu
 515 520 525
 Pro Gln His Cys Asp Ile Ala Val Thr Glu Val Asp Ala Leu Gln Ser
 530 535 540
 Ser Lys Leu Lys Leu Gln Glu Ser Ala Asn Asn Met Trp Ser Val Val
 545 550 555 560
 Val Leu His Asp Val Val Leu Ser Ala Ser Ser Leu Leu Val Ser Asn
 565 570 575
 Val Lys Ala His Ser Lys Arg Tyr Gly Ala Phe Gly Leu Tyr Ser Thr
 580 585 590
 Gly Thr Leu Thr Leu Val Arg Gly Ser Ser Leu Tyr Thr Arg Tyr Cys
 595 600 605
 Ser Phe Glu Gly Tyr Thr His Leu Phe Tyr Val Tyr Arg Leu Ser Val
 610 615 620
 Arg Asp His Ser Val Phe Ala Leu Leu Asn Asn Thr Met Ser Ser Gly
 625 630 635 640
 Arg Thr Leu Leu Phe Gln His Ser Arg Phe Ser Val Ser Asp Tyr Ser
 645 650 655
 Val Leu Arg Val Val Gly Asn Ser Gly Phe Val Ser Cys Ala Ile Ser
 660 665 670
 Ala His Ile Pro Trp Val Leu Gln Asn Ser Ser Trp Leu Asp Trp Arg
 675 680 685
 Asp Asn Asp Val Glu Val Gly Ala Met Leu Cys Cys Phe Ser Ser Ala
 690 695 700
 Thr Val Ser Ile Asp Ser Ser Ser Val Val Thr Leu Lys Gly Cys Lys
 705 710 715 720
 Met Gly Ser Thr Gly Leu Ser Val Pro Leu Leu Ser Gln Ser Asp Ala
 725 730 735
 Gly Tyr Arg Phe Val Ala Gly Cys Leu Thr Val Ala Gly Arg Leu Val
 740 745 750
 Thr Thr Ala Ala Glu Leu Glu Leu Asn Gly Ile Thr Asn Val Thr Lys
 755 760 765
 Val Val Ala Cys Gly Gln Cys Thr Lys Asp Gly Asp Cys Phe Ala Pro
 770 775 780
 Leu Thr Thr Ala Val Ile Asp Cys Lys Cys Gln Cys Ala Ala Gly Gly
 785 790 795 800
 His Gly Asp Val Cys Val Pro Ala Pro Met Pro Ala Gly Pro Pro Pro
 805 810 815
 Pro Leu Thr Pro Pro Thr Leu Pro Thr Pro Pro Pro Val Gly Glu Cys
 820 825 830
 Ile Ser Asp Met Val Tyr Pro Glu Val Ala Gln Ala Val Gly Ser Gly
 835 840 845
 Leu Ser Trp Leu Cys Tyr Arg Asn Val Thr Phe Ser Gly Gly Gly Met
 850 855 860
 Ser Leu Thr Val Leu Ile Glu Ala Met Thr Gly Asp Val Ala Asn Val
 865 870 875 880
 Thr Phe Asp Gly Cys Thr Trp Arg Asp Gly Ala Val Leu Leu Leu Leu

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885					890					895					
Gly	Asn	Ala	Tyr	Ala	Ala	Val	Gly	Ser	Leu	Asn	Ile	Val	Val	Thr	Gly
	900							905						910	
Asn	Thr	Phe	Asp	Asp	Ala	Leu	Leu	Ser	Pro	Glu	Gly	Val	Phe	Pro	Pro
	915						920					925			
His	Thr	Asn	Ile	Thr	Ile	Ser	Gly	Asn	Arg	Phe	Thr	Val	Thr	Gly	Leu
	930					935					940				
Ile	Pro	Arg	Ser	Gly	Leu	Asp	Ile	Arg	Arg	Pro	Ser	Cys	Val	Ala	Met
945					950					955					960
Asn	Ala	Leu	Ala	Ile	Ser	Asn	Gly	Ser	Ala	Val	Val	Leu	Ser	Gly	Asn
			965						970					975	
Val	Phe	Gln	Ser	Val	Arg	Ala	Ser	Ser	Ile	Ala	Ile	His	Val	Val	Arg
			980					985				990			
Ser	Ala	Leu	Arg	Val	Ser	Trp	His	Ser	Val	Phe	Ala	Val	Val	Gly	Asn
		995					1000					1005			
Thr	Phe	His	Met	Ala	Gly	Ser	Asp	Gly	Thr	Leu	Ile	His	Leu	Glu	
	1010					1015					1020				
Gly	Thr	Ser	Gln	Ser	Phe	Ser	Leu	Ser	Val	Leu	Asn	Asn	Ser	Ala	
	1025					1030					1035				
Met	Val	Ile	Arg	Gly	Asn	Ala	Val	Ser	Arg	Pro	Val	Arg	Tyr	Val	
	1040					1045					1050				
Ile	Ile	Phe	Val	Trp	Val	Phe	Cys	Val	Glu	Ser	Phe	Ser	Ala	Val	
	1055					1060					1065				
Val	Phe	Gln	Gly	Asn	Asp	Met	Arg	Gly	Ser	Val	Ala	Ala	Phe	Phe	
	1070					1075					1080				
Ser	Gly	Phe	Tyr	Ser	Tyr	Ile	Tyr	Tyr	Asn	Ser	Trp	Leu	Gln	Leu	
	1085					1090					1095				
Ser	Gly	Asn	Phe	Cys	Arg	Glu	Ser	Pro	Ser	Glu	Ala	Phe	Ala	Val	
	1100					1105					1110				
Phe	Asn	Pro	Thr	Val	Asn	Leu	Arg	Asp	Ser	Thr	Met	Ser	Val	Ser	
	1115					1120					1125				
Gly	Asn	Gln	Phe	Met	Phe	Gly	Thr	Gly	Thr	Pro	Thr	Leu	Ile	Lys	
	1130					1135					1140				
Ile	Pro	Lys	Arg	Ser	Ser	Asp	Leu	Thr	Asn	Gly	Thr	Ile	Val	Ala	
	1145					1150					1155				
Ala	Cys	Asn	Thr	Gly	Ser	Asp	Glu	Glu	Glu	Ala	Asn	Tyr	Val	Ile	
	1160					1165					1170				
Pro	Ser	Val	Tyr	Asn	Ala	Thr	Ile	Leu	Thr	Cys	Ser	Asp	Pro	Cys	
	1175					1180					1185				
Thr	Leu	Ala	Ala	Ser	Cys	Phe	Pro	Ala	Tyr	Thr	Thr	Thr	Ala	Ser	
	1190					1195					1200				
Ser	Asp	Gly	Cys	Ala	Cys	Ala	Cys	Ala	Glu	Gly	Gly	His	Gly	Asp	
	1205					1210					1215				
Ala	Cys	Leu	Pro	Val	Ala	Val	Pro	Glu	Ala	Pro	Ser	Thr	Asp	Gly	
	1220					1225					1230				
Ala	Asp	Leu	Cys	Val	Arg	Asp	Val	Arg	Val	Asp	Val	Glu	Val	Asn	
	1235					1240					1245				
Ala	Val	Leu	Gly	Thr	Ser	Val	Val	Cys	Tyr	Val	Gly	Val	Thr	Phe	
	1250					1255					1260				
Ala	Ala	Asp	Val	Val	Val	Asp	Val	Glu	Ser	Met	Ser	Gly	Ser	Val	
	1265					1270					1275				
Arg	Asn	Val	Thr	Leu	Ala	Asn	Cys	Thr	Phe	Val	Gly	Gly	Ala	Ser	
	1280					1285					1290				

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Leu Tyr 1295	Val Val Gly Trp	Arg 1300	Ser Asp	Pro Pro	Ala 1305	Gly Glu Arg
Ala Asp 1310	Ala Leu Ile Ser	Gly 1315	Leu Glu Ser	Arg Ser	Gly Gly Gly	
Val Leu 1325	Val Ala Asn Arg	Phe 1330	Pro Pro Gly	Ser Arg	Val Thr Val	
Val Asp 1340	Ser Val Leu Ile	Ala 1345	Glu Lys Arg	Val Ala	Tyr Arg Gly	
Ala Tyr 1355	Gly Leu Gly Asp	Ala 1360	Ser Ala Cys	Leu Val	Val His Asn	
Val Asn 1370	Leu Thr Gly Ser	Val 1375	Leu Thr Ile	Ala Arg	Thr His Val	
Ala Ala 1385	Val Phe Arg Asp	Ala 1390	Val Gly Val	Leu Val	Phe Gly Asp	
Val Ala 1400	Leu Ser Ser Arg	Gly 1405	Ala Leu Tyr	Leu Asp	Gly Leu Leu	
Val Gln 1415	Thr Ala Leu Gly	Leu 1420	Cys Val Ser	Val Glu	Gly Gly Val	
Ala Ala 1430	Ser Gly Gly Ser	Val 1435	Val Ala Phe	Val Asp	Ser Asp Phe	
Leu Leu 1445	Cys Lys His Ala	Val 1450	Ser Val Arg	Glu Ala	Val Ser Val	
Ser Gly 1460	Ala Ala Val Ala	Leu 1465	Val Arg Ser	Asp Phe	Ser Ser Thr	
Glu Asp 1475	Tyr Ala Val Ala	Phe 1480	Tyr Ser Thr	Val Ser	Leu Ala Asp	
Gly Ser 1490	Met Leu Leu Ala	Lys 1495	Gly Asn Val	His Asp	Gly Val Ser	
Arg Glu 1505	Met Leu Tyr Ala	Ala 1510	Gly Ala Val	Thr Ala	Ala Gly Ser	
Thr Leu 1520	Ser Phe Val Arg	Asn 1525	Arg Ala Leu	Leu Pro	Arg Met Leu	
Ser Val 1535	Ser Leu Ser Leu	Ser 1540	Ala Gly Ala	His Leu	Arg Val Ala	
Cys Asn 1550	Asp Ala Gly Gly	Arg 1555	Phe Leu Ser	Thr Ala	Glu Glu Tyr	
Ala Ala 1565	Ala Gly Phe Gly	Asp 1570	Ala Gly Ser	Ile Asp	Phe Ala Gly	
Cys Asp 1580	Ala Cys Asp Arg	Asp 1585	Thr His Cys	Tyr Ala	Pro Gly Thr	
Ala Ser 1595	Ala Ser Met Arg	Asn 1600	Gly Val Cys	Val Cys	Val Cys Gly	
Ser Gly 1610	Gly Tyr Gly Glu	Ala 1615	Cys Val Pro	Val Gly	Ala Pro Ala	
Leu Pro 1625	Pro Ala Val Gly	Thr 1630	Ala Pro Ser	Val Phe	Val Arg Glu	
Gly Val 1640	Thr Val Arg Ser	Val 1645	Phe Val Val	Pro Ala	Gly Ala Ser	
Glu Val 1655	Thr Leu Arg His	Val 1660	Val Leu Asp	Gly Val	Ser Pro Val	
Leu Tyr 1670	Val Pro Trp Met	Ala 1675	Arg Asp Gly	Met Arg	Ile Val Val	
Gln Asn 1685	Val Ser Leu Leu	Asn 1690	Gly Ala Val	Leu Tyr	Val Met Gly	

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Gly	Gly	Gly	Val	Leu	Arg	Gly	Ala	Gly	Ala	Ala	Gly	Ser	Asp	Glu
1700						1705					1710			
Ser	Gly	Pro	Val	Glu	Leu	Ser	Val	Cys	Asp	Val	Glu	Ala	Leu	Asn
1715						1720					1725			
Gly	Ala	Leu	Val	Leu	Ser	Gly	Thr	Phe	Pro	Pro	Gly	Ser	Val	Leu
1730						1735					1740			
Thr	Val	Thr	Asp	Ser	Leu	Leu	Val	Ala	Ala	Arg	Pro	Thr	Pro	Leu
1745						1750					1755			
Val	Cys	Leu	Pro	Asp	Ser	Gln	Ser	Ser	Pro	Tyr	Ala	Pro	Val	Leu
1760						1765					1770			
Val	Leu	Ser	Gly	Leu	Arg	Leu	Val	Arg	Ser	Val	Leu	Val	Val	Ser
1775						1780					1785			
Gly	Val	Ala	Leu	Val	Thr	Val	Met	Thr	Gly	Gly	Arg	Thr	Val	Val
1790						1795					1800			
Val	Asp	Gly	Ala	Val	Leu	Glu	Leu	Val	Gly	Gly	Gly	Val	Ala	Leu
1805						1810					1815			
Asp	Ala	Ala	Val	Leu	Gly	Gly	Glu	Tyr	Ala	Leu	Tyr	Ala	Ser	Ala
1820						1825					1830			
Arg	Val	Val	Ala	Ser	Glu	Gly	Ala	Val	Leu	Arg	Val	Ser	Gly	Ser
1835						1840					1845			
Gln	Val	Tyr	Ala	Ala	His	Gly	Leu	Val	Phe	Gly	Ser	Gly	Val	Val
1850						1855					1860			
Ala	Asn	Ala	Ser	Thr	Val	Val	Val	Asn	Asp	Asn	Ala	Gly	Val	Leu
1865						1870					1875			
Thr	Asp	Gly	Ala	Leu	Leu	Val	Leu	Arg	Gly	Ser	Ala	Ser	Phe	Val
1880						1885					1890			
Ser	Gly	Ser	Trp	Leu	Ser	Val	Arg	Gly	Asn	Ser	Ile	Ser	Gly	Arg
1895						1900					1905			
Leu	Leu	Ser	Val	Pro	Ser	Tyr	Pro	Arg	Ser	Ala	Asp	Leu	Val	Gln
1910						1915					1920			
Ser	Thr	Leu	Thr	Phe	His	Gly	Asn	Ala	Gly	Ser	Gly	Thr	Val	Val
1925						1930					1935			
Met	Asp	Gly	Thr	Val	Ala	Leu	Gly	Gly	Ala	Gly	Arg	Arg	Phe	Val
1940						1945					1950			
Val	Gly	Cys	Leu	Thr	Leu	Asn	Gly	Leu	Thr	Leu	Arg	Pro	Ile	Asp
1955						1960					1965			
Tyr	Arg	Ser	Ala	Gly	Ile	Ile	Gly	Glu	Phe	Arg	Pro	Val	Ala	Cys
1970						1975					1980			
Gly	Val	Cys	Asp	Ala	Glu	Val	Arg	Cys	Phe	Ala	Ala	Ala	Thr	Arg
1985						1990					1995			
Ala	Met	Thr	Val	Ser	Cys	Arg	Cys	Arg	Cys	Ala	Glu	Gly	Gly	Tyr
2000						2005					2010			
Gly	Arg	Asp	Cys	Leu	Pro	Val	Tyr	Leu	Pro	His	Val	Asp	Gly	Cys
2015						2020					2025			
Asn	Arg	Thr	Pro	Gly	Met	Pro	Leu	Leu	Ser	His	Thr	Ala	Thr	Leu
2030						2035					2040			
Thr	Glu	Thr	Arg	Ser	Pro	Thr	Pro	Thr	Trp	Thr	Pro	Ser	Pro	Thr
2045						2050					2055			
Pro	Ser	Met	Ser	Ala	Thr	His	Tyr	Ser	Pro	Thr	Arg	Tyr	Gly	Pro
2060						2065					2070			
Thr	Glu	Thr	Leu	Gln	Val	Thr	Glu	Thr	Val	Ala	Leu	Ser	Pro	Thr
2075						2080					2085			
Arg	Thr	Pro	Thr	Ala	Ser	Val	Ser	Ser	Thr	Leu	Trp	Trp	Ser	Asp

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2090					2095					2100				
Val	Ala	Cys	Pro	Thr	Leu	Thr	Val	Thr	Thr	Thr	Ala	Ala	Gly	Gly
2105						2110					2115			
Ser	Leu	Thr	Gln	Asn	Asp	Ile	Arg	Gly	Gly	Gly	Ser	Ala	Val	Pro
2120						2125					2130			
Thr	Arg	Leu	Met	Val	Ala	Leu	Pro	Pro	Pro	Phe	Arg	Trp	Ala	Arg
2135						2140					2145			
Asp	Pro	Gln	Leu	Gly	Thr	His	Leu	Ser	Phe	Val	Pro	Val	Ser	Thr
2150						2155					2160			
Ala	Gln	Pro	Ser	Gly	Phe	Gly	Gly	Pro	Trp	Gly	Ala	Met	Leu	Arg
2165						2170					2175			
Asn	Ala	Thr	Trp	Val	Arg	Asn	Ala	Thr	Asn	Pro	Ser	Thr	Val	Leu
2180						2185					2190			
Glu	Leu	Ala	Val	Pro	Val	His	Arg	Gly	Tyr	Phe	Ile	Val	Ala	Asp
2195						2200					2205			
Glu	Thr	Ile	Val	Ile	Arg	Cys	Gly	Ala	Ala	Ala	Val	Ser	Gly	Gly
2210						2215					2220			
Cys	Lys	Gly	Val	Leu	Leu	Gly	Ser	Phe	Thr	Ile	Arg	Ser	Asn	Thr
2225						2230					2235			
Leu	Pro	Ala	Ala	Ala	Ser	Ala	Leu	Ser	Ala	Ile	Thr	Gly	Val	Val
2240						2245					2250			
Ala	Gly	Ala	Ala	Ala	Val	Ala	Val	Val	Val	Thr	Gly	Gly	Leu	Gly
2255						2260					2265			
Ser	Val	Leu	Glu	Met	Gln	Ala	Leu	Gly	Val	Phe	Ala	Arg	Met	Ser
2270						2275					2280			
Cys	Ala	Ser	Ala	Gln	Glu	Arg	Ala	Ser	Thr	Ala	Ala	Leu	Pro	Tyr
2285						2290					2295			
Phe	Leu	Ser	Val	Phe	Ala	Ala	Leu	Asp	Pro	Leu	Trp	Met	Val	Val
2300						2305					2310			
Gly	Asn	Ala	Leu	Leu	Ala	Ala	Val	Phe	Gly	Cys	Val	His	Cys	Gly
2315						2320					2325			
Val	Thr	Ala	Ala	Phe	Gln	Arg	Trp	Arg	Gly	Val	Asp	Ala	Ala	Ser
2330						2335					2340			
Ala	Trp	Ala	Ala	Met	Arg	Phe	Pro	Ser	Leu	Thr	Tyr	Val	Val	Ala
2345						2350					2355			
His	Ala	Met	His	Leu	Gly	Ile	Phe	Phe	Gly	Ser	Val	Leu	Ala	Leu
2360						2365					2370			
Ala	Met	Pro	Gly	Ala	Arg	Val	Gln	His	Arg	Val	Ile	Gly	Ala	Val
2375						2380					2385			
Gly	Val	Leu	Tyr	Gly	Val	Ala	Phe	Pro	Ala	Gly	Val	Cys	Tyr	Leu
2390						2395					2400			
Ile	Ala	Arg	His	Val	Gly	Ala	Ser	Phe	Thr	Arg	Tyr	Trp	Gln	Phe
2405						2410					2415			
Ser	Arg	Lys	Pro	Leu	His	Glu	Arg	Leu	Leu	Tyr	Pro	Val	Gly	Cys
2420						2425					2430			
Trp	His	Pro	Ala	Ala	Gln	Gln	Arg	Met	Tyr	Gly	Gly	Met	Leu	Thr
2435						2440					2445			
Asn	Met	Arg	Gly	Ser	Arg	Val	Tyr	Trp	Cys	Val	Phe	Gln	Leu	Ser
2450						2455					2460			
Val	Leu	Cys	Val	Val	Gly	Leu	Ile	Ala	Ala	Val	His	Pro	Pro	Val
2465						2470					2475			
Gly	Gly	Cys	His	Val	Gln	Tyr	Phe	Cys	Met	Ala	Ala	Val	Leu	Leu
2480						2485					2490			

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Ala Gly Ala Gly Val Val Ala Phe Thr Asn Met Met Arg Ser Ala
 2495 2500 2505

Phe Leu Thr Val Met His Ala Ala Gly Phe Val Leu Leu Ala Ala
 2510 2515 2520

Leu Cys Leu Val Ser Ala Ala Asn His Leu Ala Pro Ser Asp Gly
 2525 2530 2535

Val Ala Arg Ala Tyr Ala Ala Met Val Leu Leu Leu Thr Thr Val
 2540 2545 2550

Leu Leu Ala Ile Thr Val Tyr Ser Val Val Val Trp Tyr Ala Glu
 2555 2560 2565

Asp Arg His Trp Gln Asp Leu Arg Glu Pro Gln Arg Cys Gly Leu
 2570 2575 2580

Glu Ala Leu Leu Arg Asp Asp Glu Glu Ser Asp Glu Glu Thr Gln
 2585 2590 2595

Lys Leu His Asp Met Thr Ser Leu Ser Tyr Ala Ser Glu Thr Thr
 2600 2605 2610

Gly Ala Ser Ser Tyr Arg Pro Pro Ala Pro Pro Xaa Gln Ser Val
 2615 2620 2625

Thr Gly Asp Thr Arg Ser Asp Val Leu Ser Pro Leu Asp Arg Ala
 2630 2635 2640

Ser Ser Ala Ser Gly Lys Ile Asp His Ala Leu Leu
 2645 2650 2655

<210> SEQ ID NO 27
 <211> LENGTH: 152
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 27

Met Ser Ile Glu Ser Ala Phe Tyr Ala Phe Ala Ser Phe Gly Gly Ala
 1 5 10 15

Pro Thr Lys Glu Met Asp Asn Ala His Phe Ser Lys Met Leu Lys Glu
 20 25 30

Thr Lys Val Ile Gly Lys Gln Phe Thr Ser Thr Asp Ala Asp Leu Leu
 35 40 45

Phe Asn Lys Val Lys Ala Lys Gly Ala Arg Lys Ile Thr Leu Ser Asp
 50 55 60

Phe Val Asp Lys Ala Val Pro Glu Ile Ala Ser Lys Leu Lys Lys Ser
 65 70 75 80

Val Glu Glu Leu Ile Ala Asp Ile Ser Ser Cys Ser Pro Glu Ala Arg
 85 90 95

Ala Thr Lys Ala Asp Ala Val Lys Phe His Asp Asp Lys Asn Met Tyr
 100 105 110

Thr Gly Val Tyr Lys Ala Gly Gly Pro Thr Asn Val Asp Arg Asn Ser
 115 120 125

Gly Ser Leu Ser Gly Val Val Asp Arg Arg Val Ala Gln Thr Asp Val
 130 135 140

Cys Gly Thr Thr Ala Ser Gln Lys
 145 150

<210> SEQ ID NO 28
 <211> LENGTH: 2106
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (2076)..(2076)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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<400> SEQUENCE: 28

Glu Ser Ala Asn Asn Met Trp Ser Val Val Val Leu His Asp Val Val
 1 5 10 15
 Leu Ser Ala Ser Ser Leu Leu Val Ser Asn Val Lys Ala His Ser Lys
 20 25 30
 Arg Tyr Gly Ala Phe Gly Leu Tyr Ser Thr Gly Thr Leu Thr Leu Val
 35 40 45
 Arg Gly Ser Ser Leu Tyr Thr Arg Tyr Cys Ser Phe Glu Gly Tyr Thr
 50 55 60
 His Leu Phe Tyr Val Tyr Arg Leu Ser Val Arg Asp His Ser Val Phe
 65 70 75 80
 Ala Leu Leu Asn Asn Thr Met Ser Ser Gly Arg Thr Leu Leu Phe Gln
 85 90 95
 His Ser Arg Phe Ser Val Ser Asp Tyr Ser Val Leu Arg Val Val Gly
 100 105 110
 Asn Ser Gly Phe Val Ser Cys Ala Ile Ser Ala His Ile Pro Trp Val
 115 120 125
 Leu Gln Asn Ser Ser Trp Leu Asp Trp Arg Asp Asn Asp Val Glu Val
 130 135 140
 Gly Ala Met Leu Cys Cys Phe Ser Ser Ala Thr Val Ser Ile Asp Ser
 145 150 155 160
 Ser Ser Val Val Thr Leu Lys Gly Cys Lys Met Gly Ser Thr Gly Leu
 165 170 175
 Ser Val Pro Leu Leu Ser Gln Ser Asp Ala Gly Tyr Gln Phe Val Ala
 180 185 190
 Gly Cys Leu Thr Val Ala Gly Arg Leu Val Thr Thr Ala Ala Glu Leu
 195 200 205
 Glu Leu Asn Gly Ile Thr Asn Val Thr Thr Val Ala Ala Cys Gly Gln
 210 215 220
 Cys Thr Lys Asp Gly Asp Cys Phe Ala Pro Leu Thr Thr Ala Val Ile
 225 230 235 240
 Asp Cys Lys Cys Gln Cys Ala Ala Gly Gly His Gly Asp Val Cys Val
 245 250 255
 Pro Ala Pro Met Pro Ala Gly Pro Pro Pro Pro Leu Thr Pro Pro Thr
 260 265 270
 Leu Pro Thr Pro Pro Pro Pro Pro Val Gly Glu Cys Ile Ser Asp Met
 275 280 285
 Val Tyr Pro Glu Val Ala Gln Ala Val Gly Ser Gly Leu Ser Trp Leu
 290 295 300
 Cys Tyr Arg Asn Val Thr Phe Ser Gly Gly Gly Met Ser Leu Thr Val
 305 310 315 320
 Leu Ile Glu Ala Met Thr Gly Asp Val Ala Asn Val Val Phe Asp Gly
 325 330 335
 Cys Thr Trp Arg Asp Gly Ala Leu Leu Leu Leu Gly Asn Ala Tyr
 340 345 350
 Ala Ala Val Gly Ser Leu Asn Ile Val Val Thr Gly Asn Thr Phe Asp
 355 360 365
 Asp Ala Leu Leu Ser Pro Glu Gly Val Phe Pro Pro His Thr Asn Ile
 370 375 380
 Thr Ile Ser Gly Asn Arg Phe Thr Val Thr Arg Leu Ile Pro Arg Ser
 385 390 395 400
 Gly Leu Asp Ile Met Arg Pro Ser Cys Val Ala Met Asn Ala Leu Ala
 405 410 415

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Ile Ser Asn Gly Ser Ala Val Val Leu Ser Gly Asn Val Phe Gln Ser
 420 425 430
 Val Arg Ala Ser Ser Ile Ala Ile His Val Val Arg Ser Ala Leu Arg
 435 440 445
 Val Ser Trp His Ser Val Phe Ala Val Val Gly Asn Thr Phe His Met
 450 455 460
 Ala Gly Ser Asp Gly Thr Pro Ile Asn Leu Glu Gly Thr Ser Gln Ser
 465 470 475 480
 Phe Ser Leu Ser Val Leu Asn Asn Ser Ala Met Val Ile Arg Gly Asn
 485 490 495
 Ala Val Ser Arg Pro Val Arg Tyr Phe Leu Leu Leu Ile Leu Ala Leu
 500 505 510
 His Val Glu Ser Phe Ser Ala Val Val Phe Gln Gly Asn Asp Met Arg
 515 520 525
 Gly Ser Val Ala Ala Phe Leu Ser Gly Phe Tyr Ser Tyr Ile Tyr Tyr
 530 535 540
 Asn Ser Trp Leu Gln Leu Ser Gly Asn Phe Cys Arg Glu Ser Pro Ser
 545 550 555 560
 Glu Ala Phe Ala Val Phe Asn Pro Thr Val Asn Leu Arg Asp Ser Thr
 565 570 575
 Val Ser Val Ser Gly Asn Gln Phe Met Phe Gly Thr Gly Thr Pro Thr
 580 585 590
 Leu Ile Lys Ile Pro Lys Arg Ser Ser Asp Leu Thr Asn Gly Thr Ile
 595 600 605
 Val Ala Ala Cys Asn Thr Gly Ser Asp Glu Glu Glu Ala Asn Tyr Val
 610 615 620
 Ile Pro Ser Val Tyr Asn Ala Thr Ile Leu Thr Cys Ser Asp Pro Cys
 625 630 635 640
 Thr Leu Ala Ala Ser Cys Phe Pro Ala Tyr Thr Thr Thr Ala Ser Ser
 645 650 655
 Asp Gly Cys Ala Cys Ala Cys Ala Glu Gly Gly His Gly Asp Ala Cys
 660 665 670
 Leu Pro Val Ala Val Pro Glu Pro Pro Ser Thr Asp Gly Ala Asp Leu
 675 680 685
 Cys Val Arg Glu Val Arg Val Gly Val Glu Val Ile Ala Gly Leu Gly
 690 695 700
 Thr Ser Val Val Cys Tyr Val Gly Val Thr Phe Ala Ala Asp Val Val
 705 710 715 720
 Val Asp Val Glu Ser Met Ser Gly Ser Val Arg Asn Val Thr Leu Val
 725 730 735
 Asn Cys Thr Phe Val Gly Gly Ala Ser Leu Tyr Val Val Gly Trp Arg
 740 745 750
 Ser Asp Pro Thr Ala Gly Glu Arg Ala Asp Ala Leu Ile Ser Gly Leu
 755 760 765
 Glu Ser Arg Ser Gly Gly Gly Val Leu Val Ala Asn Arg Phe Pro Pro
 770 775 780
 Gly Ser Arg Val Thr Leu Val Asp Ser Val Leu Ile Ala Glu Ala Arg
 785 790 795 800
 Val Ala Tyr Arg Gly Ala Tyr Asp Leu Gly Asp Leu Ser Ala Cys Leu
 805 810 815
 Val Val His Ser Val Asn Leu Thr Gly Ser Val Leu Thr Ile Ala Arg
 820 825 830
 Thr His Val Ala Ala Val Phe Arg Asp Ala Val Gly Val Leu Phe Phe

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835				840				845							
Gly	Gly	Val	Ala	Leu	Ser	Ser	Arg	Gly	Ala	Leu	Tyr	Val	Asp	Gly	Leu
850						855						860			
Leu	Val	Gln	Thr	Val	Leu	Gly	Leu	Cys	Val	Ser	Val	Glu	Asp	Gly	Val
865						870				875					880
Ala	Val	Ser	Gly	Gly	Ser	Val	Val	Ala	Phe	Val	Asp	Ser	Asp	Phe	Leu
						885				890				895	
Leu	Cys	Lys	His	Ala	Val	Ser	Val	Arg	Gly	Ala	Val	Gly	Val	Ser	Gly
						900				905				910	
Ser	Ala	Val	Ala	Phe	Met	Arg	Ser	Glu	Phe	Leu	Ser	Thr	Glu	Asp	Tyr
						915				920				925	
Ala	Val	Ala	Phe	Tyr	Ser	Thr	Leu	Ser	Leu	Ala	Asp	Gly	Ser	Met	Leu
930						935						940			
Leu	Ala	Lys	Gly	Asn	Val	His	Asp	Gly	Val	Ser	Arg	Glu	Met	Ile	Tyr
945						950					955				960
Ala	Ala	Gly	Ala	Val	Thr	Ala	Ala	Gly	Ser	Thr	Leu	Ser	Phe	Val	Arg
						965				970				975	
Asn	Arg	Ala	Leu	Leu	Pro	Arg	Met	Leu	Ser	Leu	Ser	Leu	Ser	Leu	Ala
						980				985				990	
Ala	Gly	Ala	His	Leu	Arg	Val	Ala	Cys	Asn	Asp	Ala	Gly	Gly	Arg	Val
						995				1000				1005	
Leu	Ser	Thr	Ala	Glu	Glu	Tyr	Ala	Ala	Ala	Ala	Gly	Phe	Gly	Asp	Ala
1010						1015								1020	
Gly	Arg	Ile	Asp	Val	Ala	Gly	Cys	Asp	Asp	Cys	Asp	Arg	Asp	Thr	
1025						1030						1035			
His	Cys	Tyr	Ala	Pro	Gly	Thr	Ala	Ser	Ala	Thr	Met	Arg	Asn	Gly	
1040						1045						1050			
Val	Cys	Val	Cys	Ala	Cys	Gly	Ser	Gly	Gly	Tyr	Gly	Glu	Ala	Cys	
1055						1060						1065			
Val	Pro	Val	Gly	Ala	Pro	Ala	Leu	Pro	Pro	Ala	Val	Gly	Thr	Ala	
1070						1075						1080			
Ser	Ser	Leu	Phe	Phe	Arg	Glu	Gly	Val	Thr	Val	Arg	Ser	Val	Phe	
1085						1090						1095			
Val	Val	Pro	Ala	Gly	Ala	Ser	Glu	Val	Thr	Leu	Arg	His	Val	Val	
1100						1105						1110			
Leu	Asp	Gly	Val	Ser	Pro	Val	Leu	Tyr	Val	Pro	Trp	Met	Ala	Arg	
1115						1120						1125			
Asp	Gly	Val	Arg	Ile	Val	Val	Gln	Ser	Val	Ser	Leu	Arg	Asn	Gly	
1130						1135						1140			
Ala	Val	Leu	Tyr	Val	Met	Gly	Gly	Gly	Ala	Leu	Arg	Gly	Ala	Val	
1145						1150						1155			
Ala	Ala	Val	Ser	Asp	Glu	Ser	Gly	Pro	Val	Glu	Leu	Ser	Val	Cys	
1160						1165						1170			
Asp	Val	Glu	Ala	Leu	Asn	Gly	Ala	Leu	Val	Leu	Ser	Gly	Thr	Phe	
1175						1180						1185			
Pro	Pro	Gly	Ser	Val	Leu	Thr	Val	Thr	Asp	Thr	Leu	Leu	Val	Ala	
1190						1195						1200			
Ala	Arg	Pro	Thr	Pro	Leu	Val	Cys	Leu	Pro	Asp	Ser	Gln	Phe	Ser	
1205						1210						1215			
Pro	Tyr	Ala	Pro	Val	Leu	Val	Leu	Ser	Gly	Leu	Arg	Leu	Val	Arg	
1220						1225						1230			
Ser	Val	Leu	Val	Val	Ser	Gly	Val	Ala	Leu	Val	Thr	Val	Met	Thr	
1235						1240						1245			

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Gly	Gly	Arg	Thr	Val	Val	Val	Asp	Gly	Ala	Val	Leu	Glu	Leu	Val
1250						1255					1260			
Gly	Gly	Gly	Val	Ala	Leu	Asp	Ala	Ala	Val	Leu	Gly	Gly	Glu	Tyr
1265						1270					1275			
Ala	Leu	Tyr	Ala	Ser	Ala	Arg	Val	Val	Ala	Leu	Glu	Gly	Ala	Val
1280						1285					1290			
Leu	Arg	Val	Ser	Gly	Ser	Gln	Val	Tyr	Ala	Ala	His	Gly	Leu	Val
1295						1300					1305			
Phe	Asp	Ser	Gly	Val	Met	Ala	Asn	Ala	Ser	Thr	Val	Val	Val	Asn
1310						1315					1320			
Asp	Asn	Ala	Gly	Val	Leu	Thr	Asp	Gly	Ala	Leu	Leu	Glu	Leu	Arg
1325						1330					1335			
Gly	Ser	Ala	Ser	Phe	Val	Ser	Gly	Ser	Trp	Leu	Ser	Val	Arg	Gly
1340						1345					1350			
Asn	Ser	Ile	Ser	Gly	Arg	Leu	Leu	Ser	Val	Pro	Ser	Tyr	Pro	Arg
1355						1360					1365			
Ser	Ala	Asp	Leu	Val	Gln	Ser	Thr	Leu	Thr	Phe	His	Gly	Asn	Ala
1370						1375					1380			
Gly	Ser	Gly	Thr	Val	Val	Met	Asp	Gly	Thr	Val	Ala	Leu	Gly	Gly
1385						1390					1395			
Ala	Gly	Arg	Arg	Phe	Val	Val	Gly	Cys	Leu	Thr	Leu	Asn	Gly	Gln
1400						1405					1410			
Ala	Leu	Arg	Pro	Ile	Asp	Tyr	Arg	Ser	Ala	Gly	Ile	Ile	Gly	Glu
1415						1420					1425			
Phe	Arg	Pro	Val	Ala	Cys	Gly	Val	Cys	Asp	Ala	Glu	Val	Arg	Cys
1430						1435					1440			
Phe	Ala	Ala	Ala	Thr	Arg	Ala	Met	Thr	Val	Ser	Cys	Arg	Cys	Arg
1445						1450					1455			
Cys	Ala	Glu	Gly	Gly	Tyr	Gly	Arg	Asp	Cys	Leu	Pro	Val	Tyr	Leu
1460						1465					1470			
Pro	His	Val	Asp	Gly	Cys	Asn	Cys	Thr	Pro	Gly	Met	Pro	Leu	Leu
1475						1480					1485			
Ser	His	Thr	Ala	Thr	Leu	Thr	Glu	Thr	Arg	Ser	Pro	Thr	Pro	Thr
1490						1495					1500			
Trp	Thr	Pro	Asn	Pro	Thr	Pro	Ser	Met	Ser	Ala	Thr	His	Tyr	Ser
1505						1510					1515			
Pro	Thr	Gln	Tyr	Gly	Pro	Thr	Lys	Thr	Leu	Gln	Met	Thr	Glu	Thr
1520						1525					1530			
Val	Ala	Leu	Ser	Pro	Thr	Arg	Thr	Pro	Thr	Ala	Ser	Val	Ser	Ser
1535						1540					1545			
Thr	Leu	Trp	Trp	Ser	Asp	Val	Ala	Cys	Pro	Thr	Leu	Thr	Val	Thr
1550						1555					1560			
Thr	Thr	Ala	Ala	Gly	Gly	Ser	Leu	Thr	Gln	Asn	Asp	Ile	Arg	Gly
1565						1570					1575			
Gly	Gly	Ser	Ala	Val	Pro	Thr	Arg	Leu	Met	Val	Ala	Leu	Pro	Pro
1580						1585					1590			
Pro	Phe	Arg	Trp	Ala	Ser	Asp	Pro	Gln	Leu	Gly	Thr	His	Leu	Ser
1595						1600					1605			
Phe	Val	Pro	Val	Ser	Thr	Ala	Gln	Pro	Ser	Gly	Phe	Gly	Gly	Pro
1610						1615					1620			
Trp	Gly	Ala	Met	Leu	Arg	Asn	Ala	Thr	Trp	Val	Arg	Asn	Ala	Thr
1625						1630					1635			
Asn	Pro	Phe	Thr	Val	Val	Glu	Leu	Ala	Val	Pro	Val	His	Arg	Gly
1640						1645					1650			

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Tyr	Phe	Ile	Val	Ala	Asp	Glu	Thr	Ile	Val	Ile	Arg	Cys	Gly	Ala
1655						1660					1665			
Ala	Ala	Val	Ser	Gly	Gly	Cys	Lys	Gly	Ala	Leu	Leu	Gly	Ser	Phe
1670						1675					1680			
Thr	Ile	Arg	Ser	Asn	Thr	Pro	Pro	Ala	Val	Ala	Ser	Ala	Leu	Ser
1685						1690					1695			
Ala	Ile	Thr	Gly	Val	Val	Ala	Gly	Ala	Ala	Ala	Val	Ala	Val	Val
1700						1705					1710			
Val	Thr	Gly	Gly	Leu	Gly	Ser	Ile	Leu	Glu	Met	Gln	Ala	Leu	Gly
1715						1720					1725			
Val	Phe	Ala	Arg	Met	Ser	Cys	Ala	Ser	Ala	Gln	Glu	Arg	Ala	Ser
1730						1735					1740			
Thr	Ala	Ala	Leu	Pro	Tyr	Phe	Leu	Ser	Val	Phe	Ala	Ala	Leu	Asp
1745						1750					1755			
Pro	Leu	Trp	Met	Val	Val	Gly	Asn	Ala	Leu	Leu	Ala	Ala	Val	Phe
1760						1765					1770			
Gly	Cys	Val	His	Cys	Gly	Val	Thr	Ala	Ala	Phe	Gln	Arg	Trp	Arg
1775						1780					1785			
Gly	Val	Asp	Ala	Ala	Ser	Ala	Trp	Ala	Ala	Met	Arg	Phe	Pro	Ser
1790						1795					1800			
Leu	Thr	Tyr	Val	Val	Ala	His	Ala	Met	His	Leu	Gly	Val	Phe	Phe
1805						1810					1815			
Gly	Ser	Val	Leu	Ala	Leu	Ala	Val	Pro	Gly	Ala	Arg	Val	Gln	His
1820						1825					1830			
Arg	Val	Ile	Gly	Val	Val	Gly	Val	Leu	Tyr	Gly	Val	Ala	Phe	Pro
1835						1840					1845			
Ala	Gly	Val	Cys	Tyr	Leu	Ile	Ala	Arg	His	Met	Gly	Ala	Ser	Phe
1850						1855					1860			
Glu	Arg	Tyr	Trp	Gln	Phe	Ser	Arg	Lys	Pro	Leu	His	Glu	Arg	Leu
1865						1870					1875			
Leu	Tyr	Pro	Val	Gly	Cys	Trp	His	Pro	Ala	Ala	Gln	Gln	Arg	Met
1880						1885					1890			
Tyr	Gly	Gly	Met	Leu	Thr	Thr	Met	Arg	Gly	Ser	Arg	Val	Tyr	Trp
1895						1900					1905			
Cys	Val	Phe	Gln	Leu	Ser	Val	Leu	Cys	Val	Val	Gly	Leu	Ile	Ala
1910						1915					1920			
Ala	Val	His	Pro	Pro	Val	Gly	Gly	Cys	His	Val	Gln	Tyr	Phe	Cys
1925						1930					1935			
Met	Ala	Thr	Leu	Leu	Leu	Ala	Gly	Ala	Gly	Val	Val	Ala	Phe	Thr
1940						1945					1950			
Asn	Met	Met	Arg	Ser	Ala	Phe	Leu	Thr	Val	Met	His	Ala	Ala	Gly
1955						1960					1965			
Phe	Val	Leu	Leu	Ala	Ala	Leu	Cys	Leu	Val	Ser	Ala	Ala	Asn	His
1970						1975					1980			
Leu	Ala	Pro	Ser	Asp	Gly	Val	Ala	Arg	Ala	Tyr	Ala	Ala	Ile	Val
1985						1990					1995			
Leu	Leu	Leu	Thr	Thr	Val	Leu	Leu	Ala	Ile	Thr	Val	Tyr	Ser	Val
2000						2005					2010			
Val	Val	Trp	Tyr	Ala	Glu	Asp	Arg	His	Trp	Gln	Glu	Leu	Arg	Glu
2015						2020					2025			
Pro	Arg	Arg	Gly	Gly	Leu	Glu	Ala	Leu	Leu	Arg	Asp	Asp	Glu	Asp
2030						2035					2040			
Ser	Asp	Glu	Glu	Thr	Gln	Lys	Leu	His	Asp	Met	Thr	Ser	Leu	Ser

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2045 2050 2055

Tyr Ala Ser Glu Thr Thr Gly Ala Ser Ser Tyr Arg Pro Pro Ala
 2060 2065 2070

Pro Pro Xaa Gln Ser Val Thr Gly Asp Thr Arg Ser Asp Val Leu
 2075 2080 2085

Ser Pro Leu Asp Arg Ala Ser Ser Ala Arg Gly Lys Ile Asp His
 2090 2095 2100

Ala Leu Leu
 2105

<210> SEQ ID NO 29
 <211> LENGTH: 318
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 29

Met Met His Asn Ser Ala Ala Cys Leu Ser Lys Val Ala Val Leu Gly
 1 5 10 15

Ala Ala Gly Gly Ile Gly Gln Pro Leu Ser Leu Leu Leu Lys Asn Asn
 20 25 30

Pro Leu Val Ser Ala Leu Ser Cys Tyr Asp Leu Arg Gly Ala Pro Gly
 35 40 45

Val Ala Ala Asp Leu Ser His Ile Cys Ser Pro Ala Lys Val Thr Gly
 50 55 60

Tyr Thr Lys Glu Glu Ile Asn Lys Ala Leu Asp Gly Ala Glu Leu Val
 65 70 75 80

Leu Ile Pro Ala Gly Val Pro Arg Lys Pro Gly Met Thr Arg Asp Asp
 85 90 95

Leu Phe Asn Thr Asn Ala Ser Ile Val Arg Asp Leu Val Ile Ala Cys
 100 105 110

Ala Lys Val Cys Pro Lys Ala Phe Ile Gly Val Val Ser Asn Pro Val
 115 120 125

Asn Ser Thr Val Pro Ile Ala Ala Glu Thr Leu Lys Lys Ala Gly Val
 130 135 140

Phe Asp Pro Ala Arg Leu Phe Gly Val Thr Thr Leu Asp Leu Val Arg
 145 150 155 160

Ala Arg Thr Phe Val Ala Glu Ala Gly Gly Lys Ser Pro Tyr Asp Val
 165 170 175

His Val Pro Val Val Gly Gly His Ser Gly Pro Thr Ile Val Pro Leu
 180 185 190

Leu Ser Gln Ser Gly Val Glu Leu Ser Asp Ser Gln Val Lys Ala Ile
 195 200 205

Thr His Arg Val Gln Tyr Gly Gly Asp Glu Val Val Gln Ala Lys Asp
 210 215 220

Gly Ala Gly Ser Ala Thr Leu Ser Met Ala Tyr Ala Gly Ala Glu Trp
 225 230 235 240

Ser Asn Ser Ile Leu Lys Ala Leu Arg Gly Asp Ser Gly Val Val Glu
 245 250 255

Tyr Thr Phe Ile Gln Thr Asp Val Trp Pro Asn Leu Pro Tyr Phe Ser
 260 265 270

Cys Ala Val Glu Ile Gly Lys Asn Gly Val Val Lys Ala His Lys Pro
 275 280 285

Gln Leu Asn Lys Phe Glu Glu Ser Leu Met Glu Lys Ala Ile Val Asp
 290 295 300

Leu Gln Lys Asn Ile Ala Arg Gly Lys Ser Phe Val Thr Asn

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305                310                315

<210> SEQ ID NO 30
<211> LENGTH: 550
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 30

Met Ser Asn Phe Pro Ala Ala Ser Pro Ser Ile Trp Val Gly Gly Leu
 1                    5                    10
Asp Pro Asn Leu Asn Glu Gln Lys Leu Tyr Asp His Phe Val Arg Leu
 20                   25
Gly Pro Val Ala Ser Val Arg Val Cys Val Asp Ser Val Thr Gln Lys
 35                   40                   45
Ser Leu Gly Tyr Gly Tyr Val Asn Phe Gln Asn Pro Ala Asp Ala Glu
 50                   55                   60
Lys Ala Leu Asp Gln Ala Gly Val Lys Leu Gly Ser Lys His Ile Arg
 65                   70                   75                   80
Ile Ala Lys Ile Gln Arg Asp Pro Ser Lys Arg Arg Ser Gly Val Thr
 85                   90                   95
Asn Ile Val Val Lys Lys Leu Pro Pro Ser Val Asp Thr Tyr Ala Leu
 100                  105                  110
Lys Glu Met Phe Ser Lys Tyr Gly Arg Leu Thr Ala Ile Gly Leu Ala
 115                  120                  125
Thr Asn Glu Asn Gly Glu Ser Arg Gly Tyr Ala Arg Ile Ser Tyr Glu
 130                  135                  140
Lys Glu Glu Ser Ala Ile Gln Ala Val Gln Glu Val Asn Gly Met Val
 145                  150                  155                  160
Ile Asp Asp Cys Ala Ile Thr Val Glu Arg Tyr Gln Pro His His Arg
 165                  170                  175
Glu Glu Gln Leu Lys Gln Phe Thr Asn Leu Tyr Val Lys Asn Leu Asp
 180                  185                  190
Pro Ser Val Asn Asp Glu Lys Leu Lys Glu Val Phe Ser Ala Phe Gly
 195                  200                  205
Glu Val Thr Ser Ala Lys Val Arg Asp Leu Gly Ala Asn Gln Thr Val
 210                  215                  220
Gly Phe Ala Tyr Val Ala Tyr Ala Thr His Glu Ala Ala Ala Lys Ala
 225                  230                  235                  240
Val Glu Glu Leu Asp Glu Lys Glu Ser Pro Leu Ala Lys Glu Gly Met
 245                  250                  255
Lys Leu Ser Val Cys Arg Phe Arg Ser Arg Asp Glu Arg Gln Arg Glu
 260                  265                  270
Arg Glu Arg Leu Arg Arg Glu Arg Gln Gln Gln His Ser Lys Tyr Pro
 275                  280                  285
Asn Leu Tyr Val Lys Asn Phe Asp Asp Thr Val Thr Ser Glu Arg Leu
 290                  295                  300
Lys Glu Leu Phe Glu Arg Cys Gly Glu Thr Val Ser Val Ser Val Met
 305                  310                  315                  320
Met Asp Arg Ala Thr Arg Val Ser Arg Cys Phe Gly Phe Val Ser Phe
 325                  330                  335
Lys Glu Gln Ser Ala Ala Ser Arg Ala Ile Gln Glu Leu His Gly Ser
 340                  345                  350
Thr Ala Leu Gly Pro Arg Pro Leu Phe Val Thr Tyr Ala Leu Arg Lys
 355                  360                  365
Asp Ala Arg Arg Gln Thr Leu Glu Asp Met Arg Asn Lys Gln Pro Arg

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370			375			380									
Met	Arg	Gln	Pro	Pro	Met	Gly	Ser	Leu	Met	Gly	Gly	Met	Met	Gly	Pro
385					390					395					400
Gln	Leu	Gly	Phe	Met	Gly	Pro	Gln	Ala	Met	Phe	Asn	Gly	Val	Pro	Phe
			405						410					415	
Val	Asn	Pro	Arg	Met	Ser	Met	Met	Pro	Thr	Pro	Met	Gly	Met	Gly	Gly
			420					425					430		
Gln	Leu	Arg	Pro	Met	Gly	Pro	Thr	Pro	Met	Asn	Gln	Val	Arg	Ala	Arg
		435					440					445			
Pro	Met	Pro	Gln	Arg	Pro	Pro	Met	Gln	Pro	Ile	Met	Ala	Pro	Pro	Pro
	450				455						460				
Gln	Pro	Gln	Ser	Leu	Ala	Ser	Gln	Gly	Gln	Asn	Leu	Ser	Thr	Val	Leu
465				470						475					480
Ala	Asn	Leu	Thr	Pro	Glu	Gln	Gln	Lys	Asn	Val	Leu	Gly	Glu	Arg	Leu
			485						490					495	
Tyr	Asn	His	Ile	Val	Ala	Ile	Asn	Pro	Ala	Ala	Ala	Ala	Lys	Val	Thr
	500							505					510		
Gly	Met	Leu	Leu	Glu	Met	Asp	Asn	Gly	Glu	Ile	Leu	Asn	Leu	Leu	Asp
	515						520					525			
Thr	Pro	Gly	Leu	Leu	Asp	Ala	Lys	Val	Gln	Glu	Ala	Leu	Glu	Val	Leu
	530					535					540				
Asn	Arg	His	Met	Asn	Val										
545				550											
<210> SEQ ID NO 31															
<211> LENGTH: 1091															
<212> TYPE: PRT															
<213> ORGANISM: Trypanosoma cruzi															
<400> SEQUENCE: 31															
Ser	Val	Pro	Cys	Arg	Trp	Lys	Ser	Lys	Arg	Met	Trp	Gly	Arg	Ala	Thr
1			5						10					15	
Leu	Ile	Pro	Thr	Thr	Ser	Ala	Arg	Arg	Leu	Arg	Thr	Arg	Thr	Gly	Pro
			20					25					30		
Leu	Ile	Pro	Arg	Arg	Thr	Ser	Ala	Pro	Cys	Arg	Arg	Lys	Ser	Lys	Arg
		35					40					45			
Met	Trp	Gly	Arg	Ala	Thr	Leu	Ile	Pro	Thr	Thr	Ser	Ala	Arg	Arg	Leu
	50					55					60				
Arg	Thr	Arg	Thr	Gly	Pro	Leu	Ile	Pro	Arg	Arg	Thr	Ser	Val	Pro	Cys
65				70					75						80
Arg	Trp	Lys	Ser	Lys	Arg	Met	Trp	Gly	Pro	Arg	His	Val	Asp	Pro	Asp
			85						90				95		
His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser
		100						105					110		
Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Leu	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro
		115					120					125			
Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr
	130					135					140				
Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Leu	Glu	Glu
145				150					155					160	
Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser
			165						170					175	
Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg
			180					185					190		
Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp

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195					200					205					
Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp
210						215					220				
Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Leu	Glu	Glu	Gln	Glu	Asp	Val
225					230					235					240
Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp
				245					250					255	
Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln
				260				265						270	
Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe
				275			280					285			
Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr
				290		295					300				
Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His
305					310					315					320
Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro
				325					330					335	
Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu
				340				345						350	
Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr
				355			360					365			
Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu
				370		375						380			
Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp
385					390					395					400
His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser
				405					410					415	
Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu	Gln	Glu	Asp	Val	Gly	Pro
				420				425					430		
Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr
				435			440						445		
Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu
				450		455					460				
Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser
465					470					475					480
Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg
				485					490					495	
Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp
				500				505						510	
Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp
				515			520					525			
Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val
				530			535					540			
Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe	Arg	Ser	Thr	Thr	Gln	Asp
545					550					555					560
Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr	Lys	Arg	Ala	Leu	Pro	Gln
				565					570					575	
Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His	Val	Asp	Pro	Asp	His	Phe
				580				585					590		
Arg	Ser	Thr	Thr	Gln	Asp	Ala	Tyr	Arg	Pro	Val	Asp	Pro	Ser	Ala	Tyr
				595			600					605			
Lys	Arg	Ala	Leu	Pro	Gln	Glu	Glu	Glu	Glu	Asp	Val	Gly	Pro	Arg	His
610						615					620				

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Val Asp Pro Asp His Phe Arg Ser Thr Thr Gln Asp Ala Tyr Arg Pro
 625 630 635 640

Val Asp Pro Ser Ala Tyr Lys Arg Ala Leu Pro Gln Glu Glu Glu Glu
 645 650 655

Asp Val Gly Pro Arg His Val Asp Pro Asp His Phe Arg Ser Thr Thr
 660 665 670

Gln Asp Ala Tyr Arg Pro Val Asp Pro Ser Ala Tyr Lys Arg Ala Leu
 675 680 685

Pro Gln Glu Glu Glu Glu Asp Val Gly Pro Arg His Val Asp Pro Asp
 690 695 700

His Phe Arg Ser Thr Thr Gln Asp Ala Tyr Arg Pro Val Asp Pro Ser
 705 710 715 720

Ala Tyr Lys Arg Ala Leu Pro Gln Glu Glu Glu Asp Val Gly Pro
 725 730 735

Arg His Val Asp Pro Asp His Phe Arg Ser Thr Thr Gln Asp Ala Tyr
 740 745 750

Arg Pro Val Asp Pro Ser Ala Tyr Lys Arg Ala Leu Pro Gln Glu Glu
 755 760 765

Glu Glu Asp Val Gly Pro Arg His Val Asp Pro Asp His Phe Arg Ser
 770 775 780

Thr Thr Gln Asp Ala Tyr Arg Pro Val Asp Pro Ser Ala Tyr Lys Arg
 785 790 795 800

Ala Leu Pro Gln Glu Glu Gln Glu Asp Val Gly Pro Arg His Val Asp
 805 810 815

Pro Asp His Phe Arg Ser Thr Thr Gln Asp Ala Tyr Arg Pro Val Asp
 820 825 830

Pro Ser Ala Tyr Lys Arg Glu Ser Pro Val Val Lys Asp Val Arg Ala
 835 840 845

Val Asn Val Arg His Ala Tyr Pro Asp Thr Leu Arg Ser Val Ser His
 850 855 860

Glu Ser Tyr Lys Ser Val Asp Ser Ser Ala Tyr Lys Arg Glu Ser Pro
 865 870 875 880

Val Val Lys Asp Leu Arg Ala Val Asn Val Arg His Ala Tyr Pro Asp
 885 890 895

Thr Leu Arg Ser Val Ser His Glu Ser Tyr Lys Leu Leu Asn Val Ala
 900 905 910

Ser Thr Arg Asp Gly Leu Ser Arg Ala Val Cys His Arg Ile Ser Asp
 915 920 925

Gly Lys Ala Ala Gln Tyr Gly Glu Ser Ser Phe Ser Ser Phe Val Ser
 930 935 940

Asn Gly Asp Arg Asn Gly Thr Asp Gly Ala Ser Ser Ser Cys Arg Gly
 945 950 955 960

Ser Ala Arg Ala Cys Phe Gly Lys Ser Ser Ser Glu Val Phe Glu Ser
 965 970 975

Asn Phe Gln Thr Pro Leu Lys Gly Thr Asp Asp Gly His Phe Ser Ser
 980 985 990

Lys Gly Tyr Phe Cys Pro Cys His Thr Asp Pro Glu Met Tyr Arg Ser
 995 1000 1005

Thr Ser His Ala Asp Tyr Lys Ala His His Lys Asp Ala Tyr Ser
 1010 1015 1020

Arg Pro Tyr Leu Lys Pro Leu Asp Arg Lys Phe Pro Leu Glu Arg
 1025 1030 1035

Arg Asp Phe Leu Ser Glu Tyr Arg Lys Asn Phe Leu Arg Pro Glu
 1040 1045 1050

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Pro Gln Ser Leu Ser Arg Pro Val Ala Ala Ser Thr Val Thr Val
1055 1060 1065

Arg His Val Asp Pro Ser Val Tyr Thr Thr Thr Asn Gln Ala Val
1070 1075 1080

Phe Lys Asp His Trp Lys Lys Phe
1085 1090

<210> SEQ ID NO 32

<211> LENGTH: 305

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 32

Met Met Met His Pro Phe Leu Cys Ala Leu Leu Phe Phe Ala Leu Cys
1 5 10 15

Cys Cys Phe Pro Asn Ser Val Cys Ala Ala Asp Asp Thr Ala Thr Asn
20 25 30

Thr Thr Glu Asn Ala Ser Ala Met Ala Pro Pro Ala Asp Met Arg Gly
35 40 45

Ala Leu Arg Glu Val Leu Gly Ala Met Gln Lys Ala Gln Glu Tyr Ala
50 55 60

Asp Glu Ala Asn Arg His Cys Val Gln Ala Arg Met Ser Ala Glu Asn
65 70 75 80

Ala Arg Glu His Glu Glu Gly Ala Lys Asn Ala Leu Arg Lys Leu Gly
85 90 95

Ser Glu Ala Thr Arg Met Ser Arg Ala Leu Gln Gln Ala Asp Glu Ala
100 105 110

Val Lys Leu Ala Asp Ala Ala Val Ala Glu Cys Lys Ala Ala Glu Glu
115 120 125

Ala Ala Gln Ala Ala Gly Ile Met Thr Leu Asp Ala Val Gly Glu Val
130 135 140

Leu Lys His Val Lys Asp Glu Lys Thr Lys Val Gly Ser Gly Pro Glu
145 150 155 160

Leu Leu Lys Arg Ala Ala Glu Gln Thr Val Leu Ser Leu Glu Lys Ala
165 170 175

Lys Glu Ala Glu Ala Glu Ala Glu Lys Ala Ala Ala Ala Ala Glu Lys
180 185 190

Thr Arg Glu Ala Ala Glu Lys Ala Ala Ala Ala Arg Thr Leu Ala Gln
195 200 205

Asp Val Ala Ala Thr Ala Ser Ala Leu Leu Arg Gln Arg Glu Lys Glu
210 215 220

Glu Glu Arg Arg Arg Ala Ser Asp Arg Glu Val Ala Glu Ala Ala Lys
225 230 235 240

Lys Ala Ala Val Ala Glu Val Met Asn Lys Phe Ala Ala Lys Lys Gly
245 250 255

Asn Asp Ala Ala Pro Gly Arg Asn Ser Thr Ser Thr Arg Phe Gln Arg
260 265 270

Thr Arg Pro Arg Val Asp Gly Gly Gly Ile Pro Leu Leu Leu Arg Ala
275 280 285

Pro Leu Leu Met Leu Ala Ala Val Ala Ser Val Ser Gly Phe Leu Ser
290 295 300

Cys
305

<210> SEQ ID NO 33

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<211> LENGTH: 204
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 33
Met Ser Arg Ala Leu Gln Gln Ala Glu Glu Ala Val Lys Leu Ala Asp
1          5          10          15
Ala Ala Val Ala Glu Cys Lys Ala Ala Glu Glu Ala Ala Gln Ala Ala
20          25          30
Gly Ile Met Thr Leu Asp Ala Val Gly Glu Val Leu Lys His Val Lys
35          40          45
Asp Glu Lys Thr Lys Val Gly Ser Gly Pro Glu Leu Lys Arg Ala
50          55          60
Ala Glu Gln Thr Val Leu Ser Leu Glu Lys Ala Lys Glu Ala Glu Ala
65          70          75          80
Glu Thr Glu Lys Ala Ala Ala Ala Ala Gln Lys Thr Arg Glu Ala Ala
85          90          95
Glu Lys Ala Ala Ala Ala Gln Thr Leu Ala Gln Asp Val Ala Ala Thr
100         105         110
Ala Ile Ala Leu Leu Arg Gln Arg Glu Lys Glu Glu Glu Arg Arg Arg
115         120         125
Ala Arg Asp Arg Glu Glu Ala Glu Ala Ala Lys Lys Ala Ala Val Ala
130         135         140
Glu Val Met Asn Lys Phe Ala Ala Lys Lys Gly Asn Asp Ala Ala Pro
145         150         155         160
Gly Arg Asn Ser Thr Ala Thr Arg Ile Gln Arg Thr Arg Pro Arg Val
165         170         175
Asp Gly Gly Gly Ile Pro Leu Leu Leu Arg Ala Pro Leu Leu Met Leu
180         185         190
Ala Ala Val Ala Ser Val Phe Gly Phe Leu Ser Cys
195         200

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<210> SEQ ID NO 34
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 34
Met Leu Ala Val Met Val Met Arg Pro Phe Leu Cys Ala Leu Leu Phe
1          5          10          15
Phe Ala Leu Cys Cys Cys Phe Pro Asn Ser Val Cys Ala Ala Asp Asp
20          25          30
Thr Ala Thr Asn Thr Thr Glu Asp Val Asn Ala Ser Ala Ile Pro Thr
35          40          45
Asn Met Lys Glu Ala Phe Asp Trp Ala Phe Lys Ala Met Phe Lys Ala
50          55          60
Arg Glu Glu Val Asp Glu Ala Asn Gln His Cys Val Gln Ala Lys Leu
65          70          75          80
Ser Ala Thr Lys Ala Ala Gly Leu Glu Lys Glu Ala Glu Met Ala Leu
85          90          95
Lys Lys Leu Gly Ala Glu Ala Val Thr Leu Ser Lys Ala Leu Gln Asp
100         105         110
Ala Arg Glu Ala Asn Arg Glu Ala Glu Ala Ala Val Thr Glu Cys Glu
115         120         125
Ala Ala Glu Ala Ala Ala Gln Gln Ala Glu Ile Ala Thr Leu Asp Ala
130         135         140

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Ala Tyr Glu Val Leu Asn His Val Lys Thr Asp Arg Lys Asp Lys Asn
 145 150 155 160

Ser Lys Thr Arg Asp Leu Leu Asp Lys Ala Ala Asn His Thr Ala Ile
 165 170 175

Ala Val Lys Lys Ala Lys Glu Ala Glu Ala Glu Ser Glu Lys Ala Ala
 180 185 190

Ala Ala Ala Gln Lys Thr Leu Glu Ala Ala Glu Lys Ala Ala Ala Ala
 195 200 205

Arg Thr Leu Ala Gln Asp Val Ala Ala Thr Ala Ser Ala Leu Leu Arg
 210 215 220

Gln Arg Glu Arg Glu Glu Ile Arg Arg Ala Arg Asp Arg Glu Val
 225 230 235 240

Ala Glu Ala Ala Lys Lys Ala Ala Val Ala Glu Val Met Lys Lys Phe
 245 250 255

Ala Ala Arg Lys Gly Asn Asp Ala Ala Leu Gly Arg Asn Ser Thr Ser
 260 265 270

Thr Arg Leu Gln Arg Thr Arg Pro Arg Val Asp Gly Gly Gly Ile Pro
 275 280 285

Leu Leu Leu Arg Ala Pro Leu Leu Met Leu Ala Ala Val Ala Ser Phe
 290 295 300

Phe Gly Phe Leu Leu Cys
 305 310

<210> SEQ ID NO 35
 <211> LENGTH: 230
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 35

Met Pro Ala Tyr Pro Ser Arg Glu Glu Tyr Arg Arg Val Arg Lys Thr
 1 5 10 15

Asp Gly Glu Lys Glu Lys Gly Gln Gly Pro Glu Asn Glu Ile Arg Val
 20 25 30

Thr Ala Lys His Gly Gln Arg Ser Tyr Ile Thr Tyr Ala Ile Ala Leu
 35 40 45

Leu Arg Gly Glu Asp Gly Lys Val Gln Asn Asp Thr Ile Lys Ile Ser
 50 55 60

Ala Met Gly Ala Ala Ile His Asn Ala Val Asn Ile Ala Glu Ile Val
 65 70 75 80

Lys Arg Arg Val Val Gly Leu His Gln Thr Thr Asp Val Ser Ser Glu
 85 90 95

Ile Ile His Asp Glu Tyr Glu Ala Ile Asp Gly Lys Lys Glu Asn Met
 100 105 110

Lys Val Glu Arg Lys Val Ser Thr Ile Leu Ile Thr Leu Ser Leu Lys
 115 120 125

Pro Leu Asp Arg Asn His Val Gly Tyr Gln Pro Pro Leu Pro Glu Ser
 130 135 140

Glu Val Lys Glu Gln Asp Asp Pro Glu Pro Gly Glu Ser Ala Gly Glu
 145 150 155 160

Asn Arg Asn Arg Gly Glu Arg Gly Glu Arg Thr Asp Arg Gly Gly Arg
 165 170 175

Gly Gly Gly Arg Gly Asn Asn Ser Gly Gly Arg Gly Arg Gly Tyr Ser
 180 185 190

Arg Gly Gly Gln Arg Gly Gly Ser Asn Ile Gly Ala Ser Arg Ser Gly
 195 200 205

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Arg Gly Gly Asn Gly Arg Gly Gly Ser Arg Gly Gly Pro Asp Arg Arg
210 215 220

Asn Asp Arg Arg Val Asp
225 230

<210> SEQ ID NO 36
<211> LENGTH: 272
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 36

Met Pro Pro Met Leu Ala Ala Ala Lys Ser Leu Thr Leu Tyr Lys Asp
1 5 10 15

Phe Asn Lys Asp Val Lys Asp Met Leu Thr Lys Ser Tyr Ser Asp Ala
20 25 30

Gln Lys Trp Lys Leu Glu Ser Lys Phe Lys Gly Pro Glu Asp Lys Leu
35 40 45

Phe Val Asn Pro Met Ala Thr Ser Asp Gly Lys Phe Ser Val Asp Val
50 55 60

Glu Tyr Ala Pro Arg Cys Gly Ala Ala Leu Lys Ala Thr Leu Glu Pro
65 70 75 80

Ser Asn Cys Asn Ala Asn Leu Thr Ala Ser Tyr Leu Cys Gln Gly His
85 90 95

Lys Val Glu Ala Val Gly Lys Lys Asn Gly Glu Tyr Glu Leu Ser His
100 105 110

Glu Phe Val Met Pro Ser Arg Met Ser Ser His Ala Lys Leu Val Asn
115 120 125

Lys Thr Val Glu Val Gly Val Ala Thr Ala Val Ala Pro His Cys Gln
130 135 140

Val Gly Cys Gly Ala Leu Tyr Thr Leu Asp Gly Lys Lys Asp Tyr Asp
145 150 155 160

Leu Thr Leu Gly Cys Arg Tyr Ala His Ala Gly Tyr Ala Leu Ala Val
165 170 175

Arg Thr Asn Lys Leu Arg Ser Tyr Thr Thr Ser Phe Val Thr Pro Ile
180 185 190

Pro Lys Cys Pro His His Val Leu Val Gly Ala Glu Val Val Cys Gly
195 200 205

Arg Gly Gln Gly Trp Thr Gly Thr Leu Gly Phe Glu Thr Ala Cys Val
210 215 220

Leu Phe Lys Gly Asn Thr Leu Lys Ala Arg Val Asn Lys Asn Lys Glu
225 230 235 240

Trp Ala Val Val Tyr Ile Ala Lys Leu Val Asp Asn Trp Thr Ala Ala
245 250 255

Val Thr Leu Asp Lys Asn Leu Lys Pro Gly Val Leu Leu Thr His Ser
260 265 270

<210> SEQ ID NO 37
<211> LENGTH: 534
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 37

Met Arg Val Ile Leu Asp Gly Cys Ser Leu Thr Pro Asp Val Leu Tyr
1 5 10 15

Ala Leu Gly Tyr Glu Lys Gly Ala Thr Ile Glu Ile Ser Asp Glu Ala
20 25 30

Val Ala Arg Ile Thr Ala Ala Arg Ala Val Ile Asp Lys Ile Val Asn

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35					40					45					
Asp	Arg	Gln	Thr	Val	Tyr	Gly	Ile	Asn	Thr	Gly	Phe	Gly	Lys	Phe	Glu
50					55					60					
Ser	Thr	Ile	Ile	Pro	Pro	His	Gln	Leu	Glu	Glu	Leu	Gln	Leu	Asn	Leu
65					70					75					80
Ile	Arg	Ser	His	Ser	Ala	Cys	Val	Gly	Glu	Pro	Leu	Thr	Pro	Glu	Arg
				85					90					95	
Ala	Arg	Met	Met	Leu	Ala	Leu	Arg	Val	Asn	Val	Leu	Cys	Lys	Gly	His
				100					105					110	
Ser	Gly	Ile	Arg	Leu	Glu	Thr	Val	Gln	Lys	Tyr	Val	Lys	Ala	Phe	Asn
				115					120					125	
Ala	Gly	Val	Val	Pro	Tyr	Ile	Pro	Glu	Gln	Gly	Thr	Val	Gly	Ala	Ser
				130					135					140	
Gly	Asp	Leu	Gly	Pro	Leu	Ser	His	Leu	Ala	Leu	Gly	Met	Leu	Gly	Glu
145					150					155					160
Gly	Leu	Leu	Ala	Thr	Leu	Asn	Asn	Lys	Lys	Phe	Arg	Asp	Ala	Gly	Ser
				165					170					175	
Val	Leu	Arg	Glu	Leu	Gly	Val	Glu	Pro	Ile	Thr	Leu	Ala	Ala	Lys	Glu
				180					185					190	
Gly	Leu	Ala	Leu	Ile	Asn	Gly	Thr	Gln	Phe	Ile	Ser	Ala	Leu	Gly	Ala
				195					200					205	
Glu	Ala	Val	Val	Arg	Ala	Arg	Lys	Ile	Ala	Arg	Leu	Ala	Asp	Val	Ala
				210					215					220	
Leu	Ala	Met	Ser	His	Glu	Ala	Leu	Arg	Ala	Thr	Asn	Ser	Thr	Leu	Asn
225					230					235					240
Pro	Asp	Ile	His	Arg	Val	Arg	Pro	His	Lys	Gly	Gln	Gln	Leu	Val	Ala
				245					250						255
Gln	Arg	Leu	Arg	Ala	Leu	Leu	His	Ser	Glu	Glu	Tyr	Pro	Ser	Met	Ile
				260					265					270	
Asn	Glu	Ser	His	Val	Asn	Cys	Gly	Arg	Val	Gln	Asp	Ala	Tyr	Ser	Ile
				275					280					285	
Arg	Cys	Ala	Pro	Gln	Val	His	Gly	Ile	Ser	Asn	Glu	Val	Ile	Glu	Trp
				290					295					300	
Val	Tyr	Gly	Ile	Leu	Thr	Thr	Glu	Leu	Asn	Cys	Ala	Thr	Asp	Asn	Pro
305					310					315					320
Leu	Val	Phe	Pro	Asp	Gly	Val	Lys	Lys	Val	Val	Ser	Gly	Gly	Asn	Phe
				325					330					335	
His	Gly	Glu	Tyr	Pro	Ala	Lys	Ala	Leu	Asp	Met	Leu	Ala	Ile	Gly	Val
				340					345					350	
His	Glu	Leu	Gly	Asn	Ile	Ser	Glu	Arg	Arg	Ile	Glu	Arg	Leu	Asn	Asn
				355					360					365	
Pro	Thr	Leu	Ser	Arg	Leu	Pro	Ala	Phe	Leu	Val	Lys	Asn	Gly	Gly	Leu
				370					375					380	
Asn	Ser	Gly	Phe	Met	Ile	Ala	His	Cys	Thr	Ala	Ala	Ala	Leu	Val	Ser
385					390					395					400
Glu	Asn	Lys	Val	Tyr	Cys	His	Pro	Ala	Ser	Ala	Asp	Ser	Ile	Ser	Thr
				405					410					415	
Ser	Ala	Ala	Gln	Glu	Asp	His	Val	Ser	Met	Gly	Gly	Phe	Ser	Ala	Arg
				420					425					430	
Lys	Ala	Ile	Lys	Val	Val	Glu	Asn	Val	Glu	Arg	Ile	Ile	Ala	Ile	Glu
				435					440					445	
Leu	Leu	Gly	Ala	Cys	Gln	Gly	Ile	Asp	Leu	Leu	Arg	Pro	Leu	Arg	Thr
				450					455					460	

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Thr Glu Pro Met Glu Lys Val Trp Ser Leu Val Arg Ser Val Ser Pro
 465 470 475 480

Pro Trp Glu Glu Asp Arg Val Ile Asn Thr Asp Ile Asp Asn Val Thr
 485 490 495

Lys Leu Leu Arg Ser Gly Ala Val Trp Lys Thr Val Lys Pro Tyr Val
 500 505 510

Pro Glu Glu Ala Arg Phe Leu Gly Val Leu Thr Val Lys Lys Pro Phe
 515 520 525

Glu Leu Lys Ser Lys Met
 530

<210> SEQ ID NO 38
 <211> LENGTH: 185
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 38

Ala Gly Arg Gly Lys Met Arg Asn Arg Arg Tyr Val Ala Arg Arg Gly
 1 5 10 15

Pro Met Leu Val Met Pro Asp Asn Lys Gly Thr Arg Ala Phe Arg Asn
 20 25 30

Ile Phe Gly Leu Asp Leu Ala Asn Val Asn Ala Leu Asn Leu Leu His
 35 40 45

Leu Ala Pro Gly Gly His Val Gly Arg Phe Val Val Trp Thr Lys Gly
 50 55 60

Ala Phe Glu Lys Leu Asp Ser Ile Phe Gly Thr Phe Thr Lys Ala Ser
 65 70 75 80

Met Val Lys Lys Gly Phe Met Leu Pro Ala Pro Met Leu Thr Asn Thr
 85 90 95

Asp Val Thr Arg Ile Met Gln Ser Glu Glu Val Arg Arg Val Leu Lys
 100 105 110

Pro Lys Lys Leu Gln Pro Lys Lys Ala Ser Arg Leu Thr Gln Pro Thr
 115 120 125

Asn Gly Phe Arg Asn Arg Arg Leu Arg Leu Arg Leu Asn Pro Phe Gln
 130 135 140

Lys Lys Glu Ser Ser Met Leu Arg Gly Leu Arg Asn Arg Lys Asn Arg
 145 150 155 160

Glu Val Arg His Lys Ser Lys Ala Val Arg Ile Ala Lys Ala Lys Lys
 165 170 175

Ala Ile Ala Gly Gly Ala Lys Lys Lys
 180 185

<210> SEQ ID NO 39
 <211> LENGTH: 372
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 39

Met Ala Gln Arg Val Glu Val Leu Gln Thr Gln Leu Pro Ala Tyr Asn
 1 5 10 15

Arg Leu Lys Thr Pro Tyr Glu Ala Glu Leu Ile Ala Thr Ala Lys Lys
 20 25 30

Met Thr Ala Pro Gly Lys Gly Leu Leu Ala Ala Asp Glu Ser Ile Gly
 35 40 45

Ser Cys Ala Lys Arg Phe Ala Pro Leu Gly Leu Ser Asn Thr Glu Glu
 50 55 60

His Arg Arg Gln Tyr Arg Ala Leu Met Leu Glu Cys Glu Gly Met Glu

-continued

65	70	75	80
Gln Tyr Ile Ser Gly Val Ile Leu His Asp Glu Thr Val Tyr Gln Lys 85 90 95			
Ala Ser Thr Gly Glu Thr Phe Val Gln Leu Leu Gln Arg Lys Gly Val 100 105 110			
Val Pro Gly Ile Lys Thr Asp Met Gly Leu Asn Pro Leu Val Glu Gly 115 120 125			
Ala Glu Gly Glu Gln Met Thr Gly Gly Leu Asp Gly Tyr Val Glu Arg 130 135 140			
Ala Arg Lys Tyr Tyr Ser Leu Gly Cys Arg Phe Cys Lys Trp Arg Asn 145 150 155 160			
Val Tyr Lys Ile Gln Asn Gly Thr Val Ser Glu Ala Ala Val Arg Phe 165 170 175			
Asn Ala Glu Thr Leu Ala Arg Tyr Ala Val Leu Ser Gln Leu Ser Gly 180 185 190			
Leu Val Pro Ile Val Glu Pro Glu Val Met Ile Asp Gly Thr His Ser 195 200 205			
Ile Glu Thr Cys Gln Arg Val Ser Gln His Val Trp Ala Glu Val Val 210 215 220			
Thr Ala Leu His Arg His Gly Val Met Trp Glu Gly Cys Leu Leu Lys 225 230 235 240			
Pro Asn Met Val Val Pro Gly Ala Glu Ser Gly Val Lys Ala Thr Pro 245 250 255			
Gly Gln Val Ala Gln Tyr Thr Val Ser Thr Leu Ala Arg Val Leu Pro 260 265 270			
Pro Ala Leu Pro Gly Val Thr Phe Leu Ser Gly Gly Leu Ser Glu Val 275 280 285			
Gln Ala Ser Glu Tyr Leu Asn Ala Met Asn Ile Cys Asn Leu Pro Arg 290 295 300			
Pro Trp Lys Leu Thr Phe Ser Tyr Ala Arg Ala Leu Gln Ser Ser Ala 305 310 315 320			
Leu Lys Ala Trp Gly Gly Lys Asp Ser Gly Ile Ala Ala Gly Arg Arg 325 330 335			
Ala Phe Met His Arg Ala Lys Met Asn Ser Leu Ala Gln Leu Gly Arg 340 345 350			
Tyr Asn Arg Ala Glu Asp Asp Lys Glu Ser His Ser Leu Tyr Val Ala 355 360 365			
Gly Asn Ser Tyr 370			

<210> SEQ ID NO 40

<211> LENGTH: 477

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 40

Met Phe Arg Arg Cys Ala Val Lys Leu Asn Pro Tyr Asp Val Val Val 1 5 10 15
Ile Gly Gly Gly Pro Gly Gly Tyr Val Ala Ser Ile Lys Ala Ala Gln 20 25 30
Leu Gly Met Lys Thr Ala Cys Val Glu Lys Arg Gly Thr Leu Gly Gly 35 40 45
Thr Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala Leu Leu His Ala 50 55 60
Thr His Leu Tyr His Asp Ala His Ala Ser Phe Ala Arg Tyr Gly Leu

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65	70	75	80
Met Gly Gly Glu Gly Val Thr Met Asp Ser Ala Lys Met Gln Gln Gln	85	90	95
Lys Glu Arg Ala Val Lys Gly Leu Thr Gly Gly Val Glu Tyr Leu Phe	100	105	110
Lys Lys Asn Lys Val Thr Tyr Tyr Lys Gly Glu Gly Ser Phe Glu Thr	115	120	125
Ala His Ser Ile Arg Val Asn Gly Leu Asp Gly Lys Gln Glu Met Leu	130	135	140
Glu Thr Lys Lys Thr Ile Ile Ala Thr Gly Ser Glu Pro Thr Glu Leu	145	150	160
Pro Phe Leu Pro Phe Asp Glu Lys Val Val Leu Ser Ser Thr Gly Ala	165	170	175
Leu Ala Leu Pro Arg Val Pro Lys Thr Met Val Val Ile Gly Gly Gly	180	185	190
Val Ile Gly Leu Glu Leu Gly Ser Val Trp Ala Arg Leu Gly Ala Glu	195	200	205
Val Thr Val Val Glu Phe Ala Pro Arg Cys Ala Pro Thr Leu Asp Glu	210	215	220
Asp Val Thr Asn Ala Leu Val Gly Ala Leu Ala Lys Asn Glu Lys Met	225	230	240
Lys Phe Met Thr Ser Thr Lys Val Val Gly Gly Lys Asn Asn Gly Asp	245	250	255
Ser Val Ser Leu Glu Val Glu Gly Lys Asn Gly Lys Arg Glu Thr Leu	260	265	270
Thr Cys Glu Ala Leu Leu Val Ser Val Gly Arg Arg Pro Phe Thr Gly	275	280	285
Gly Leu Gly Leu Asp Lys Ile Asn Ala Ala Lys Asn Glu Arg Gly Phe	290	295	300
Val Lys Ile Gly Asp His Phe Glu Thr Ser Ile Pro Asp Val Tyr Ala	305	310	315
Ile Gly Asp Val Val Asp Lys Gly Pro Met Leu Ala His Lys Ala Glu	325	330	335
Asp Glu Gly Val Ala Cys Ala Glu Ile Leu Ala Gly Lys Pro Gly His	340	345	350
Val Asn Tyr Gly Val Ile Pro Ala Val Ile Tyr Thr Met Pro Glu Val	355	360	365
Ala Ser Val Gly Lys Ser Glu Asp Glu Leu Lys Lys Glu Gly Val Ala	370	375	380
Tyr Lys Val Gly Lys Phe Pro Phe Asn Ala Asn Ser Arg Ala Lys Ala	385	390	395
Val Ser Thr Glu Asp Gly Phe Val Lys Val Leu Val Asp Lys Ala Thr	405	410	415
Asp Arg Ile Leu Gly Val His Ile Val Cys Thr Thr Ala Gly Glu Leu	420	425	430
Ile Gly Glu Ala Cys Leu Ala Met Glu Tyr Gly Ala Ser Ser Glu Asp	435	440	445
Val Gly Arg Thr Cys His Ala His Pro Thr Met Ser Glu Ala Leu Lys	450	455	460
Glu Ala Cys Met Ala Cys Phe Ala Lys Thr Ile Asn Phe	465	470	475

<210> SEQ ID NO 41

<211> LENGTH: 195

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<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 41

Met Val Phe Ser Ile Pro Pro Leu Pro Trp Gly Tyr Asp Gly Leu Ala
1           5           10           15

Ala Lys Gly Leu Ser Lys Gln Gln Val Thr Leu His Tyr Asp Lys His
20           25           30

His Gln Gly Tyr Val Thr Lys Leu Asn Ala Ala Ala Gln Thr Asn Ser
35           40           45

Ala Leu Ala Thr Lys Ser Ile Glu Glu Ile Ile Arg Thr Glu Lys Gly
50           55           60

Pro Ile Phe Asn Leu Ala Ala Gln Ile Phe Asn His Thr Phe Tyr Trp
65           70           75           80

Glu Ser Met Cys Pro Asn Gly Gly Gly Glu Pro Thr Gly Lys Val Ala
85           90           95

Asp Glu Ile Asn Ala Ser Phe Gly Ser Phe Ala Lys Phe Lys Glu Glu
100          105          110

Phe Thr Asn Val Ala Val Gly His Phe Gly Ser Gly Trp Ala Trp Leu
115          120          125

Val Lys Asp Thr Asn Ser Gly Lys Leu Lys Val Tyr Gln Thr His Asp
130          135          140

Ala Gly Cys Pro Leu Thr Glu Pro Asn Leu Lys Pro Leu Leu Thr Cys
145          150          155          160

Asp Val Trp Glu His Ala Tyr Tyr Val Asp Tyr Lys Asn Asp Arg Ala
165          170          175

Ala Tyr Val Gln Thr Phe Trp Asn Val Val Asn Trp Lys Asn Val Glu
180          185          190

Arg Gln Leu
195

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<210> SEQ ID NO 42
<211> LENGTH: 464
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 42

Met Glu Asn Glu Met Pro Arg Leu Pro Val Trp Phe Gly Gly Ala Ala
1           5           10           15

Leu Leu Gly Gly Gly Tyr Tyr Ala Phe Tyr Tyr His Phe Tyr Thr Pro
20           25           30

Arg His Thr Thr Arg Ser Glu Ile Glu Thr Ala Ile Glu Ala Glu Lys
35           40           45

Leu Lys Trp Gln Ser Val Met Gln Arg Tyr Ala Ile Leu His His Met
50           55           60

Val Gln Lys Leu Arg Asp Gly Gly Thr Asp Ala Thr Asn Arg Glu Arg
65           70           75           80

Leu Tyr Ile Tyr Cys Glu Leu Gln Lys Leu Arg Glu Ala Phe Tyr Asn
85           90           95

Leu Ser Ile Arg Ala Gln Ser Ala Ser Glu Lys Gln Thr Val Leu Arg
100          105          110

Asp Leu Asp Glu Val Glu Thr Ala Asn Cys Thr Val Leu Asn Phe Arg
115          120          125

Glu Glu Ala Leu Thr Val Gly Gln Ile Ile Thr Cys Gln Leu Cys Phe
130          135          140

Ser Leu Tyr Val Leu Cys Phe Cys Leu Ile Asp Pro Val Leu Arg Arg

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145		150		155		160									
Val	His	Cys	Glu	Ala	Val	Glu	Arg	Val	Arg	Arg	Arg	Gly	Leu	His	Ile
				165					170					175	
Val	Ser	Trp	Trp	Met	Met	Arg	Leu	Leu	Arg	Ile	Pro	Val	Thr	Met	Thr
			180						185					190	
Leu	Glu	Asn	Thr	Leu	Pro	Pro	Pro	Asp	Gly	Asp	Val	Asn	Glu	Val	Thr
		195					200					205			
Arg	Glu	Asp	Glu	Glu	Tyr	Ile	Val	Phe	Ser	Pro	Gln	His	Trp	Ile	Glu
		210				215						220			
Val	Val	Gly	Phe	Trp	Ala	Cys	Pro	Thr	Asn	Pro	Leu	Leu	Thr	Asn	Ile
		225				230				235					240
Arg	Val	Arg	Trp	Leu	Leu	Pro	Cys	Asp	Ala	Val	Pro	Phe	Glu	Ile	His
			245						250						255
Trp	Arg	Gly	Arg	Trp	Gln	Gln	Met	Gln	Gln	Glu	Leu	Arg	Asp	Pro	Ile
			260						265					270	
Leu	Val	Val	Lys	Lys	Glu	Glu	Glu	Asn	Arg	Arg	Pro	Val	Val	Gln	Ser
			275					280					285		
Ser	Phe	Gly	Tyr	Pro	Leu	Ala	Ser	Arg	Leu	Thr	Cys	Gly	Gly	Gly	Met
		290				295						300			
Gln	Asp	Asp	Ser	Asn	Ser	Asp	His	Val	Leu	Ala	Thr	Thr	Asp	Val	Tyr
		305			310						315				320
Thr	Pro	Arg	Phe	Phe	Pro	Val	Val	Ser	Thr	Gly	Met	Pro	Arg	Val	Leu
				325						330					335
Phe	Val	Ser	Asp	Ala	Val	Ala	Arg	Arg	Asp	Val	Arg	Thr	Arg	Arg	Thr
			340						345						350
Leu	His	Glu	Val	Tyr	Thr	Asn	Val	Lys	His	Arg	Gln	Phe	Pro	Val	Thr
			355					360					365		
His	Asn	Val	Val	Lys	Asn	Asn	Lys	Ala	Asp	Asp	Cys	Val	Lys	Gly	Tyr
		370					375					380			
Asp	Val	Thr	Tyr	Glu	Gln	Leu	His	Ala	Trp	Arg	Lys	His	Gly	Gly	Val
		385				390					395				400
Pro	Val	Trp	His	Arg	Val	Glu	Trp	Gly	Ser	Val	Tyr	Gly	Asp	Val	Gly
				405						410					415
Ser	Gly	Gly	Asn	Arg	Asn	Arg	Arg	Glu	Leu	Thr	Phe	Arg	Met	Gly	Arg
			420						425					430	
Pro	Cys	Ser	Ala	Asn	Glu	Leu	Met	Val	Arg	Gln	Leu	Glu	Leu	Leu	Gln
			435					440					445		
Asn	Leu	Leu	Leu	Gln	Thr	Glu	Ser	Asn	Gly	Glu	Glu	Val	Ser	Leu	Leu
			450				455					460			

<210> SEQ ID NO 43
 <211> LENGTH: 416
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 43

Met	Ser	Ser	Trp	Asp	Val	Ser	Met	Ser	Asn	His	Ala	Gly	Leu	Val	Phe
1				5					10					15	
Asn	Pro	Ile	Arg	Thr	Val	Ser	Asp	Asn	Ala	Lys	Pro	Ser	Pro	Ser	Pro
		20						25					30		
Lys	Pro	Ile	Ile	Lys	Leu	Ser	Val	Gly	Asp	Pro	Thr	Leu	Asp	Lys	Asn
		35					40						45		
Leu	Leu	Thr	Ser	Ala	Ala	Gln	Ile	Lys	Lys	Leu	Lys	Glu	Ala	Ile	Asp
	50					55					60				
Ser	Gln	Glu	Cys	Asn	Gly	Tyr	Phe	Pro	Thr	Val	Gly	Ser	Pro	Glu	Ala

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65	70	75	80
Arg Glu Ala Val	Ala Thr Trp Trp	Arg Asn Ser Phe	Val His Lys Glu
	85	90	95
Glu Leu Lys Ser	Thr Ile Val Lys	Asp Asn Val Val	Leu Cys Ser Gly
	100	105	110
Gly Ser His Gly	Ile Leu Met Ala	Ile Thr Ala Ile	Cys Asp Ala Gly
	115	120	125
Asp Tyr Ala Leu	Val Pro Gln Pro	Gly Phe Pro His	Tyr Glu Thr Val
	130	135	140
Cys Lys Ala Tyr	Gly Ile Gly Met	His Phe Tyr Asn	Cys Arg Pro Glu
	145	150	155
Asn Asp Trp Glu	Ala Asp Leu Asp	Glu Ile Arg Arg	Leu Lys Asp Asp
	165	170	175
Lys Thr Lys Leu	Leu Ile Val Thr	Asn Pro Ser Asn	Pro Cys Gly Ser
	180	185	190
Asn Phe Ser Arg	Lys His Val Glu	Asp Ile Val Arg	Leu Ala Glu Glu
	195	200	205
Leu Arg Leu Pro	Leu Phe Ser Asp	Glu Ile Tyr Ala	Gly Met Val Phe
	210	215	220
Lys Gly Lys Asp	Pro Asn Ala Thr	Phe Thr Ser Val	Ala Asp Phe Glu
	225	230	235
Thr Thr Val Pro	Arg Val Ile Leu	Gly Gly Thr Ala	Lys Asn Leu Val
	245	250	255
Val Pro Gly Trp	Arg Leu Gly Trp	Leu Leu Tyr Val	Asp Pro His Gly
	260	265	270
Asn Gly Pro Ser	Phe Leu Asp Gly	Leu Lys Arg Val	Gly Met Leu Val
	275	280	285
Cys Gly Pro Cys	Thr Val Val Gln	Ala Ala Leu Gly	Glu Ala Leu Leu
	290	295	300
Asn Thr Pro Gln	Glu His Leu Asp	Gln Ile Val Ala	Lys Ile Glu Glu
	305	310	315
Ser Ala Met Tyr	Leu Tyr Asn His	Ile Gly Glu Cys	Ile Gly Leu Ala
	325	330	335
Pro Thr Met Pro	Arg Gly Ala Met	Tyr Leu Met Ser	Arg Ile Asp Leu
	340	345	350
Glu Lys Tyr Arg	Asp Ile Lys Thr	Asp Val Glu Phe	Phe Glu Lys Leu
	355	360	365
Leu Glu Glu Glu	Asn Val Gln Val	Leu Pro Gly Thr	Ile Phe His Ala
	370	375	380
Pro Gly Phe Thr	Arg Leu Thr Thr	Thr Arg Pro Val	Glu Val Tyr Arg
	385	390	395
Glu Ala Val Glu	Arg Ile Lys Ala	Phe Cys Gln Arg	His Ala Ala Val
	405	410	415

<210> SEQ ID NO 44

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 44

Leu Leu Lys Lys	Leu Asp Lys Thr	Val Lys Ser Glu	Glu Pro Ser Gly
1	5	10	15
Leu Glu Leu Val	Asp Leu Arg Asp	Phe Glu Ser His	Asp Leu Cys Leu
	20	25	30
Glu Gly Met Gly	Ala Met Asn Phe	Ser Tyr Asn Gly	Glu Phe Val Tyr

-continued

35	40	45																			
Met	Ala	Leu	Ser	Asp	Arg	Ser	Ser	Glu	Lys	Leu	Leu	Asp	Val	Val	Cys						
50						55						60									
Ser	Pro	Glu	Asn	Leu	Asn	Ile	Pro	Lys	Glu	Lys	Arg	Phe	Val	Phe	Thr						
65						70						75						80			
Ala	Val	Leu	Pro	Arg	Phe	Ser	Gly	Glu	Asn	Lys	Arg	Cys	Val	Gly	Glu						
				85						90						95					
Asp	Val	Val	His	His	Thr	Asn	Leu	Ile	Gly	Trp	Cys	Gly	Lys	Gly	Ile						
				100						105						110					
Cys	Ala	Trp	Gly	Leu	Asn	Phe	Leu	Arg	Phe	Ser	Ser	Glu	Glu	Lys	Lys						
			115						120						125						
Gln	Ala	Phe	Phe	Glu	His	Leu	Glu	Ala	Thr	Tyr	Lys	Lys	Ile	Ile	Asn						
				130						135						140					
Leu	Ser	Ala	Glu	Glu	Ile	Arg	Ala	Phe	Ala	Gly	Asn	Ala	Cys	Glu	Ile						
145						150						155						160			
Ala	Leu	Ser	Ser	Glu	Glu	Glu	Glu	Arg	His	Val	Leu	Cys	Ile	Ser	Asn						
				165						170						175					
Glu	Ala	Leu	Asn	Ser	Leu	His	His	Arg	Asn	Tyr	Gln	Ile	Leu	Glu	Glu						
				180						185						190					
Trp	Tyr	Gly	Arg	Glu	Asn	Ile	Phe	Val	Phe	Tyr	Ala	Glu	Thr	Leu	Glu						
			195						200						205						
Arg	Arg	Ser	Gly	Thr	Ser	Ile	Ser	Ser	Leu	Ile	Ser	Cys	Pro	Val	Thr						
		210						215						220							
His	Gly	Glu	Val	Leu	Pro	Ala	Pro	Gly	Glu	Val	Thr	Ala	Leu	Glu	Val						
				225						230						235			240		
Ala	His	Val	Asp	Glu	Lys	Val	Ile	Ala	Asn	Leu	Leu	Asn	Arg								
				245						250											

<210> SEQ ID NO 45
 <211> LENGTH: 376
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 45

Met	Phe	Gln	Leu	Ser	Ser	Gly	Thr	Thr	Ser	Phe	Leu	Val	Leu	Leu	Leu						
1						5						10						15			
Ala	Val	Ala	Thr	Leu	Ala	Ser	Val	Ala	Arg	Ala	Gly	Asp	Pro	Ser	Val						
				20						25						30					
Ser	Leu	Glu	Gly	Ile	Val	Asp	Leu	Thr	Ala	Ser	Asn	Phe	Asp	Glu	His						
			35						40						45						
Val	Gly	Lys	Gly	Val	Pro	Ala	Leu	Val	Glu	Phe	Tyr	Ala	Pro	Trp	Cys						
				50						55						60					
Gly	His	Cys	Lys	Lys	Met	Val	Pro	Glu	Phe	Glu	Lys	Val	Gly	Gln	Ala						
					65						70						75			80	
Val	Lys	Thr	Ala	Arg	Asp	Lys	Val	Leu	Val	Gly	Lys	Val	Asp	Ala	Thr						
				85						90						95					
Gln	Asn	Arg	Asp	Leu	Ala	Glu	Arg	Phe	Gly	Val	Asn	Gly	Tyr	Pro	Thr						
				100						105						110					
Ile	Leu	Phe	Phe	Pro	Ala	Asp	Ser	Gln	Thr	Lys	Gln	Gln	Tyr	Ser	Glu						
				115						120						125					
Ala	Arg	Glu	Ala	Ala	Ala	Phe	Leu	Ser	Phe	Leu	Asn	Arg	Gln	Val	Pro						
				130						135						140					
Gly	Leu	Asn	Ile	Gly	Val	Pro	His	Glu	His	Thr	Tyr	Ala	Val	Glu	Leu						
					145						150						155			160	
Thr	Lys	Arg	Asn	Phe	Asp	Ala	Val	Val	Met	Asp	Glu	Ala	Lys	Asp	Ala						

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165					170					175					
Leu	Val	Met	Phe	Tyr	Ala	Pro	Trp	Cys	Gly	His	Cys	Lys	Lys	Leu	His
			180					185					190		
Pro	Val	Phe	Glu	Arg	Leu	Ala	Thr	Ala	Phe	Lys	Glu	Glu	Ala	Asp	Ile
		195					200					205			
Val	Ile	Gly	Lys	Leu	Asn	Ala	Asp	Asp	Ala	Ser	Asn	Gly	Ala	Val	Arg
	210					215					220				
Asn	Arg	Tyr	Lys	Val	Asp	Gly	Tyr	Pro	Thr	Leu	Ala	Phe	Phe	Gln	Lys
	225					230					235				240
Arg	Ser	Lys	Ser	Glu	Pro	Gln	Tyr	Tyr	Ser	Gly	Gly	Arg	Ser	Leu	Glu
				245					250					255	
Glu	Leu	Val	Glu	Tyr	Val	Asn	Glu	Arg	Thr	Gly	Lys	Asn	Arg	Leu	Pro
			260					265						270	
Ser	Gly	Asp	Leu	Ser	Glu	Lys	Val	Gly	Val	Asn	Asp	Glu	Leu	Ser	Lys
		275					280					285			
Val	Leu	Arg	Asp	Met	Met	Leu	Lys	Glu	Lys	Ser	Val	Asp	Glu	Lys	Lys
	290					295					300				
Gln	Tyr	Leu	Glu	Lys	Val	Lys	Lys	Ala	Ala	Ala	Asp	Leu	Thr	Gly	Val
	305					310					315				320
Glu	Ala	Val	His	Tyr	Pro	Arg	Ile	Ala	Glu	Lys	Ile	Leu	Gln	Leu	Gly
			325					330						335	
Ala	Glu	Tyr	Val	Glu	Met	Glu	Leu	Gly	Arg	Ile	Ala	Arg	Leu	Lys	Gln
			340					345						350	
Gly	Asp	Val	Lys	Gly	Glu	Lys	Arg	Asp	Met	Leu	Thr	Ile	Arg	Asn	Asn
		355					360						365		
Ile	Leu	Ala	Ser	Leu	Lys	Asp	Glu								
	370					375									
<210> SEQ ID NO 46															
<211> LENGTH: 410															
<212> TYPE: PRT															
<213> ORGANISM: Trypanosoma cruzi															
<400> SEQUENCE: 46															
Met	Leu	Gly	Asp	Pro	Val	Asn	Arg	Leu	Glu	Glu	Leu	Gln	Ala	Glu	Leu
1				5					10					15	
Leu	Ser	Arg	Gln	Asn	Ala	Leu	Ser	Ser	Arg	Ile	Gln	Ser	Ile	Leu	Ser
			20					25					30		
Glu	Thr	Gly	Glu	Lys	Glu	Leu	Pro	Glu	Leu	Gln	Val	Leu	Ser	Glu	Asp
		35					40					45			
Arg	Ala	Gln	Leu	Ile	Lys	Glu	Ile	Gln	His	Thr	Lys	Asp	Glu	Gly	Asp
	50					55					60				
Ala	Leu	Leu	Leu	Gln	Val	Glu	Gly	Asn	Gly	Glu	Ala	Ala	Ser	Met	Tyr
	65			70					75						80
Arg	Val	Ala	Ala	Arg	Val	Gln	Arg	Phe	Glu	Glu	Phe	Ala	Asn	Arg	Ala
			85						90					95	
Asn	Glu	Tyr	Val	Lys	Asn	Val	Leu	Pro	Glu	Glu	Ile	Glu	Arg	Val	Ala
			100					105					110		
Asp	Ala	Asp	Arg	His	His	Ser	Asp	Asp	Glu	Val	Ala	Ala	Tyr	Ile	Lys
		115					120					125			
Glu	Leu	Gln	Ala	Lys	Arg	Glu	Glu	Glu	Leu	Arg	Lys	Ile	Gly	Ile	Leu
		130				135					140				
Lys	Glu	Arg	Thr	Thr	Lys	Arg	Ala	Asn	Asp	Leu	Arg	Asn	Gly	Pro	Arg
	145					150			155					160	
Val	Glu	Asn	Gly	Glu	Leu	Asn	Ala	Leu	Cys	Arg	Val	Ser	Arg	Ala	Lys

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165					170					175					
Glu	Glu	Glu	Leu	Asp	Lys	Glu	Thr	Lys	Glu	Thr	Gln	Glu	Tyr	Val	Asp
			180					185					190		
Glu	Ala	Arg	Leu	Lys	Lys	Ala	Asp	Leu	Ile	Ala	Lys	Ile	Arg	Glu	Leu
		195					200					205			
Arg	Gln	Ala	Lys	Ser	Lys	Leu	Gln	Val	Glu	Leu	Leu	Asp	Thr	Lys	His
		210				215						220			
Lys	Asn	Glu	Lys	Lys	Val	Asn	Glu	Met	Lys	Ala	Arg	Ile	Arg	His	Ala
225					230					235					240
Glu	Leu	Ala	Asn	Lys	Arg	Asp	Ile	Arg	Val	Cys	Gln	Gln	Leu	Asn	Thr
			245						250					255	
Thr	Asn	Ala	Ala	Leu	Thr	Thr	Asn	Ala	Gln	Thr	Leu	Leu	Gly	Gln	Leu
			260					265					270		
Asn	Val	Glu	His	Tyr	Gly	Ile	Glu	Gly	Ala	Pro	Ser	Glu	Lys	Ala	Leu
		275					280					285			
Ile	Thr	Met	Arg	Lys	Glu	Glu	Glu	Arg	Arg	Ala	Ala	Ala	Val	Leu	Ala
		290				295					300				
Asn	Gly	Arg	Gly	Asn	Asn	Asn	Asn	Asn	Asn	Gly	Ser	His	Pro	Ala	Gly
305				310						315					320
Asn	Glu	Thr	Glu	Gly	Leu	Cys	Pro	Arg	Arg	Asp	Thr	Gly	Asn	Phe	Asn
			325					330						335	
Gly	Asp	Ala	Ser	Ala	Ala	Ser	Ser	Arg	Arg	Gln	Ser	Asn	Gln	Gln	Asn
			340					345					350		
Thr	Gly	Leu	Ala	Ala	Met	Gln	Arg	Thr	Ala	Ser	Gln	Arg	Ser	Arg	Asp
		355					360					365			
Ala	Ser	Gln	Lys	Ala	Asp	Leu	Asp	Gln	Arg	Arg	Gln	Ser	Gly	Gln	Ser
		370				375						380			
Ser	Gln	Ala	Ser	Arg	His	Ser	Ala	Thr	Ser	Lys	Asp	Arg	Gln	Ala	Gly
385					390					395					400
Arg	Thr	Asn	Ser	Arg	Gly	Ser	Ser	Ala	Pro						
			405						410						

<210> SEQ ID NO 47

<211> LENGTH: 518

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 47

Met	Leu	Thr	Arg	Leu	Arg	Ser	Ala	Ala	Leu	Arg	Gly	Ala	Ala	Gly	Thr
1				5					10					15	
Arg	Ala	Ala	Ser	Gly	Met	Pro	Thr	Ala	Asp	His	Lys	Gly	Arg	Val	Gly
			20					25					30		
Tyr	Val	Ser	Gln	Val	Ile	Gly	Ala	Val	Val	Asp	Val	His	Phe	Ala	Glu
		35					40					45			
Gly	Val	Pro	Pro	Val	Leu	Thr	Ala	Leu	Asp	Val	Val	Glu	Lys	Leu	Gly
		50				55						60			
Arg	Asp	Glu	Pro	Leu	Thr	Leu	Glu	Ile	Val	Gln	His	Leu	Asp	Ala	His
65					70					75					80
Thr	Gly	Arg	Cys	Ile	Ala	Met	Gln	Thr	Thr	Asp	Leu	Leu	Lys	Leu	Lys
			85						90					95	
Ser	Lys	Val	Val	Ser	Ser	Gly	Gly	Asn	Ile	Ser	Val	Pro	Val	Gly	Arg
			100					105					110		
Glu	Thr	Leu	Gly	Arg	Ile	Phe	Asn	Val	Leu	Gly	Asp	Ala	Ile	Asp	Gln
		115					120					125			
Arg	Gly	Pro	Val	Gly	Glu	Lys	Gln	Arg	Met	Pro	Ile	His	Ala	Val	Ala

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130			135			140									
Pro	Lys	Leu	Ala	Asp	Gln	Ala	Ala	Glu	Asp	Thr	Ile	Leu	Thr	Thr	Gly
145					150					155					160
Ile	Lys	Val	Ile	Asp	Leu	Ile	Leu	Pro	Tyr	Cys	Lys	Gly	Gly	Lys	Ile
				165						170					175
Gly	Leu	Phe	Gly	Gly	Ala	Gly	Val	Gly	Lys	Thr	Val	Ile	Ile	Met	Glu
				180						185				190	
Leu	Ile	Asn	Asn	Val	Ala	Lys	Gly	His	Gly	Gly	Phe	Ser	Val	Phe	Ala
		195						200						205	
Gly	Val	Gly	Glu	Arg	Thr	Arg	Glu	Gly	Thr	Asp	Leu	Tyr	Leu	Glu	Met
		210						215						220	
Met	Gln	Ser	Lys	Val	Ile	Asp	Leu	Lys	Gly	Asp	Ser	Lys	Cys	Val	Leu
225					230						235				240
Val	Tyr	Gly	Gln	Met	Asn	Glu	Pro	Pro	Gly	Ala	Arg	Ala	Arg	Val	Ala
				245						250				255	
Gln	Ser	Ala	Leu	Thr	Met	Ala	Glu	Tyr	Phe	Arg	Asp	Val	Glu	Gly	Gln
				260						265				270	
Asp	Val	Leu	Leu	Phe	Ile	Asp	Asn	Ile	Phe	Arg	Phe	Thr	Gln	Ala	Asn
		275						280						285	
Ser	Glu	Val	Ser	Ala	Leu	Leu	Gly	Arg	Ile	Pro	Ala	Ala	Val	Gly	Tyr
		290						295						300	
Gln	Pro	Thr	Leu	Ala	Glu	Asp	Leu	Gly	Gln	Leu	Gln	Glu	Arg	Ile	Thr
		305			310						315				320
Ser	Thr	Thr	Lys	Gly	Ser	Ile	Thr	Ser	Val	Gln	Ala	Val	Tyr	Val	Pro
				325						330				335	
Ala	Asp	Asp	Ile	Thr	Asp	Pro	Ala	Pro	Ala	Thr	Thr	Phe	Ser	His	Leu
				340				345						350	
Asp	Ala	Thr	Thr	Val	Leu	Asp	Arg	Ala	Val	Ala	Glu	Ser	Gly	Ile	Tyr
		355						360						365	
Pro	Ala	Val	Asn	Pro	Leu	Glu	Cys	Ala	Ser	Arg	Ile	Met	Asp	Pro	Asp
		370						375						380	
Val	Ile	Ser	Val	Asp	His	Tyr	Asn	Val	Ala	Gln	Asp	Val	Val	Gln	Met
		385			390						395				400
Leu	Thr	Lys	Tyr	Lys	Glu	Leu	Gln	Asp	Ile	Ile	Ala	Val	Leu	Gly	Ile
				405							410			415	
Asp	Glu	Leu	Ser	Glu	Glu	Asp	Lys	Leu	Ile	Val	Asp	Arg	Ala	Arg	Lys
				420				425						430	
Val	Thr	Lys	Phe	Leu	Ser	Gln	Pro	Phe	Gln	Val	Ala	Glu	Val	Phe	Thr
		435						440						445	
Gly	Met	Thr	Gly	His	Tyr	Val	Gln	Leu	Glu	Glu	Thr	Ile	Glu	Ser	Phe
		450						455			460				
Ser	Gly	Leu	Leu	Met	Gly	Thr	Tyr	Asp	Gln	Val	Pro	Glu	Met	Ala	Phe
		465			470						475				480
Tyr	Met	Val	Gly	Gly	Ile	Thr	Ser	Val	Leu	Glu	Lys	Gly	Lys	Lys	Met
				485						490				495	
Ala	Glu	Glu	Ala	Ala	Glu	Leu	Glu	Lys	Leu	Arg	Arg	Ala	Arg	Ala	Ala
				500				505						510	
Gln	Ala	Gly	Gln	Lys	Glu										
		515													

<210> SEQ ID NO 48

<211> LENGTH: 415

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

-continued

<400> SEQUENCE: 48

His Thr Tyr Ala Leu Ser Leu Thr Leu Trp Ser Gly Val Asn Pro Glu
 1 5 10 15
 Asn Ala Arg Thr His Lys Leu Leu Ala Ala Ala Ala Leu Ala Asn Val
 20 25 30
 Ala Val Thr Leu Lys Ala Cys Glu Tyr Gly Arg Glu Asn Glu Thr Ala
 35 40 45
 Glu Tyr Cys Arg Asn Cys Ser Pro Cys Gly Arg Tyr Pro Val Leu Gln
 50 55 60
 Thr Glu Glu Gly Cys Val Phe Glu Ser Asn Ala Ile Leu Arg His Ile
 65 70 75 80
 Ala Arg Leu Asp Arg Ser Gly Gly Phe Leu Tyr Gly Arg Thr Pro Leu
 85 90 95
 Glu Gly Ser Gln Val Asp Met Trp Leu Asp Phe Ser Ala Thr Glu Leu
 100 105 110
 Asp Ala Ala Ser Ala Pro Phe Val His His Ala Phe Arg Gly Glu Pro
 115 120 125
 Leu Pro Ala Asn Ala Met Asp Arg Val His Glu Val Leu Arg Ala Leu
 130 135 140
 Glu Ala Trp Leu Glu Thr Arg Thr Phe Leu Val Gly Glu Arg Met Thr
 145 150 155 160
 Val Ala Asp Val Ala Val Ala Phe Ala Leu Gln Trp His Tyr Arg Leu
 165 170 175
 Asn Gly Ala Glu Gly Glu Ala Leu Thr Lys Lys Tyr Arg Asn Ala Tyr
 180 185 190
 Arg Leu Tyr Asn Thr Val Met Gln Gln Pro Lys Thr Val Glu Val Leu
 195 200 205
 Arg Ser Gln Gly Ala Thr Phe Gly Pro Val Lys Ala Glu Arg Lys Gly
 210 215 220
 Lys Asp Ala Ala Ala Pro Ala Arg Ala Glu Lys Lys Pro Lys Ala Ala
 225 230 235 240
 Ala Ala Ala Ala Asp Gly Ala Glu Glu Glu Asp Glu Ala Pro Arg Glu
 245 250 255
 Lys Lys Lys Pro Asn Pro Leu Asp Glu Leu Pro Pro Ser Pro Phe Val
 260 265 270
 Leu Asp Ala Phe Lys Arg Glu Tyr Ser Asn Thr Asp Thr Arg Thr Val
 275 280 285
 Ala Ala Pro Tyr Phe Phe Gln His Tyr Asp Ala Ala Gly Tyr Thr Thr
 290 295 300
 Phe Trp Cys Arg Tyr Lys Tyr Asn Glu Asp Asn Lys Met Gln Phe Met
 305 310 315 320
 Thr Ala Asn Leu Ile Arg Gly Trp Phe Gln Arg Met Glu His Val Arg
 325 330 335
 Lys Tyr Ala Phe Gly Val Ala Leu Ile Ile Gly Glu Glu Arg Arg His
 340 345 350
 Asp Ile Val Ala Leu Trp Val Phe Arg Gly Arg Gly Met Pro Ala Ile
 355 360 365
 Val Glu Asp Val Glu Asp Thr Glu Leu Phe Asp Trp Glu Glu Val Ala
 370 375 380
 Asp Val Ala Ala Gln Arg Glu Arg Ile Thr Asp Tyr Leu Cys Trp Glu
 385 390 395 400
 Gly Pro Thr Ile Pro Arg Pro Val Leu Glu Gly Arg Val Phe Lys
 405 410 415

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<210> SEQ ID NO 49
<211> LENGTH: 153
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 49
Met Thr Ser Glu Arg Thr Phe Ile Ala Val Lys Pro Asp Gly Val Gln
1          5          10          15
Arg Cys Leu Val Gly Glu Ile Ile Gln Arg Phe Glu Lys Lys Gly Tyr
          20          25          30
Lys Leu Val Ala Leu Lys Met Leu Gln Pro Ser Ala Glu Gln Ala Gln
          35          40          45
Gln His Tyr Ile Asp Leu Ala Ser Lys Pro Phe Tyr Lys Asp Leu Val
          50          55          60
Ala Tyr Phe Ser Ser Gly Pro Ile Val Gly Met Val Trp Glu Gly Lys
65          70          75          80
Gly Val Val Lys Gly Gly Arg Val Leu Leu Gly Ala Thr Asn Pro Ala
          85          90          95
Asp Ser Leu Pro Gly Thr Ile Arg Gly Asp Phe Ala Val Asp Val Gly
          100          105          110
Arg Asn Val Cys His Gly Ser Asp Ser Val Asp Ser Ala Lys Arg Glu
          115          120          125
Ile Ala Phe Trp Phe Lys Pro Glu Glu Leu Val Asn Trp Thr Ser His
          130          135          140
Ser Val Lys Gln Val Tyr Glu Ser Ala
145          150

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<210> SEQ ID NO 50
<211> LENGTH: 260
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 50
Met Gly Lys Thr Val Leu Thr Cys Arg Lys Gly Asn Gly Ser Val Tyr
1          5          10          15
Gln Leu His Gly His Lys Arg Leu Gly Pro Ala Lys Leu Arg Ile Leu
          20          25          30
Asp Tyr Ala Glu Arg His Gly Phe Met Arg Gly Val Val Lys Thr Ile
          35          40          45
Glu His Glu Pro Gly Arg Gly Ala Pro Leu Ala Arg Val Glu Phe Arg
          50          55          60
His Pro Tyr Lys Tyr Arg Arg Val Lys Glu Leu Met Val Ala Pro Glu
65          70          75          80
Gly Met Phe Thr Gly Gln Ser Val Leu Cys Gly Val Lys Ala Pro Leu
          85          90          95
Ala Ile Gly Asn Val Leu Pro Leu Gly Gln Ile Thr Glu Gly Cys Ile
          100          105          110
Val Cys Asn Val Glu Ala Lys Val Gly Asp Arg Gly Thr Ile Ala Arg
          115          120          125
Ala Ser Gly Asp Tyr Cys Ile Ile Ile Ser His Asn His Glu Thr Gly
          130          135          140
Arg Thr Arg Leu Lys Leu Pro Ser Gly Gln Lys Lys Thr Val Pro Ser
145          150          155          160
Asn Cys Arg Ala Met Ile Gly Ile Ile Ala Gly Gly Gly Arg Ile Glu
          165          170          175
Lys Pro Val Leu Lys Ala Gly Asn Ser Phe Tyr Arg Phe Arg Gly Lys

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180          185          190
Arg Asn Cys Trp Pro Lys Val Arg Gly Val Ala Arg Asn Pro Val Glu
   195          200          205

His Pro His Gly Gly Gly Asn His Gln His Ile Gly His Pro Ser Thr
   210          215          220

Val Ser Arg His Ala Pro Pro Gly Gln Lys Val Gly Leu Ile Ala Ala
   225          230          235          240

Arg Arg Thr Gly Arg Ile Arg Gly Ser Arg Ala Val Lys Gly Ala Trp
   245          250          255

His Pro Glu Glu
   260
    
```

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<210> SEQ ID NO 51
<211> LENGTH: 852
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (129)..(129)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (211)..(211)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (218)..(218)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
    
```

<400> SEQUENCE: 51

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Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu
 1      5      10      15

Val Glu Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp
 20     25     30

Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
 35     40     45

Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu
 50     55     60

Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Val Met Glu Pro
 65     70     75     80

Thr Leu Glu Ala Leu Ala Lys Lys Tyr Asn Trp Glu Lys Lys Val Cys
 85     90     95

Arg Arg Cys Tyr Ala Arg Leu Pro Val Arg Ala Ser Asn Cys Arg Lys
100    105    110

Lys Ala Cys Gly His Cys Ser Asn Leu Arg Met Lys Lys Lys Leu Arg
115    120    125

Xaa Ser Ala Met Leu Trp Thr Asp Ala Leu Lys Tyr Thr Pro Ser Leu
130    135    140

Ala Phe Leu Phe Phe Phe Asp Leu Phe Phe Leu Leu Arg Cys Phe
145    150    155    160

Val Leu Phe Phe Ser Val Phe Met Ile Ser Ala Val Cys Pro Pro Ala
165    170    175

Phe Met Gln Cys Leu Val Ile Leu Ser Ile Phe Trp Asn Tyr Gly Asp
180    185    190

Asn Ile Leu Val Phe Lys Leu Leu Ile Thr Asn Cys Ala Leu Glu Phe
195    200    205

Pro Ala Xaa Leu Leu Leu Thr His Cys Xaa Glu Arg Glu Thr Met Gln
210    215    220

Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu Val Glu
    
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225	230	235	240
Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp Lys Glu 245 250 255			
Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu 260 265 270			
Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr 275 280 285			
Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln Ile Phe Val Lys 290 295 300			
Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu Val Glu Ser Ser Asp Thr 305 310 315 320			
Ile Glu Asn Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro 325 330 335			
Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg 340 345 350			
Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val 355 360 365			
Leu Arg Leu Arg Gly Gly Met Gln Ile Phe Val Lys Thr Leu Thr Gly 370 375 380			
Lys Thr Ile Ala Leu Glu Val Glu Ser Ser Asp Thr Ile Glu Asn Val 385 390 395 400			
Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg 405 410 415			
Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp 420 425 430			
Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg 435 440 445			
Gly Gly Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala 450 455 460			
Leu Glu Val Glu Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile 465 470 475 480			
Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala 485 490 495			
Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln 500 505 510			
Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln 515 520 525			
Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu Val Glu 530 535 540			
Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp Lys Glu 545 550 555 560			
Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu 565 570 575			
Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr 580 585 590			
Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln Ile Phe Val Lys 595 600 605			
Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu Val Glu Ser Ser Asp Thr 610 615 620			
Ile Glu Asn Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro 625 630 635 640			
Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg 645 650 655			

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Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val
 660 665 670
 Leu Arg Leu Arg Gly Gly Met Gln Ile Phe Val Lys Thr Leu Thr Gly
 675 680 685
 Lys Thr Ile Ala Leu Glu Val Glu Ser Ser Asp Thr Ile Glu Asn Val
 690 695 700
 Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg
 705 710 715 720
 Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp
 725 730 735
 Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg
 740 745 750
 Gly Gly Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala
 755 760 765
 Leu Glu Val Glu Ser Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile
 770 775 780
 Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala
 785 790 795 800
 Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln
 805 810 815
 Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln
 820 825 830
 Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Ala Leu Glu Val Glu
 835 840 845
 Ser Ser Asp Thr
 850

<210> SEQ ID NO 52
 <211> LENGTH: 225
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 52

Met Arg Arg Val Leu Phe Ser Ala Ala Leu Ser Leu Val Pro Lys Ala
 1 5 10 15
 Pro Leu Phe Arg Arg Cys Val Pro Ser Val Ala Lys Ala Ala Ser Met
 20 25 30
 Gly Pro Leu Val Ala Met Glu Gln Arg Arg Leu Ala Ser His Ala Ala
 35 40 45
 Leu Ser Ser Ala Leu Arg His Glu Leu Glu Glu Glu Gln Gln Arg Ser
 50 55 60
 Glu Lys Pro Ala Lys Pro Glu Leu Pro Ala Gly Trp Thr Leu Glu Arg
 65 70 75 80
 Lys Pro Gly Gln Met Leu Phe Thr Met Arg Lys Lys His Glu Asp Glu
 85 90 95
 Glu Ile Ile Ile Arg Cys Leu Gly Glu Glu Ser Gly Asp Asp Asp Val
 100 105 110
 Val Ser Leu Asp Phe Asp Ala Tyr Ile Thr Cys Asn Asn Lys Ala Leu
 115 120 125
 Val Cys Arg Met Ser Phe Glu Ser Glu Glu Val Ile Met Gly Gln Val
 130 135 140
 Ser Phe Leu Asp Asp Ala Lys Leu Ala Leu Asp Asp Ser Val Glu Gly
 145 150 155 160
 Asn Arg Lys Arg Gln Trp Leu Tyr Lys Gly Pro Lys Leu Asp Glu Leu
 165 170 175

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Asp Glu Arg Leu Val Asp Ser Leu Thr Ser Tyr Leu Lys Asp Arg Gly
 180 185 190

Val Asn Glu Asp Leu Cys Arg Phe Met Glu Glu Tyr Phe Phe Trp Ala
 195 200 205

Glu Gln Ala Glu Tyr Glu Glu Trp Leu Ser Ala Ile Asn Arg Phe Val
 210 215 220

Ser
 225

<210> SEQ ID NO 53
 <211> LENGTH: 651
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 53

Met Leu Leu Gln Ala Leu Leu Val Leu Ser Ala Val Val Val Ala Val
 1 5 10 15

Ala Ala Pro Asp Gly Thr Gly Lys Val Glu Ala Pro Cys Val Gly Ile
 20 25 30

Asp Leu Gly Thr Thr Tyr Ser Val Val Gly Val Trp Gln Lys Gly Asp
 35 40 45

Val His Ile Ile Pro Asn Asp Met Gly Asn Arg Ile Thr Pro Ser Val
 50 55 60

Val Ala Phe Thr Glu Thr Glu Arg Leu Ile Gly Asp Gly Ala Lys Asn
 65 70 75 80

Gln Leu Pro Gln Asn Pro His Asn Thr Ile Tyr Ala Ile Lys Arg Leu
 85 90 95

Ile Gly Arg Lys Tyr Ser Asp Ala Thr Val Gln Thr Asp Lys Lys Leu
 100 105 110

Leu Ser Tyr Glu Val Val Ala Asp Lys Asp Gly Lys Pro Lys Val Gln
 115 120 125

Val Glu Val Gly Gly Lys Lys Lys Gln Phe Thr Pro Glu Glu Val Ser
 130 135 140

Ala Met Val Leu Gln Lys Met Lys Glu Ile Ala Glu Thr Tyr Leu Gly
 145 150 155 160

Glu Lys Val Lys Asn Ala Val Val Thr Val Pro Ala Tyr Phe Asn Asp
 165 170 175

Ala Gln Arg Gln Ser Thr Lys Asp Ala Gly Thr Ile Ala Gly Leu Asn
 180 185 190

Val Val Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala Tyr Gly
 195 200 205

Leu Asn Lys Ala Gly Glu Lys Asn Ile Leu Val Phe Asp Leu Gly Gly
 210 215 220

Gly Thr Phe Asp Val Ser Leu Leu Thr Ile Asp Glu Gly Phe Phe Glu
 225 230 235 240

Val Val Ala Thr Asn Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp
 245 250 255

Asn Asn Met Met Arg Tyr Phe Val Asp Met Leu Lys Lys Lys Lys Asn
 260 265 270

Val Asp Val Ser Lys Asp Gln Lys Ala Leu Ala Arg Leu Arg Lys Ala
 275 280 285

Cys Glu Ala Ala Lys Arg Gln Leu Ser Ser His Pro Glu Ala Arg Val
 290 295 300

Glu Val Asp Ser Leu Thr Glu Gly Phe Asp Phe Ser Glu Lys Ile Thr
 305 310 315 320

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Arg Ala Lys Phe Glu Glu Leu Asn Met Glu Leu Phe Lys Gly Thr Leu
 325 330 335

Val Pro Val Gln Arg Val Leu Glu Asp Ala Lys Leu Lys Lys Ser Asp
 340 345 350

Ile His Glu Ile Val Leu Val Gly Gly Ser Thr Arg Val Pro Lys Val
 355 360 365

Gln Gln Leu Ile Arg Asp Phe Phe Gly Gly Lys Glu Pro Asn Arg Gly
 370 375 380

Ile Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Ala
 385 390 395 400

Val Leu Thr Gly Glu Ser Glu Val Gly Gly Arg Val Val Leu Val Asp
 405 410 415

Val Ile Pro Leu Ser Leu Gly Ile Glu Thr Val Gly Gly Val Met Thr
 420 425 430

Lys Leu Ile Glu Arg Asn Thr Gln Ile Pro Thr Lys Lys Ser Gln Val
 435 440 445

Phe Ser Thr Tyr Gln Asp Asn Gln Pro Gly Val Leu Ile Gln Val Tyr
 450 455 460

Glu Gly Glu Arg Gln Met Thr Lys Asp Asn Arg Leu Leu Gly Lys Phe
 465 470 475 480

Glu Leu Ser Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu
 485 490 495

Val Thr Phe Asp Val Asp Glu Asn Ser Ile Leu Gln Val Ser Ala Val
 500 505 510

Asp Lys Ser Ser Gly Lys Lys Glu Glu Ile Thr Ile Thr Asn Asp Lys
 515 520 525

Gly Arg Leu Ser Glu Glu Glu Ile Glu Arg Met Val Arg Glu Ala Ala
 530 535 540

Glu Phe Glu Asp Glu Asp Arg Lys Val Arg Glu Arg Val Asp Ala Arg
 545 550 555 560

Asn Ser Leu Glu Ser Val Ala Tyr Ser Leu Arg Asn Gln Val Asn Asp
 565 570 575

Lys Glu Lys Leu Gly Gly Lys Leu Ser Ala Asp Asp Lys Ser Ala Val
 580 585 590

Glu Ala Ala Val Lys Glu Ala Met Gln Phe Leu Asp Asp Asn Pro Asn
 595 600 605

Ala Asp Lys Glu Glu Tyr Asp Glu Ala Arg Asp Lys Leu Gln Ser Val
 610 615 620

Thr Asn Pro Ile Ile Gln Lys Val Tyr Gln Ser Gly Gly Gly Ala Asp
 625 630 635 640

Gly Asp Glu Arg Pro Glu Pro Met Asp Asp Leu
 645 650

<210> SEQ ID NO 54
 <211> LENGTH: 3481
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 54

Met Val Asp Gly Ala Ser Ala Thr Arg His Asp Ser Pro Val Asp Thr
 1 5 10 15

Cys Leu Gly Trp Trp Met Arg Ser Ala Val Gly Ala Cys Pro Arg Ser
 20 25 30

Arg His Arg Val Gln Arg Arg Ala Ile Ala Ala Val Arg Val Ala Leu
 35 40 45

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Leu Val Val Leu Ala Leu Val Ala Ala Ala Ala Trp Met Pro Ala Val
 50 55 60
 His Ala Val Val Leu Arg Leu Arg Gly Gly Thr Val Asp Arg Ala Ile
 65 70 75 80
 Thr Val Gly Arg Ala Val Asp Thr Val Leu Met Asp Gly Val His Ile
 85 90 95
 Thr Asn Gly Val Ala Val Val Leu Asp Val Pro Ala Met Leu Pro Gly
 100 105 110
 Ala Leu Arg Ile Glu Leu Arg Asn Cys Val Cys Asp Gly Gly Ala Gln
 115 120 125
 Ile Tyr Val Arg Gly Tyr Ser Gly Glu Pro Ala Ser Asp Arg Ser Leu
 130 135 140
 Glu Val Ser Val Ser Gly Leu Ser Gly Ser Tyr Cys Ser Leu Val Phe
 145 150 155 160
 Val His Asn Leu Pro Ala His Thr Asn Val Thr Val Arg Asp Ser Thr
 165 170 175
 Ile Val Thr Ala Gly Pro Met Arg Tyr Ser Gln Leu Ser Gly Leu Thr
 180 185 190
 Asp Ala Val Ala Ser Pro Leu Val Leu His Ala Thr Ser Leu Leu Gln
 195 200 205
 Thr Gln Leu Arg Val Ser Lys Thr Val Leu Arg Ser Leu Gln Ala Gly
 210 215 220
 Gly Ser Ala Val Tyr Val Gly Gly Gly Val Asp Leu Leu Ser Ser Ala
 225 230 235 240
 Val Val Leu Asp Gly Val Ser Leu Glu Ala Ser Gly Gly Pro Thr Ala
 245 250 255
 Ser Ala Met His Val Ala Ser Ser Ser Arg Leu Ser Leu Trp Ser His
 260 265 270
 Ser Val Phe Ser Val Thr Asn Val Ser Val Val Ser Ser Gly Gly Gly
 275 280 285
 Ile Val Leu Gly Glu Arg Leu Ala Val Phe Tyr Ser Val Leu Arg Phe
 290 295 300
 Val Gly Val Glu Gly Pro Val Ala Ser Ser Leu Val Arg Cys Asp Gly
 305 310 315 320
 Gly Thr Val Gly Ala Gly Gly Trp Leu Glu Leu Arg Asp Val Trp Ala
 325 330 335
 Val Gly Glu Ala Ser Ser Val Ala Ser Leu Leu Gly Val Thr Leu Ser
 340 345 350
 Gly Gly Thr Val Ser Ile Val Arg Cys Ala Ala Thr Gly Ala Thr Leu
 355 360 365
 Val Ser Gly Leu Ala Ile Thr Ser Gly Thr Val Thr Val Gln Cys Asn
 370 375 380
 Arg Ala Gly Gly Arg Val Leu Gln Ser Gly Gly Asp Tyr Arg Met Ala
 385 390 395 400
 Gly Leu Pro Ser Val Ser Val Val Pro Cys Asp Gly Cys Ala Ser Ala
 405 410 415
 Leu Ala Cys Phe Asp Ala Leu Thr Ala Ser Phe Val Asp Cys Val Cys
 420 425 430
 Gly Cys Arg Asp Gly Gly Val Gly Glu Ala Cys Leu Pro Phe Asp Val
 435 440 445
 Pro Pro Ala Arg Ser Gly Gly Gly Gly Gly Ala Gln Asp Cys Val
 450 455 460
 Ser Gly Val Thr Leu Thr Glu Ser Val Thr Val Gly Gly Gly Arg Ala
 465 470 475 480

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Arg Ala Cys Phe Asp Ser Val Met Phe Ser Gly Pro Ile Ile Val Ala
 485 490 495
 Val Asp Leu Arg Ser Met Asp Ala Phe Ala Asp Ala Leu Asn Val Thr
 500 505 510
 Leu Arg His Cys Val Leu Glu Gly Gly Ala Gln Leu Arg Ile Gly Gly
 515 520 525
 Leu Ser Glu Ser Thr Ala Arg Leu Met Pro His Ala Leu Val Asn Met
 530 535 540
 Thr Asn Val Thr Ser Leu Glu Gly Thr Ile Val Leu His Gly Ala Met
 545 550 555 560
 Pro Leu His Ser Thr Val Leu Leu Ala Asn Ser Thr Leu Arg Ala Thr
 565 570 575
 Val Gly Gly Ser Gln Tyr Val Pro Thr Thr Arg Gly His Glu Gly Phe
 580 585 590
 Arg Tyr Asp Pro Ala Leu Val Leu Asp Gly Val Arg Leu Leu Ser Thr
 595 600 605
 Arg Phe Val Met Thr Arg Ser Thr Leu Val Cys Gly Gly Gly Ser Cys
 610 615 620
 Ala Ala Ile Leu Val Glu Arg Ala Phe Val Val Asn Leu Ser Ser Ala
 625 630 635 640
 Phe Tyr Met Asp Asn Cys Ala Val Arg Ser Gln Met His Val Met Tyr
 645 650 655
 Ala Leu Ala Ser Asp Leu Arg Val Gly Gly Gly Ser Val Phe Ser Ile
 660 665 670
 Gln Asn Ser Ser Trp Ser Ala Pro Ser Thr Glu Tyr Gly Lys Ala Ala
 675 680 685
 Cys Val Phe Glu Asp Ile Val Val Ala Gly Gly Ser Val Leu Gln Ile
 690 695 700
 Val Ser Ser Thr Phe Arg Leu Gly Phe Ala Met Leu Met Ala Asn Thr
 705 710 715 720
 Leu Thr Val Thr Gly Gly Ser Trp Leu Val His Arg Asp Ser Glu Phe
 725 730 735
 His Thr Ala Tyr Val Val Tyr Ile Val Lys Glu Asn Gly Val Lys Phe
 740 745 750
 Arg Asp Arg Ser Val Trp Ser Ile Ile Asp Asn Asn Phe Thr Tyr Gly
 755 760 765
 Ser Phe Ser Pro Phe Thr Tyr Met Thr Ser Asn Trp Ser Pro Pro Ser
 770 775 780
 Asp Thr His Pro Ile Ile Tyr Gly Val Cys Asn Glu Leu Arg Gly Ser
 785 790 795 800
 Pro Val Thr Asp Tyr Gln Tyr Asp Leu Asn Ile Gly Ala Ser Val Met
 805 810 815
 Val Leu Asp Cys Gly Ala Cys Thr Met Glu Ala Val Cys Phe Ala Ala
 820 825 830
 Arg Thr Ser Ser Ile Ser Gly Cys Glu Cys Val Cys Ala Ala Gly Gly
 835 840 845
 Tyr Gly Asp Thr Cys Leu Pro Ala Ala Val Pro Asp Gly Leu Gly Pro
 850 855 860
 Leu Pro Pro Pro Asp Ala Lys Asp Thr Glu Val Arg Cys Val His Gly
 865 870 875 880
 Gly Ser Ile Asp Ser Leu Asp Val Pro Asp Pro Gly Val Arg Gly Leu
 885 890 895
 Cys Phe Val Asn Val Thr Phe Thr Ala Ala Ile Val Leu Asp Leu Ser

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900				905				910							
Tyr	Phe	Asp	Ala	Pro	Gln	Gln	Thr	Leu	Asn	Ile	Thr	Leu	Leu	His	Cys
	915						920					925			
Val	Leu	Ile	Gly	Leu	Ser	Ile	Lys	Gly	Ser	Gly	Ala	Arg	Val	His	Val
	930					935					940				
Asn	Val	Val	Ser	Ser	Met	Leu	Asp	Ser	Gly	Glu	Leu	Glu	Phe	Arg	Gly
	945			950						955					960
Val	Phe	Gly	Val	Ser	Ser	Gln	Ile	Leu	Leu	Val	Gly	Ser	Thr	Leu	Val
			965							970				975	
Thr	Thr	Ser	Gly	His	Ala	Ile	Leu	Phe	Val	Lys	Phe	Ala	Leu	Ser	Ala
			980							985				990	
Asn	Met	Thr	Leu	Leu	Leu	Leu	Asp	Asn	Tyr	Ile	Glu	Gly	Asn	Trp	Tyr
		995					1000					1005			
Ala	Val	Tyr	Phe	Ser	Ser	Gly	Val	Val	Val	Val	Asp	Gly	Gly	Gly	Ile
	1010					1015						1020			
Ile	Val	Lys	Gly	Asn	Thr	Leu	Ser	Thr	Thr	Lys	Asp	Asp	Asp	Gly	
	1025					1030					1035				
Val	Glu	Ser	Ser	Val	Cys	Val	Tyr	Ala	Val	Asp	Val	Arg	Asn	Gly	
	1040				1045						1050				
Gly	His	Phe	Asp	Val	Glu	Asn	Asn	Thr	Met	Ser	Ala	Val	Asn	Gly	
	1055				1060						1065				
Val	Ile	Leu	Phe	Gly	Asp	Thr	Thr	Val	Ser	Ser	Ala	Gly	Leu	Leu	
	1070					1075					1080				
Arg	Val	Ala	Asp	Cys	Val	Phe	Val	Gly	Gly	Thr	Glu	Ile	Phe	Asp	
	1085					1090					1095				
Ser	Ala	Leu	Val	Tyr	Leu	Ser	Gly	Ser	Ala	Thr	Leu	Glu	Gly	Gly	
	1100					1105					1110				
Ala	Gln	Trp	Arg	Val	Glu	Gly	Asn	Ser	Val	Gly	Ala	Ala	Ser	Val	
	1115					1120					1125				
Leu	Ser	Ile	Pro	Tyr	Ser	Gln	Tyr	Lys	Ile	Gln	Leu	Ser	Gly	Ser	
	1130					1135					1140				
Gly	Thr	Thr	Val	Ala	Leu	Ala	His	Asn	Arg	Gln	Val	Glu	Gly	Ser	
	1145					1150					1155				
Ala	Val	Phe	Val	Lys	Leu	Phe	Arg	Pro	Asn	Thr	Ile	Val	Glu	Leu	
	1160					1165					1170				
Pro	Ala	Arg	Phe	Val	Val	Gly	Cys	Asn	Leu	Gln	Gly	Asp	Gly	Glu	
	1175					1180					1185				
Leu	Ser	Tyr	Asp	Gly	Leu	Phe	Pro	Glu	Glu	Leu	Val	Leu	Phe	Arg	
	1190					1195					1200				
Cys	Gly	Thr	Cys	Asn	Asp	Asp	Ala	Ala	Cys	Tyr	Met	Pro	Gly	Thr	
	1205					1210					1215				
Glu	Ser	Val	Asp	Arg	Gly	Ser	Cys	Ser	Cys	Ser	Cys	Lys	Asp	Gly	
	1220					1225					1230				
Trp	His	Gly	Ala	Ser	Cys	Leu	Pro	Phe	Glu	Val	Pro	Asp	Thr	Val	
	1235					1240					1245				
Val	Pro	Pro	Val	Ala	Glu	Arg	Ala	Val	Asp	Gly	Asp	Thr	Ser	Cys	
	1250					1255					1260				
Val	Val	Asn	Gln	Thr	Leu	Thr	Ser	Leu	Ala	Leu	Asn	Leu	Trp	Lys	
	1265					1270					1275				
Thr	His	His	Cys	Tyr	Val	Gly	Val	Thr	Phe	Ser	Gly	Val	Gly	Ala	
	1280					1285					1290				
Val	Leu	Thr	Phe	Phe	Leu	Asn	Ser	Met	Pro	Leu	His	Leu	Pro	Ile	
	1295					1300					1305				

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Asn Ile	Thr Leu	Thr Gly	Cys	Thr Phe	Arg Asp	Gly	Ala Ala	Leu			
1310			1315			1320					
Gln Phe	Val Gly	Gly Val	Gly	Ala Ala	Glu Ser	Ser	Gly Val	Leu			
1325			1330			1335					
Ile Arg	Val Ser	Gln Thr	Val	Met Arg	Ser Ser	Thr	Val Ala	Phe			
1340			1345			1350					
Ile His	Ala Leu	Pro Gln	His	Cys Asp	Ile Ala	Ile	Thr Glu	Val			
1355			1360			1365					
Asp Ala	Val Leu	Ser Phe	Asp	Phe Glu	Leu Ser	Gly	Thr Val	Asn			
1370			1375			1380					
Asn Met	Trp Ser	Val Phe	Leu	Leu Gly	Asn Val	Val	Leu Ser	Ala			
1385			1390			1395					
Ser Thr	Leu Leu	Val Ser	Asn	Val Lys	Ala His	Ala	Thr Asn	Arg			
1400			1405			1410					
Asp Ala	Leu Gly	Leu Tyr	Ser	Thr Gly	Thr Leu	Thr	Leu Val	Gly			
1415			1420			1425					
Gly Ser	Ser Leu	Tyr Leu	Arg	Tyr Cys	Ser Phe	Glu	Gly Tyr	Lys			
1430			1435			1440					
Tyr Leu	Phe Tyr	Val His	Ser	Leu Ser	Val Ser	Asp	His Ser	Val			
1445			1450			1455					
Phe Ala	Leu Leu	Asn Asn	Thr	Met Leu	Phe Gly	Ala	Ser Leu	Leu			
1460			1465			1470					
Tyr Gln	His Gln	Gly Phe	Ser	Val Ser	Asp His	Ser	Val Leu	Arg			
1475			1480			1485					
Val Val	Gly Asn	Ser Gly	Ser	Ala Arg	Tyr Ala	Ile	Cys Asn	Asp			
1490			1495			1500					
Asp Leu	Trp Thr	Val Gln	Arg	Ser Ser	Trp Leu	Asp	Trp Arg	Asp			
1505			1510			1515					
Asn Asp	Val Glu	Val Gly	Ala	Met Leu	Tyr Asp	Ser	Gly Phe	Ala			
1520			1525			1530					
Phe Val	Thr Ile	Asp Ser	Ser	Ser Ala	Val Thr	Leu	Thr Gly	Cys			
1535			1540			1545					
Arg Met	Gly Ser	Thr Gly	Leu	Ser Val	Ser Leu	Leu	Lys Arg	Ile			
1550			1555			1560					
Glu Ala	Gly Tyr	Arg Phe	Val	Ala Gly	Cys Leu	Thr	Val Ala	Gly			
1565			1570			1575					
Arg Glu	Val Thr	Thr Ala	Ala	Glu Leu	Glu Leu	Asn	Gly Ile	Thr			
1580			1585			1590					
Gly Val	Thr Thr	Ala Ala	Val	Cys Gly	Gln Cys	Thr	Lys Glu	Gly			
1595			1600			1605					
Asp Cys	Phe Ala	Pro Leu	Thr	Thr Ala	Val Ile	Asp	Cys Lys	Cys			
1610			1615			1620					
Gln Cys	Ala Ala	Gly Gly	His	Gly Asp	Val Cys	Val	Pro Ala	Pro			
1625			1630			1635					
Val Pro	Ala Gly	Pro Pro	Pro	Pro Pro	Leu Pro	Pro	Ala Pro	Pro			
1640			1645			1650					
Thr Pro	Leu Pro	Pro Pro	Val	Gly Glu	Cys Ile	Ser	Asp Met	Val			
1655			1660			1665					
Tyr Pro	Glu Val	Ala Gln	Ala	Val Gly	Gly Gly	Leu	Ser Trp	Leu			
1670			1675			1680					
Cys Tyr	Arg Asn	Val Thr	Phe	Ser Gly	Gly Gly	Met	Ser Leu	Thr			
1685			1690			1695					
Val Leu	Ile Gly	Ala Met	Thr	Gly Asp	Val Ala	Asn	Val Thr	Phe			
1700			1705			1710					

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Asp	Gly	Cys	Thr	Trp	Arg	Asp	Gly	Ala	Val	Leu	Leu	Leu	Gly	
1715						1720					1725			
Asn	Ala	Tyr	Ala	Ala	Val	Gly	Ser	Leu	Asn	Ile	Leu	Val	Ala	Gly
1730						1735					1740			
Asn	Thr	Phe	Ser	Asp	Ala	Leu	Leu	Ser	Pro	Glu	Gly	Val	Phe	Pro
1745						1750					1755			
Pro	Gln	Thr	Asn	Ile	Thr	Ile	Ser	Gly	Asn	Arg	Phe	Thr	Val	Thr
1760						1765					1770			
Arg	Leu	Ile	Pro	Arg	Ser	Gly	Leu	Asp	Ile	Trp	Ser	Pro	Ser	Cys
1775						1780					1785			
Val	Met	Met	Asn	Gly	Leu	Ala	Ile	Ser	Asn	Asp	Ser	Val	Val	Val
1790						1795					1800			
Leu	Ser	Gly	Asn	Val	Phe	Gln	Ser	Val	Thr	Ala	Ser	Ser	Ile	Ala
1805						1810					1815			
Ile	Tyr	Val	Val	Arg	Ser	Ala	Leu	Arg	Val	Ser	Trp	His	Ser	Val
1820						1825					1830			
Phe	Ala	Val	Val	Gly	Asn	Thr	Phe	His	Met	Ala	Gly	Gly	Asp	Gly
1835						1840					1845			
Thr	Leu	Ile	His	Leu	Glu	Gly	Ser	Ser	Gln	Ser	Ser	Ser	Leu	Ser
1850						1855					1860			
Val	Leu	Asn	Asn	Ser	Ala	Val	Val	Ile	Arg	Gly	Asn	Leu	Val	Thr
1865						1870					1875			
Arg	Pro	Val	Arg	Tyr	Phe	Leu	Leu	Leu	Thr	Leu	Ala	Leu	Arg	Val
1880						1885					1890			
Glu	Ser	Arg	Ser	Ala	Val	Val	Phe	Gln	Gly	Asn	Asp	Met	Gln	Arg
1895						1900					1905			
Ser	Ser	Val	Val	Phe	Phe	Pro	Gly	Phe	Ser	Ser	Tyr	Ile	Tyr	Tyr
1910						1915					1920			
Asn	Ser	Trp	Leu	Gln	Leu	Ser	Gly	Asn	Leu	Cys	His	Met	Ser	Pro
1925						1930					1935			
Ser	Glu	Ala	Phe	Ala	Phe	Leu	Tyr	Pro	Lys	Val	Asn	Leu	Arg	Asp
1940						1945					1950			
Ser	Thr	Val	Ser	Val	Ser	Gly	Asn	Arg	Phe	Met	Cys	Ser	Thr	Asp
1955						1960					1965			
Lys	Pro	Thr	Asp	Leu	Arg	Val	Ser	Thr	Ala	Ser	Arg	Asp	Ile	Thr
1970						1975					1980			
Asn	Gly	Ala	Ile	Val	Ala	Ala	Cys	Asn	Thr	Val	Asn	Gly	Glu	Glu
1985						1990					1995			
Gly	Val	Glu	Tyr	Ile	Ile	Pro	Ser	Val	Tyr	Asn	Val	Thr	Ile	Leu
2000						2005					2010			
Thr	Cys	Ser	Asp	Pro	Cys	Ala	Leu	Ala	Ala	Ser	Cys	Phe	Pro	Ala
2015						2020					2025			
Tyr	Thr	Thr	Thr	Ala	Ser	Ser	Asp	Gly	Cys	Ala	Cys	Thr	Cys	Ala
2030						2035					2040			
Glu	Gly	Gly	Arg	Gly	Asp	Ala	Cys	Leu	Pro	Ile	Ala	Val	Pro	Glu
2045						2050					2055			
Pro	Pro	Ser	Thr	Asp	Gly	Ala	Asp	Leu	Cys	Val	Arg	Glu	Val	Arg
2060						2065					2070			
Val	Asp	Val	Glu	Val	Asn	Ala	Gly	Leu	Gly	Thr	Ser	Val	Ala	Cys
2075						2080					2085			
Tyr	Val	Gly	Val	Thr	Phe	Ala	Ala	Asp	Val	Val	Val	Asp	Val	Glu
2090						2095					2100			
Leu	Met	Ser	Gly	Ser	Val	Arg	Asn	Val	Thr	Leu	Ala	Asn	Cys	Thr

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2105	2110	2115
Phe Val Gly Gly Ala Ser 2120	Leu Tyr Val Val Gly Trp 2125	Arg Ser Asp 2130
Pro Pro Ala Gly Glu Arg 2135	Ala Asp Val Leu Ile Ser 2140	Gly Leu Glu 2145
Ser Arg Ser Gly Gly Gly 2150	Val Leu Val Ala Asn Arg 2155	Phe Ser Pro 2160
Gly Ser Arg Val Thr Leu 2165	Val Asp Ser Val Leu Ile 2170	Ala Glu Lys 2175
Arg Val Val Tyr Arg Gly 2180	Ala Tyr Gly Leu Gly Asp 2185	Val Ser Ala 2190
Cys Leu Val Leu His Asn 2195	Val Asn Leu Thr Gly Ser 2200	Val Leu Thr 2205
Ile Ala Arg Thr His Val 2210	Ala Ala Val Phe Arg Gly 2215	Ala Val Gly 2220
Val Leu Phe Phe Gly Gly 2225	Val Ala Leu Ser Ser Arg 2230	Gly Ala Leu 2235
Tyr Val Asp Gly Leu Ser 2240	Val Gln Thr Ala Leu Gly 2245	Leu Cys Val 2250
Ser Val Glu Gly Gly Val 2255	Ser Ala Ser Gly Gly Ser 2260	Val Val Ala 2265
Phe Val Asp Ser Asp Phe 2270	Leu Leu Cys Lys His 2275	Ala Val Ser Val 2280
Arg Gly Ile Val Ser Val 2285	Ser Gly Ser Ala Val Ala 2290	Leu Val Arg 2295
Asn Glu Phe Ser Ser Thr 2300	Glu Asp Asn Ala Val Lys 2305	Phe Tyr Ser 2310
Thr Val Ser Leu Ala Gly 2315	Gly Ser Met Leu Leu Ala 2320	Lys Gly Asn 2325
Val His Asp Gly Val Ser 2330	Arg Glu Met Leu Tyr Ala 2335	Ala Gly Ala 2340
Val Thr Ala Ala Gly Ser 2345	Thr Leu Ser Phe Val Arg 2350	Asn Arg Ala 2355
Leu Leu Pro Arg Met Val 2360	Ser Leu Ser Leu Ser Leu 2365	Ala Ala Gly 2370
Ala His Leu Arg Val Ala 2375	Cys Asn Tyr Ala Gly Gly 2380	Arg Val Leu 2385
Ser Thr Ala Glu Glu Tyr 2390	Ala Ala Ala Gly Phe Gly 2395	Asp Ala Gly 2400
Ser Ile Asp Val Val Gly 2405	Cys Asp Ala Cys Asp Arg 2410	Asp Thr His 2415
Cys Tyr Ala Pro Gly Thr 2420	Ala Ser Ala Ser Met Thr 2425	Asn Gly Val 2430
Cys Val Cys Asp Cys Asp 2435	Ser Gly Gly Tyr Gly Glu 2440	Ala Cys Val 2445
Pro Val Gly Ala Pro Ala 2450	Leu Pro Pro Ala Val Gly 2455	Thr Ala Pro 2460
Ser Val Phe Phe Arg Glu 2465	Ser Val Thr Val Arg Ser 2470	Val Phe Val 2475
Val Pro Ala Gly Ala Ser 2480	Glu Val Thr Leu Arg His 2485	Val Val Leu 2490
Asp Gly Val Ser Pro Val 2495	Leu Tyr Val Pro Trp Met 2500	Ala Arg Asp 2505

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Gly Val	Arg Ile Val Val	Gln Asn Val Ser Leu Arg	Asn Gly Ala
2510		2515	2520
Val Leu	Tyr Val Met Gly	Gly Gly Gly Leu Arg Gly	Ala Val Ala
2525		2530	2535
Ala Gly	Ser Asp Glu Ser	Gly Pro Val Glu Leu Ser	Met Cys Glu
2540		2545	2550
Val Glu	Ala Leu Asn Gly	Ala Leu Val Leu Thr Gly	Thr Phe Pro
2555		2560	2565
Ala Gly	Ser Val Leu Thr	Val Thr Asp Ser Leu Leu	Val Ala Ala
2570		2575	2580
Arg Pro	Thr Pro Leu Val	Tyr Leu Pro Gly Ser Arg	Ser Ser Pro
2585		2590	2595
Tyr Ala	Pro Val Leu Val	Leu Ser Gly Leu Arg Leu	Val Arg Ser
2600		2605	2610
Val Leu	Val Val Ser Gly	Val Ala Leu Val Thr Val	Val Thr Gly
2615		2620	2625
Gly Arg	Thr Val Ala Val	Asp Gly Ala Val Leu Glu	Leu Val Gly
2630		2635	2640
Gly Gly	Val Ala Leu Asp	Ala Ala Val Leu Gly Gly	Asp Phe Ala
2645		2650	2655
Leu Tyr	Ala Ser Ala Arg	Val Val Ala Ser Gly Gly	Ala Val Leu
2660		2665	2670
Arg Val	Ser Gly Ser Gln	Val Tyr Ala Ala His Gly	Leu Val Phe
2675		2680	2685
Asp Ser	Gly Val Glu Ala	Asn Ala Ser Ala Val Val	Val Asn Asp
2690		2695	2700
Asn Ala	Gly Ala Leu Thr	Asp Gly Ala Leu Leu Glu	Leu Arg Gly
2705		2710	2715
Ser Ala	Ser Phe Gly Ser	Gly Ser Trp Leu Ser Val	Arg Gly Asn
2720		2725	2730
Ser Ile	Ser Gly Arg Leu	Leu Ser Val Pro Ser His	Pro Arg Ser
2735		2740	2745
Ala Lys	Leu Val Gln Ser	Thr Leu Thr Leu His Gly	Asn Thr Gly
2750		2755	2760
Ser Gly	Pro Val Val Met	Asp Gly Thr Val Ala Leu	Val Gly Ala
2765		2770	2775
Gly Arg	Arg Phe Val Val	Gly Cys Leu Thr Leu Asn	Gly Gln Val
2780		2785	2790
Leu Arg	Pro Met Asp Tyr	Arg Ser Ala Gly Ile Ile	Gly Glu Phe
2795		2800	2805
Arg Pro	Val Ala Cys Gly	Val Cys Asp Ala Asp Val	Arg Cys Phe
2810		2815	2820
Ala Ala	Ala Thr Arg Ala	Met Ser Gly Ser Cys Gly	Cys Arg Cys
2825		2830	2835
Ala Glu	Gly Gly Tyr Gly	Arg Asp Cys Leu Pro Val	Tyr Leu Pro
2840		2845	2850
His Val	Asp Gly Cys Asn	Arg Thr Pro Ala Met Pro	Pro Leu Ser
2855		2860	2865
His Thr	Ala Thr Leu Thr	Glu Thr Arg Ser Pro Thr	Pro Thr Trp
2870		2875	2880
Thr Pro	Ser Leu Ser Thr	Pro His Tyr Ser Pro Thr	His Tyr Gly
2885		2890	2895
Pro Thr	Lys Thr Leu Gln	Val Thr Gly Thr Val Ala	Leu Ser Pro
2900		2905	2910

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Thr	Arg	Thr	Pro	Thr	Ala	Ser	Val	Ser	Ser	Thr	Leu	Trp	Trp	Ser
2915						2920					2925			
Asp	Val	Ala	Cys	Pro	Thr	Leu	Thr	Val	Thr	Thr	Thr	Ala	Ala	Gly
2930						2935					2940			
Gly	Ser	Leu	Thr	Gln	Asn	Asp	Ile	Arg	Gly	Gly	Gly	Ser	Ala	Val
2945						2950					2955			
Pro	Thr	Trp	Leu	Met	Val	Ala	Leu	Pro	Pro	Pro	Phe	Arg	Trp	Ala
2960						2965					2970			
Arg	Asp	Pro	Gln	Ile	Gly	Thr	His	Leu	Ser	Phe	Val	Pro	Val	Ser
2975						2980					2985			
Thr	Ala	Gln	Pro	Ser	Gly	Phe	Gly	Gly	Pro	Trp	Gly	Ala	Met	Leu
2990						2995					3000			
Ser	Asn	Ala	Thr	Trp	Val	His	Asn	Ala	Thr	Asn	Pro	Ser	Thr	Val
3005						3010					3015			
Leu	Glu	Leu	Ala	Val	Pro	Val	His	Arg	Gly	Tyr	Phe	Ile	Ala	Ala
3020						3025					3030			
Asp	Glu	Thr	Ile	Val	Ile	Arg	Cys	Asp	Ala	Val	Ala	Val	Ser	Gly
3035						3040					3045			
Gly	Cys	Lys	Gly	Val	Leu	Leu	Gly	Ser	Phe	Thr	Ile	Arg	Ser	Ala
3050						3055					3060			
Thr	Leu	Pro	Ala	Ala	Ala	Ser	Val	Leu	Ser	Ala	Ile	Thr	Gly	Val
3065						3070					3075			
Val	Ala	Gly	Ala	Thr	Ala	Val	Ala	Val	Val	Val	Thr	Gly	Gly	Leu
3080						3085					3090			
Gly	Ser	Ile	Leu	Glu	Met	Gln	Ala	Leu	Gly	Val	Phe	Ala	Arg	Met
3095						3100					3105			
Pro	Cys	Ala	Ser	Ala	Gln	Glu	Arg	Ala	Ser	Thr	Val	Ala	Leu	Pro
3110						3115					3120			
Tyr	Phe	Leu	Ser	Val	Phe	Ala	Ala	Leu	Asp	Pro	Leu	Trp	Met	Val
3125						3130					3135			
Val	Gly	Asn	Ala	Leu	Leu	Ala	Ala	Val	Phe	Gly	Cys	Val	His	Cys
3140						3145					3150			
Gly	Val	Thr	Ala	Ala	Phe	Gln	Arg	Trp	Arg	Gly	Val	Asp	Ala	Ala
3155						3160					3165			
Ser	Ala	Trp	Ala	Ala	Met	Arg	Phe	Pro	Ser	Leu	Thr	Tyr	Val	Val
3170						3175					3180			
Gly	His	Ala	Met	His	Leu	Gly	Ile	Phe	Phe	Gly	Ser	Val	Leu	Ser
3185						3190					3195			
Leu	Ala	Thr	Pro	Gly	Ala	Arg	Val	Gln	His	Arg	Val	Ile	Gly	Val
3200						3205					3210			
Val	Gly	Val	Leu	Tyr	Gly	Val	Ala	Phe	Pro	Ala	Gly	Val	Cys	Tyr
3215						3220					3225			
Phe	Ile	Ala	Arg	His	Thr	Gly	Ala	Ser	Phe	Thr	Lys	Tyr	Trp	Gln
3230						3235					3240			
Phe	Ser	Arg	Lys	Pro	Leu	His	Glu	Arg	Leu	Leu	Tyr	Pro	Val	Gly
3245						3250					3255			
Tyr	Trp	His	Pro	Ala	Ala	Gln	Gln	Arg	Met	Tyr	Gly	Gly	Met	Leu
3260						3265					3270			
Thr	Asn	Met	Arg	Gly	Ser	His	Val	Tyr	Trp	Cys	Val	Phe	Gln	Leu
3275						3280					3285			
Ser	Val	Leu	Cys	Val	Val	Cys	Leu	Ile	Ala	Ala	Val	His	Ser	Pro
3290						3295					3300			
Val	Gly	Gly	Cys	His	Val	Gln	Tyr	Phe	Cys	Met	Ala	Ala	Val	Leu

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3305	3310	3315
Leu Ala Gly Ala Gly Val	Val Ala Phe Thr Asn Met	Met Arg Ser
3320	3325	3330
Ala Phe Leu Thr Val Met	Cys Thr Ala Ser Phe Val	Leu Leu Ala
3335	3340	3345
Val Leu Cys Val Val Ser	Ala Ala Asn His Leu Ala	Pro Ser Asp
3350	3355	3360
Gly Gly Ala Arg Ala Tyr	Ala Ala Ile Val Leu Leu	Leu Thr Thr
3365	3370	3375
Val Leu Leu Ala Val Thr	Val Tyr Ser Val Val Val	Trp His Val
3380	3385	3390
Glu Asp Arg His Trp Gln	Glu Leu Arg Glu Pro Leu	Arg Gly Gly
3395	3400	3405
Leu Glu Ala Leu Leu Arg	Asp Asp Glu Glu Ser Gly	Asp Glu Thr
3410	3415	3420
Gln Lys Pro His Glu Met	Ala Ser Ser Ser Tyr Ala	Ser Gly Thr
3425	3430	3435
Thr Ala Ala Ser Ser Tyr	Gln Pro Pro Ala Pro Pro	Leu Gln Pro
3440	3445	3450
Met Ala Gly Gly Thr Arg	Ser Asp Ala Leu Ser Leu	Phe Asp Arg
3455	3460	3465
Ala Ser Ser Ala Ser Arg	Met Phe Asp Tyr Ala Ala	Met
3470	3475	3480

<210> SEQ ID NO 55
 <211> LENGTH: 262
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 55

Met Ala Glu Gly Ile Lys Trp His Asn Ala Ala Ile Gln Asp Glu Leu	1	5	10	15
Val Pro Lys Lys Ser Pro Leu Glu Phe Lys Leu Pro Asp Ser Ala Lys	20	25	30	
Glu Leu Val Phe Met Ala Lys Leu Thr Glu Glu Ala Glu Arg Tyr Asp	35	40	45	
Glu Met Val Leu Cys Met Arg Lys Leu Val Lys Leu Asn Ser Glu Leu	50	55	60	
Asp Thr Glu Glu Arg Asn Leu Leu Ser Met Ala Tyr Lys Asn Val Ile	65	70	75	80
Gly Ser Arg Arg Asn Ala Trp Arg Ile Ile Thr Ser Ile Glu Ser Arg	85	90	95	
Glu Ser Ala Arg Glu Lys Ser Glu Asn Leu Gln Leu Ile Ala Ser Leu	100	105	110	
Arg Lys Glu Phe Glu Ala Glu Leu Ala Ala Ile Cys Asp Asp Leu Leu	115	120	125	
Ala Leu Leu Asp Thr Tyr Leu Ile Pro Ala Ser Gln Gly Gly Glu Val	130	135	140	
Lys Val Phe Tyr Leu Lys Met Lys Gly Asp Tyr His Arg Tyr Tyr Ala	145	150	155	160
Glu Ile Ala Pro Glu Ala Gly Gln Arg Gln Ala Ala Leu Asp Ala Tyr	165	170	175	
Ala Lys Ala Thr Glu Val Ala Asn Ser Ser Leu Ala Ser Thr His Pro	180	185	190	
Ile Arg Leu Gly Leu Ala Leu Asn Phe Ser Val Phe Tyr Tyr Glu Ile				

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195	200	205
Met Lys Glu His Glu Lys Gly Phe Gln Leu Ala Arg Gln Ala Tyr Asp 210 215 220		
Glu Ala Val Thr Glu Leu Glu Thr Leu Asp Asp Glu Ala Tyr Arg Glu 225 230 235 240		
Ser Asn Leu Ile Val Arg Leu Leu Arg Asp Asn Leu Asn Leu Trp Thr 245 250 255		
Asp Glu Gln Pro Ser Ser 260		

<210> SEQ ID NO 56
 <211> LENGTH: 252
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 56

Met Ser Ser Phe Thr Val Pro Glu Lys Arg Glu Gln Leu Ile Ile Met 1 5 10 15
Ala Lys Leu Ala Glu Gln Cys Glu Arg Tyr Asp Glu Ile Leu Val Cys 20 25 30
Met Lys Arg Val Val Lys Leu Asn Pro Val Leu Ser Ser Glu Glu Arg 35 40 45
Asn Leu Leu Ser Val Ala Tyr Lys Asn Val Ile Gly Ala Arg Arg Ala 50 55 60
Cys Trp Arg Ser Ile Thr Ala Leu Glu Gln Lys Glu Asp Ile Lys Lys 65 70 75 80
Glu Lys Asn Ile Thr Leu Ile Lys Gly Phe Lys Arg Gln Ile Glu Lys 85 90 95
Glu Leu Ser Asp Val Cys Ser Asp Ile Leu Glu Leu Ile Glu Glu His 100 105 110
Leu Leu Pro Asn Ala Ser Thr Glu Thr Lys Val Tyr Tyr Leu Lys 115 120 125
Met Lys Gly Asp Tyr His Arg Tyr Tyr Ala Glu Ile Glu Thr Asn Thr 130 135 140
Glu Glu Gln Lys Asn Lys Ala Leu Glu Ala Tyr Thr Gln Ala Met Gln 145 150 155 160
Tyr Asn Ser Ser Leu Lys Pro Thr Ser Pro Ile Arg Leu Gly Leu Ala 165 170 175
Leu Asn Phe Ser Val Phe Tyr Tyr Glu Ile Leu Lys Ser Pro Asp Arg 180 185 190
Gly Cys Gln Leu Ala Arg Gln Ala Phe Glu Glu Ala Leu Ser Asp Pro 195 200 205
Ser Val Leu Asp Glu Glu Gln His Lys Glu Ala Ala Leu Ile Met Gln 210 215 220
Leu Leu Arg Asp Asn Leu Ala Leu Trp Thr Glu Asp Ala His Pro Glu 225 230 235 240
Gly His Asp Asp Gly Thr Ala Met Glu Glu Leu Glu 245 250

<210> SEQ ID NO 57
 <211> LENGTH: 357
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 57

Met Ala Ser Ala Glu Val Val Ser Lys Leu Glu Ala Ala Phe Ala Lys 1 5 10 15
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Leu Gln Asn Ala Ser Asp Cys His Ser Leu Leu Lys Lys Tyr Leu Thr
 20 25 30
 Lys Glu Val Phe Asp Gln Leu Lys Gly Lys Gln Thr Lys Met Gly Ala
 35 40 45
 Thr Leu Met Asp Val Ile Gln Ser Gly Val Glu Asn Leu Asp Ser Gly
 50 55 60
 Ile Gly Val Tyr Ala Pro Asp Ala Glu Ser Tyr Thr Leu Phe Ala Ala
 65 70 75 80
 Leu Phe Asp Pro Ile Ile Glu Asp Tyr His Lys Gly Phe Lys Pro Ser
 85 90 95
 Asp Lys Gln Pro Pro Lys Asp Phe Gly Asp Leu Asn Thr Phe Ile Asp
 100 105 110
 Val Asp Pro Asp Lys Lys Tyr Val Ile Ser Thr Arg Val Arg Cys Gly
 115 120 125
 Arg Ser Leu Glu Gly Tyr Pro Phe Asn Pro Cys Leu Lys Lys Gln Gln
 130 135 140
 Tyr Glu Glu Met Glu Ser Arg Val Lys Gly Gln Leu Glu Ser Met Ser
 145 150 155 160
 Gly Glu Leu Arg Gly Lys Tyr Tyr Pro Leu Thr Gly Met Thr Lys Glu
 165 170 175
 Thr Gln Lys Gln Leu Ile Asp Asp His Phe Leu Phe Lys Glu Gly Asp
 180 185 190
 Arg Phe Leu Gln Ala Ala His Ala Cys Glu Phe Trp Pro Thr Gly Arg
 195 200 205
 Gly Ile Tyr His Asn Asp Ala Lys Thr Phe Leu Val Trp Val Asn Glu
 210 215 220
 Glu Asp His Leu Arg Ile Ile Ser Met Gln Lys Gly Gly Asn Leu Lys
 225 230 235 240
 Glu Val Phe Gly Arg Leu Val Thr Ala Val Gly Val Ile Glu Glu Lys
 245 250 255
 Val Lys Phe Ser Arg Asp Asp Arg Leu Gly Phe Leu Thr Phe Cys Pro
 260 265 270
 Thr Asn Leu Gly Thr Thr Ile Arg Ala Ser Val His Ile Lys Leu Pro
 275 280 285
 Lys Leu Gly Ala Asp Arg Lys Lys Leu Glu Glu Val Ala Ala Lys Tyr
 290 295 300
 Asn Leu Gln Val Arg Gly Thr Ala Gly Glu His Ser Asp Ser Pro Asp
 305 310 315 320
 Gly Val Tyr Asp Ile Ser Asn Lys Arg Arg Leu Gly Leu Ser Glu Tyr
 325 330 335
 Glu Ala Val Lys Glu Met Gln Asp Gly Ile Leu Glu Leu Ile Lys Ala
 340 345 350
 Glu Glu Ser Ala Arg
 355

<210> SEQ ID NO 58
 <211> LENGTH: 181
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <400> SEQUENCE: 58

Met Ser Thr Pro Gln Val Gln Asn Pro Pro Val Glu Asp Asp Glu Val
 1 5 10 15
 Pro Thr Leu Glu Ala Ala Glu Val Pro Gln Val Ala Lys Gln Thr Lys
 20 25 30

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Arg Tyr Ala Lys Ala Met Ala Lys Met Gly Leu Lys Pro Glu Pro Asn
 35 40 45
 Val Val Lys Val His Ile Arg Lys His Gly Ser Leu Ser Phe Leu Val
 50 55 60
 Asn Gln Pro Glu Leu Tyr Arg Phe Pro Gly Thr Asn Thr Phe Leu Val
 65 70 75 80
 Phe Gly Glu Ala Gln Leu Gly Asp Thr Ala Met Glu Ala Gln Glu Ala
 85 90 95
 Ala Ala Arg Ala Val Ser Gly Val Val Pro Glu Thr Glu Ser Arg Val
 100 105 110
 Glu Glu Val Pro Thr Thr Ala Glu Thr Pro Glu Thr Pro Val Pro Ala
 115 120 125
 Glu Lys Thr Asp Asp Ala Asp Ala Glu Asp Gly Gly Glu Leu Asp Glu
 130 135 140
 Arg Glu Ile Lys Val Val Met Ser Gln Gly Asn Thr Asp Arg Ala Gly
 145 150 155 160
 Ala Ile Arg Ala Leu Lys Asn Asn Lys Gly Asp Ile Val Asn Ala Ile
 165 170 175
 Leu Glu Leu Thr Met
 180

<210> SEQ ID NO 59
 <211> LENGTH: 177
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 59

Met Ser Tyr Lys Pro His His Ala Thr Val Pro Thr Asn Pro Lys Val
 1 5 10 15
 Phe Phe Asp Val Ser Ile Gly Gly Gln Ser Ala Gly Arg Val Val Phe
 20 25 30
 Glu Leu Phe Ala Asp Ala Val Pro Lys Thr Ala Glu Asn Phe Arg Ala
 35 40 45
 Leu Cys Thr Gly Glu Lys Asn Phe Gly Tyr Ala Gly Ser Gly Phe His
 50 55 60
 Arg Ile Ile Pro Gln Phe Met Cys Gln Gly Gly Asp Phe Thr Asn His
 65 70 75 80
 Asn Gly Thr Gly Gly Arg Ser Ile Tyr Gly Glu Lys Phe Ala Asp Glu
 85 90 95
 Ser Phe Ala Gly Lys Ala Gly Lys His Phe Gly Leu Gly Thr Leu Ser
 100 105 110
 Met Ala Asn Ala Gly Pro Asn Thr Asn Gly Ser Gln Phe Phe Ile Cys
 115 120 125
 Thr Ala Pro Thr Gln Trp Leu Asp Gly Lys His Val Val Phe Gly Gln
 130 135 140
 Val Leu Glu Gly Ile Glu Val Val Lys Ala Met Glu Ala Val Gly Ser
 145 150 155 160
 Gln Thr Gly Lys Thr Ser Lys Pro Val Lys Ile Glu Ala Ser Gly Gln
 165 170 175
 Leu

<210> SEQ ID NO 60
 <211> LENGTH: 340
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

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<400> SEQUENCE: 60

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Met Ser Leu Arg Leu Cys Val Cys Thr Ala Thr Ala Asn Gly Glu Tyr
1           5           10           15
Tyr Met Ser Met Phe Asn Lys His Leu Ser Ser Phe Phe Lys Thr Ile
          20           25           30
Thr Leu Gly Ser Thr Val Asp Asp Phe Lys Gln Asp Lys Asp Ser Lys
          35           40           45
Asp Ser Ile Ile Leu Tyr Val Glu Gly Pro Val Gly His Asp Ala Ile
          50           55           60
Lys Tyr Leu Cys Asp Asp Tyr His Leu Pro Lys Glu Gln Arg Arg Val
          65           70           75           80
Leu Trp Ile Tyr Ser Leu Thr Ala Gly Val Asp Val Tyr Arg Leu Gly
          85           90           95
Glu Leu Val Lys Glu Leu Gln Asp Ile Pro Phe Ala Asn Ala Arg Gly
          100          105          110
Cys Tyr Ser Ser Val Leu Ala Glu His Val Met Tyr Ser Met Leu Tyr
          115          120          125
Phe Tyr Arg Gln Thr Trp Arg Ser Leu Ala Ser Arg Ala Glu His Lys
          130          135          140
Trp Asp Pro Phe Leu Met Val Glu Leu Arg Gly Lys Lys Val Gly Ile
          145          150          155          160
Ile Gly Tyr Gly Asp Ile Gly Gln Ala Ser Ala Lys Leu Leu Ser Ala
          165          170          175
Phe Gly Met Glu Val Thr Gly Val Lys Arg Ser Ala Ser Thr Lys Glu
          180          185          190
Val Asp Glu Tyr Gly Val Arg Leu Val His Gly Asp Ala Glu Arg Glu
          195          200          205
Arg Val Leu Arg Glu Ser Asp Phe Val Val Asn Ile Leu Pro Gly Thr
          210          215          220
Glu Glu Thr Lys Arg Phe Phe Asn Lys Glu Leu Phe Ser Met Met Lys
          225          230          235          240
Pro Ser Ala Val Tyr Ile Ser Ile Gly Arg Gly Ile Thr Gln Asn Glu
          245          250          255
Asp Asp Leu Ala Cys Ala Leu Arg Asp Gly Val Ile Arg Gly Ala Ser
          260          265          270
Val Asp Val Phe Glu Arg Glu Pro Leu Pro Ala Glu Ser Pro Leu Trp
          275          280          285
Asp Ile Ser Asp Asp Lys Leu Leu Leu Thr Ala His Ser Ala Asp Arg
          290          295          300
Thr Ala Asn Leu Val Pro Asp Ser Val Arg Arg Phe Ile Gly Leu Val
          305          310          315          320
Asn Glu Tyr Ala Lys Thr Lys Arg Leu Asp Thr Tyr Leu Val Asp Pro
          325          330          335
Val Arg Gly Tyr
          340

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<210> SEQ ID NO 61

<211> LENGTH: 273

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 61

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Met Ser Ala Glu Arg Ala Ile Gly Ser Thr Ile Thr Leu Ile Thr Asn
1           5           10           15
Ser Leu Ile Arg Tyr Glu Gly Thr Leu Gly Gln Ile Asp Gly Pro Asn

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20					25					30					
Asn	Thr	Val	Ser	Leu	Thr	Asn	Val	Arg	Val	Phe	Gly	Thr	Glu	Gly	Arg
		35					40					45			
Gly	Gln	Glu	Ala	Gly	Leu	Ala	Gln	Ile	Pro	Pro	Ala	Asp	Gln	Leu	Phe
		50					55					60			
Asp	Gln	Ile	Val	Phe	Arg	Gly	Ser	Asp	Ile	Lys	Glu	Leu	Thr	Val	Phe
							70								80
Glu	Glu	Pro	His	Asn	Ala	Met	Met	Asp	Pro	Ala	Val	Val	Thr	Ala	Leu
				85					90					95	
Pro	Ala	Arg	Asn	Asn	Ser	Ala	Lys	Thr	Val	Ser	Ile	Asn	Gln	Arg	Asn
			100					105					110		
Gly	Pro	Ser	Ser	Gly	Asn	Thr	Ser	Ala	Gln	Tyr	His	Gln	Thr	His	His
		115					120					125			
Gln	His	Gln	His	Gln	His	Gln	His	Gln	Gln	Gln	Arg	Tyr	Gly	Gly	
		130					135					140			
Gly	Gly	Tyr	Arg	Arg	Gly	Gly	Gly	Ser	Gly	Tyr	Arg	Gly	Ser	Arg	Arg
				150								155			160
Val	Asp	Gly	His	Thr	Gly	Gln	Asp	Phe	Arg	Pro	Ala	Thr	Gly	Ala	Ala
				165					170					175	
Lys	Glu	Glu	Phe	Lys	Asp	Asp	Phe	Asp	Phe	Ser	Lys	Ser	Arg	Glu	Glu
				180				185					190		
Phe	Glu	Lys	Lys	Lys	Ser	Glu	Phe	Glu	Lys	Ala	Lys	Glu	Asp	Ala	Lys
			195				200					205			
Val	His	Ser	Lys	Ala	Tyr	Asp	Lys	Ser	Ser	Phe	Phe	Asp	Lys	Ile	Ser
				210			215					220			
Cys	Asp	Gln	Gln	Asp	Arg	Ala	Leu	Arg	Met	Asp	Arg	Glu	Gly	Val	Lys
							230					235			240
Arg	Ala	Asp	Ala	Glu	Thr	Phe	Gly	Ser	Glu	Met	Val	Gly	Asn	Met	Arg
				245					250					255	
Gly	Pro	Arg	Arg	Gly	Arg	Gly	Gly	Arg	Gly	Arg	Tyr	Asn	Gly	Arg	Tyr
				260				265					270		

Asn

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<210> SEQ ID NO 62
<211> LENGTH: 304
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (41)..(41)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (52)..(52)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (76)..(76)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (114)..(114)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (126)..(126)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (195)..(195)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (245)..(245)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (274)..(274)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (291)..(291)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 62

Cys Phe Phe Ala Leu Leu Ser Ala Ala Gly Leu Ile Ile Thr Arg Arg
 1 5 10 15
 Ser Ser Ala Lys Val Leu Leu Val Ser Met Ala Pro Thr Thr His Ser
 20 25 30
 Arg Leu Leu Ser Met Glu Gln Lys Xaa Leu Val Val Ser Ile Pro Lys
 35 40 45
 Arg Pro Glu Xaa Thr Thr Leu Asp Tyr Leu Ser Leu Val Pro Ser Arg
 50 55 60
 Arg Leu Leu Arg Gln Gln Ala Leu Thr Pro Leu Xaa Ser Thr Ser Leu
 65 70 75 80
 His Leu Thr Val Arg Pro Leu Leu Arg Arg Pro Leu Met Pro Lys Phe
 85 90 95
 Pro Leu Leu Leu Ser Leu Leu Arg Glu Phe Arg Ser Gly Ile Cys Phe
 100 105 110
 Ala Xaa Ser Pro Cys Phe Phe Leu Arg Thr Arg Arg Val Xaa Leu Asp
 115 120 125
 Pro Thr Val Leu Val Leu Ser Val Arg Lys Ser Ala Lys Leu Val Ser
 130 135 140
 Cys Leu Val Ile Ser Thr Arg Lys Ala Ala Leu Val Trp Tyr Pro Gly
 145 150 155 160
 Ala Ala His Ser His Met Arg Arg Leu Leu Arg Pro Gln Arg Arg Val
 165 170 175
 Ser Ala Asn Arg Cys Ala Leu Val Leu Val Ala Ile Arg Ser Met Val
 180 185 190
 Arg Thr Xaa Leu Thr Val Ser Asn Phe Ile Leu Arg Ile Leu Arg Arg
 195 200 205
 Arg Val Leu Phe Leu Leu Val Arg Leu Val Glu Leu Gln Arg Arg Lys
 210 215 220
 Pro Gln Ser Leu Ser Arg Thr Thr Pro Ser Arg Ser Arg Trp Tyr His
 225 230 235 240
 Ser Leu Ala Ala Xaa Leu Leu Arg Gln Ala Val Val Trp Ala Met Gln
 245 250 255
 Val Pro Trp Phe Leu Val Asp Arg Val Gln Gln Arg Ala Lys Trp Lys
 260 265 270
 Arg Xaa Lys Leu Arg Ala Ser Leu Phe Leu Ser Pro Leu Pro Ser Leu
 275 280 285
 Val Ser Xaa Trp Pro Arg Leu Ser Val Arg Ser Ala Glu Gln Arg Asn
 290 295 300

<210> SEQ ID NO 63
 <211> LENGTH: 3032
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1571)..(1571)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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<400> SEQUENCE: 63

Met Pro Phe Asp Val Pro Ser Ala Arg Thr Gly Gly Gly Gly Gly Gly
 1 5 10 15
 Ala Gln Asp Cys Val Ser Gly Val Thr Leu Thr Glu Ser Val Thr Ile
 20 25 30
 Gly Gly Gly Arg Ala Thr Ala Cys Leu Asp Ser Val Val Phe Ser Gly
 35 40 45
 Pro Ile Ile Val Ala Val Asp Leu Arg Ser Met Asp Ala Phe Ala Asp
 50 55 60
 Ala Leu Asn Val Thr Leu Arg His Cys Val Leu Ala Gly Gly Ala Gln
 65 70 75 80
 Leu Arg Ile Gly Gly Leu Ser Glu Ser Thr Ala Arg Leu Met Pro His
 85 90 95
 Ala Leu Val Asn Met Thr Asn Val Thr Ser Leu Glu Gly Thr Ile Val
 100 105 110
 Leu His Gly Ala Met Pro Pro Asn Ser Ser Val Leu Leu Ala Asn Ser
 115 120 125
 Thr Leu Arg Ala Thr Val Gly Gly Ser Gln Tyr Val Pro Thr Thr Leu
 130 135 140
 Asp Tyr Glu Gly Phe Arg Tyr Gly Pro Ala Leu Val Leu Asp Gly Val
 145 150 155 160
 Arg Leu Leu Ser Thr Arg Phe Val Met Thr Arg Ser Thr Leu Leu Cys
 165 170 175
 Gly Gly Gly Ser Cys Ala Ala Ile Leu Val Glu Arg Gly Leu Gly Ala
 180 185 190
 Asn Leu Ser Ser Val Phe Tyr Met Asp Asn Cys Ala Val Ser Ser Arg
 195 200 205
 Thr His Val Met Tyr Ala Leu Ala Ser Asp Leu Arg Val Gly Gly Gly
 210 215 220
 Ser Val Phe Ser Ile Gln Asn Ser Ser Trp Ser Ala Pro Ser Asn Glu
 225 230 235 240
 Phe Tyr Lys Gly Ala Cys Val Phe Glu Asp Val Ala Val Asp Gly Gly
 245 250 255
 Ser Val Leu Gln Ile Val Ser Ser Thr Phe Arg Leu Gly Phe Ala Met
 260 265 270
 Leu Ile Ala Asn Thr Leu Thr Val Ala Asp Gly Ser Trp Leu Val His
 275 280 285
 Arg Asn Ser Glu Phe Arg Thr Ala Tyr Val Val Tyr Val Val Lys Glu
 290 295 300
 Asn Gly Val Ser Phe Arg Asp Arg Ser Val Trp Ser Ile Leu Asp Asn
 305 310 315 320
 Asn Phe Thr Tyr Gly Ser Tyr Pro Ser Thr His Ala His Ile Thr Asn
 325 330 335
 Asp Trp Ser Pro Pro Ser Asp Ser Arg Pro Ile Ile Tyr Gly Met Cys
 340 345 350
 Asn Glu Ala Arg Gly Ser Pro Val Thr Asp Tyr Arg Asp Asp Leu Asn
 355 360 365
 Ile Gly Thr Ser Val Thr Val Leu Asp Cys Gly Thr Cys Thr Val Asp
 370 375 380
 Ala Val Cys Phe Ala Ala Arg Thr Ser Ser Ile Ser Gly Cys Glu Cys
 385 390 395 400
 Val Cys Ala Ala Gly Gly His Gly Asp Pro Cys Leu Pro Ala Ala Val
 405 410 415

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Pro	Asp	Gly	Leu	Gly	Pro	Leu	Pro	Leu	Pro	Asp	Ala	Lys	Asp	Thr	Glu
			420					425					430		
Val	Arg	Cys	Val	His	Gly	Gly	Ser	Ile	Ser	Ser	Val	Asp	Asp	Pro	Asp
		435					440					445			
Pro	Gly	Val	Arg	Gly	Leu	Cys	Phe	Val	Asn	Val	Thr	Phe	Thr	Ala	Ala
	450					455					460				
Ile	Val	Leu	Asn	Leu	Trp	Ser	Phe	Asp	Ala	Pro	Gln	Gln	Thr	Leu	Asn
465					470					475					480
Ile	Thr	Leu	Leu	Gln	Cys	Val	Leu	Met	Gly	Leu	Leu	Ile	Arg	Gly	Ser
				485					490					495	
Gly	Ala	Arg	Val	His	Val	Ser	Val	Val	Ser	Ser	Ile	Leu	Asp	Ala	Gly
			500						505				510		
Asp	Leu	Glu	Phe	Arg	Gly	Asp	Phe	Asp	Ser	Arg	Ser	Gln	Ile	Leu	Val
		515					520					525			
Ala	Gly	Ser	Lys	Leu	Val	Met	Thr	Ser	Gly	His	Thr	Ile	Gln	Phe	Gln
	530					535					540				
Arg	Phe	Ser	Leu	Gly	Ala	Asn	Ser	Thr	Leu	Leu	Leu	Leu	Asp	Asn	Leu
545					550					555					560
Ile	Glu	Gly	Glu	Ser	Tyr	Ala	Val	Arg	Leu	Ser	Phe	Val	Val	Leu	Asp
				565					570					575	
Gly	Gly	Gly	Ile	Leu	Ile	Lys	Gly	Asn	Thr	Leu	Arg	Gly	Ala	Glu	Glu
			580					585					590		
Asn	Phe	Leu	Leu	Glu	Ser	Ala	Val	Leu	Phe	Glu	Phe	Ala	Val	Leu	Lys
		595					600					605			
Asp	Gly	Gly	His	Phe	Asp	Val	Glu	Asn	Asn	Thr	Met	Ser	Ala	Val	Ser
	610				615						620				
Gly	Ile	Tyr	Phe	Tyr	Gly	Asp	Ile	Ile	Val	Ser	Ser	Ala	Gly	Leu	Leu
625				630					635						640
Arg	Val	Ala	Asp	Cys	Thr	Phe	Val	Gly	Ile	Thr	Leu	Phe	Phe	Glu	Pro
			645					650						655	
Ala	Leu	Val	His	Leu	Asp	Gly	Thr	Val	Thr	Leu	Gln	Gly	Gly	Ala	Gln
			660					665					670		
Trp	Arg	Val	Glu	Gly	Asn	Asp	Val	Ser	Ala	Ala	Ser	Val	Met	Gly	Val
	675					680						685			
Pro	Lys	Ser	Leu	Tyr	Lys	Met	Arg	Leu	Leu	Gly	Ser	Gly	Thr	Ala	Val
	690					695					700				
Val	Leu	Ala	Asn	Asn	Arg	Gln	Val	Asp	Asp	Ser	Cys	Pro	Phe	Ala	Asp
705				710						715					720
Leu	Ala	Leu	Pro	Asn	Met	Ile	Val	Val	Ser	Pro	Ala	Arg	Phe	Val	Val
			725					730						735	
Gly	Cys	Asn	Leu	Gln	Gly	Asp	Glu	Glu	Val	Leu	Tyr	Asp	Gly	Leu	Phe
			740					745					750		
Pro	Gly	Glu	Val	Val	Val	Phe	Arg	Cys	Gly	Thr	Cys	Asn	Asp	Asn	Ala
		755					760					765			
Ala	Cys	Tyr	Met	Pro	Gly	Thr	Glu	Ser	Val	Asp	Arg	Asp	Ser	Cys	Ser
	770					775					780				
Cys	Ser	Cys	Lys	Asp	Gly	Trp	Arg	Gly	Ala	Ser	Cys	Leu	Pro	Phe	Glu
785					790					795					800
Val	Pro	Asp	Thr	Val	Val	Pro	Leu	Leu	Pro	Glu	Arg	Ala	Val	Asp	Gly
			805						810					815	
Asp	Thr	Ser	Cys	Val	Val	Asn	Gln	Thr	Leu	Thr	Asn	Leu	Thr	Leu	Asn
			820					825					830		
Met	Trp	Lys	Thr	His	His	Cys	Tyr	Val	Gly	Val	Thr	Phe	Asn	Gly	Val
		835					840					845			

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Gly Ala Val Leu Lys Phe Phe Phe Asn Gly Met Pro Leu His Leu Pro
 850 855 860
 Ile Asn Ile Thr Leu Ser Gly Cys Thr Phe Arg Glu Gly Ala Ala Leu
 865 870 875 880
 Lys Phe Val Gly Gly Ala Glu Ala Ala Glu Ser Ser Gly Val Leu Ile
 885 890 895
 Arg Val Ser Gln Thr Val Met Arg Arg Ser Ser Val Val Val Phe Ala
 900 905 910
 Leu Ala Leu Pro Gln His Cys Asp Ile Ala Val Thr Glu Val Asp Ala
 915 920 925
 Val Gln Phe Ser Val Val Gln Leu Leu Glu Ser Val Asn Asn Met Trp
 930 935 940
 Ser Val Met Met Leu Arg Asn Val Val Leu Ser Ala Ser Ser Leu Leu
 945 950 955 960
 Val Ser Asn Val Lys Ala Arg Ala Phe Gly Tyr Gly Gly Phe Gly Leu
 965 970 975
 Tyr Ser Thr Gly Thr Leu Thr Leu Val Ser Gly Ser Ser Leu Tyr Val
 980 985 990
 Arg Tyr Cys Ser Leu Asp Gly Tyr Glu His Leu Phe Tyr Val His Gly
 995 1000 1005
 Leu Ser Val Arg Asp Tyr Ser Val Phe Ala Leu Leu Asn Asn Thr
 1010 1015 1020
 Met Val Ser Gly Thr Ser Phe Leu Tyr Gln Lys Gln Asn Phe Ser
 1025 1030 1035
 Val Ser Asp His Ser Val Leu Arg Val Val Gly Asn Ser Gly Ser
 1040 1045 1050
 Val Ser Asn Ala Ile Cys Ala Tyr Asn Leu Trp Ile Val Glu Gln
 1055 1060 1065
 Ser Ser Trp Leu Asp Trp Arg Asp Asn Asp Val Gly Val Gly Ala
 1070 1075 1080
 Met Phe Arg Tyr Ser Leu Ile Thr Ala Phe Ile Ile Asp Asp Ser
 1085 1090 1095
 Ser Val Thr Thr Leu Thr Gly Cys Lys Met Gly Leu Thr Gly Leu
 1100 1105 1110
 Ser Gly Pro Leu Leu Ser Gln Ala Asp Ala Gly Tyr Arg Phe Val
 1115 1120 1125
 Ser Gly Cys Leu Thr Val Ala Gly Arg Val Leu Thr Thr Ala Ala
 1130 1135 1140
 Glu Leu Glu Leu Asn Gly Ile Thr Asn Val Thr Met Val Ala Ala
 1145 1150 1155
 Cys Gly Gly Cys Thr Lys Glu Val Asp Cys Phe Ala Pro Leu Thr
 1160 1165 1170
 Thr Ala Val Ile Asp Cys Lys Cys Gln Cys Ala Ala Gly Gly His
 1175 1180 1185
 Gly Asp Val Cys Val Pro Ala Pro Val Pro Ala Gly Ser Leu Ser
 1190 1195 1200
 Pro Pro Pro Val Pro Pro Pro Pro Pro Pro Pro Arg Thr Pro
 1205 1210 1215
 Pro Pro Pro Pro Phe Gly Glu Cys Ile Ser Asp Met Val Tyr Pro
 1220 1225 1230
 Glu Val Ala Gln Ala Val Gly Gly Gly Leu Ser Trp Leu Cys Tyr
 1235 1240 1245
 Arg Asn Val Thr Phe Ser Gly Gly Gly Met Ser Leu Thr Val Leu

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1250	1255	1260
Ile Gly Ala Met Thr Gly Glu Val Ala Asn Ile Thr Phe Asp Gly 1265 1270 1275		
Cys Thr Trp Arg Asp Gly Ala Val Leu Leu Leu Leu Gly Asn Ala 1280 1285 1290		
Tyr Ala Ala Val Gly Ser Leu Asn Ile Val Val Thr Gly Asn Thr 1295 1300 1305		
Phe Ser Asp Ala Leu Leu Ser Pro Glu Gly Gly Phe Pro Pro His 1310 1315 1320		
Thr Asn Ile Thr Ile Ser Gly Asn Arg Phe Thr Val Thr Arg Leu 1325 1330 1335		
Ile Pro Arg Phe Cys Leu Gly Val Arg Arg Pro Ser Cys Val Ala 1340 1345 1350		
Met Asn Gly Leu Ala Val Ser Asn Asp Ser Ala Val Val Leu Ser 1355 1360 1365		
Gly Asn Val Phe Gln Thr Val Thr Ala Ser Ser Ser Ala Ile Tyr 1370 1375 1380		
Val Val Gly Ser Ser Leu Arg Val Ser Trp His Ser Val Phe Ala 1385 1390 1395		
Val Val Gly Asn Thr Phe His Met Ala Gly Ala Asn Gly Thr Leu 1400 1405 1410		
Ile Tyr Ile Gly Gly Ser Arg His Ser Leu Ser Leu Ser Val Leu 1415 1420 1425		
Asn Asn Ser Ala Val Val Ile Arg Gly Asn Val Val Thr Arg Pro 1430 1435 1440		
Val Lys Tyr Phe Ile Val Phe Leu Trp Ser Leu Arg Val Glu Ser 1445 1450 1455		
Gln Cys Ala Val Val Phe Gln Gly Asn Asp Met Gln Gly Ser Leu 1460 1465 1470		
Val Val Phe Tyr Ser Val Phe Ser Ser Tyr Ile Phe Tyr Asn Ser 1475 1480 1485		
Trp Leu Gln Leu Ser Gly Asn Leu Cys Arg Val Ser Pro Ser Glu 1490 1495 1500		
Gly Leu Thr Val Phe Asn Pro Thr Val Asn Leu Arg Asp Ser Thr 1505 1510 1515		
Val Ser Val Ser Gly Asn Arg Phe Met Ser Ser Thr Val Ser Pro 1520 1525 1530		
Thr Val Leu Leu Ile Pro Lys Ser Ser Arg Asp Leu Thr Asn Gly 1535 1540 1545		
Ala Ile Val Ala Ala Cys Asn Thr Val Asn Gly Gly Glu Glu Ala 1550 1555 1560		
Asn Tyr Val Ile Pro Phe Val Xaa Asn Ala Thr Ile Leu Thr Cys 1565 1570 1575		
Ser Gly Pro Cys Ala Leu Ala Ala Ser Cys Phe Pro Pro Tyr Thr 1580 1585 1590		
Ser Thr Ala Ser Ser Asp Gly Cys Ala Cys Thr Cys Ala Glu Gly 1595 1600 1605		
Gly His Gly Asp Ala Cys Leu Pro Val Ala Val Pro Glu Pro Pro 1610 1615 1620		
Ser Thr Asp Gly Ala Asp Leu Cys Val Gln Asp Val Arg Val Gly 1625 1630 1635		
Val Glu Val Ser Ala Gly Leu Gly Thr Ser Val Ala Cys Tyr Val 1640 1645 1650		

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Gly	Val	Thr	Phe	Ala	Ala	Asp	Val	Val	Val	Asp	Val	Gly	Leu	Met
1655						1660					1665			
Ser	Gly	Ser	Val	Arg	Asn	Val	Thr	Leu	Ala	Asn	Cys	Thr	Phe	Val
1670						1675					1680			
Asp	Gly	Ala	Ser	Leu	Tyr	Val	Val	Gly	Trp	Leu	Ser	Asp	Pro	Thr
1685						1690					1695			
Ala	Gly	Gln	Arg	Ala	Asp	Val	Leu	Ile	Ser	Gly	Leu	Glu	Ser	Arg
1700						1705					1710			
Ser	Gly	Gly	Gly	Val	Val	Val	Ala	Asn	Arg	Phe	Pro	Pro	Gly	Ser
1715						1720					1725			
Arg	Val	Thr	Val	Val	Asp	Ser	Val	Leu	Ile	Ala	Glu	Arg	Arg	Val
1730						1735					1740			
Ala	Tyr	Arg	Gly	Val	Tyr	Asp	Leu	Gly	Asp	Ala	Ser	Ala	Cys	Leu
1745						1750					1755			
Val	Val	His	Asn	Val	Asn	Leu	Thr	Gly	Ser	Val	Leu	Thr	Ile	Ala
1760						1765					1770			
Arg	Thr	Gln	Val	Val	Ala	Val	Phe	Arg	Asp	Ala	Val	Gly	Val	Leu
1775						1780					1785			
Val	Val	Gly	Gly	Val	Ala	Leu	Ser	Ser	Arg	Gly	Ala	Leu	Tyr	Val
1790						1795					1800			
Asp	Gly	Leu	Ala	Val	Gln	Thr	Ala	Leu	Gly	Leu	Cys	Val	Ser	Val
1805						1810					1815			
Glu	Gly	Gly	Val	Ala	Ala	Ser	Gly	Gly	Ser	Val	Val	Ala	Phe	Val
1820						1825					1830			
Asp	Ser	Asp	Phe	Leu	Leu	Cys	Lys	His	Ala	Val	Thr	Val	Arg	Gly
1835						1840					1845			
Ala	Val	Ser	Val	Ser	Gly	Ser	Met	Val	Ala	Phe	Val	Arg	Ser	Asp
1850						1855					1860			
Phe	Leu	Ser	Thr	Glu	Asn	His	Ala	Val	Ala	Phe	Tyr	Ser	Ala	Val
1865						1870					1875			
Ser	Leu	Ala	Gly	Gly	Ser	Met	Leu	Leu	Val	Lys	Gly	Asn	Val	His
1880						1885					1890			
Asp	Gly	Val	Ser	Arg	Glu	Met	Leu	Tyr	Ala	Ala	Gly	Ala	Val	Thr
1895						1900					1905			
Ala	Ala	Gly	Ser	Thr	Leu	Ser	Phe	Val	Arg	Asn	Arg	Ala	Leu	Leu
1910						1915					1920			
Pro	Arg	Met	Leu	Thr	Val	Ser	Leu	Ser	Leu	Val	Ala	Gly	Ala	His
1925						1930					1935			
Leu	Arg	Val	Ala	Cys	Asn	Asp	Ala	Gly	Gly	Arg	Val	Leu	Leu	Thr
1940						1945					1950			
Ala	Glu	Glu	Tyr	Ala	Ala	Ala	Gly	Phe	Gly	Asp	Ala	Gly	Arg	Ile
1955						1960					1965			
Asp	Val	Ala	Gly	Cys	Asp	Ala	Cys	Asn	Met	Asp	Thr	His	Cys	Tyr
1970						1975					1980			
Ala	Pro	Gly	Thr	Ala	Ser	Ala	Ser	Val	Thr	Asp	Gly	Val	Cys	Val
1985						1990					1995			
Cys	Ala	Cys	Ser	Ser	Gly	Gly	Tyr	Gly	Glu	Ala	Cys	Val	Pro	Val
2000						2005					2010			
Gly	Val	Pro	Ala	Leu	Pro	Pro	Pro	Val	Gly	Thr	Ala	Ser	Ser	Val
2015						2020					2025			
Phe	Val	Arg	Glu	Gly	Val	Thr	Val	Arg	Ser	Val	Phe	Val	Val	Pro
2030						2035					2040			
Ala	Gly	Ala	Ser	Glu	Val	Thr	Leu	Arg	His	Val	Val	Leu	Asp	Gly
2045						2050					2055			

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Val	Ser	Pro	Val	Leu	Tyr	Val	Pro	Trp	Met	Ala	Arg	Asp	Gly	Val
2060						2065					2070			
Arg	Ile	Val	Val	Gln	Asn	Val	Ser	Leu	Leu	Asn	Gly	Ala	Val	Leu
2075						2080					2085			
Tyr	Val	Met	Gly	Gly	Gly	Gly	Leu	Arg	Gly	Ala	Gly	Ala	Ala	Gly
2090						2095					2100			
Ser	Asp	Glu	Gly	Gly	Pro	Val	Glu	Leu	Ser	Val	Cys	Asp	Val	Glu
2105						2110					2115			
Ala	Leu	Asn	Gly	Ala	Leu	Val	Leu	Thr	Asp	Thr	Phe	Pro	Ala	Gly
2120						2125					2130			
Ser	Val	Leu	Thr	Val	Thr	Asp	Ser	Leu	Leu	Val	Ala	Ala	Arg	Pro
2135						2140					2145			
Thr	Pro	Leu	Val	Tyr	Leu	Pro	Gly	Ser	Gln	Ser	Ser	Pro	Tyr	Ala
2150						2155					2160			
Pro	Val	Leu	Val	Leu	Ser	Gly	Leu	Arg	Leu	Val	Arg	Ser	Val	Leu
2165						2170					2175			
Val	Val	Tyr	Gly	Val	Ala	Leu	Val	Thr	Val	Met	Thr	Gly	Gly	Arg
2180						2185					2190			
Thr	Val	Leu	Val	Asp	Gly	Ala	Val	Leu	Gly	Leu	Val	Gly	Gly	Gly
2195						2200					2205			
Val	Ala	Leu	Asp	Ala	Ala	Val	Leu	Gly	Gly	Asp	Val	Ala	Leu	Tyr
2210						2215					2220			
Ala	Ser	Ala	Arg	Val	Val	Ala	Ser	Glu	Gly	Ala	Val	Leu	Arg	Val
2225						2230					2235			
Ser	Gly	Ser	Arg	Val	Tyr	Ala	Ala	His	Gly	Leu	Val	Phe	Asp	Ser
2240						2245					2250			
Gly	Val	Glu	Ser	Asn	Ala	Ser	Ala	Val	Val	Val	Asn	Asp	Asn	Ala
2255						2260					2265			
Gly	Ala	Leu	Thr	Asp	Gly	Ala	Leu	Leu	Val	Leu	Arg	Gly	Ser	Ala
2270						2275					2280			
Ser	Phe	Ala	Ser	Gly	Ser	Trp	Leu	Ser	Val	Arg	Gly	Asn	Ser	Ile
2285						2290					2295			
Ser	Gly	Arg	Leu	Leu	Ser	Val	Pro	Ser	Tyr	Pro	Arg	Ser	Ala	Glu
2300						2305					2310			
Leu	Val	Gln	Ser	Thr	Leu	Thr	Leu	His	Gly	Asn	Ala	Gly	Ser	Gly
2315						2320					2325			
Pro	Val	Val	Met	Asp	Gly	Thr	Val	Ala	Leu	Val	Gly	Ala	Gly	Gly
2330						2335					2340			
Lys	Phe	Val	Leu	Gly	Cys	Leu	Thr	Leu	Asn	Gly	Gln	Val	Leu	Arg
2345						2350					2355			
Pro	Met	Asp	Tyr	Arg	Ser	Ala	Gly	Ile	Ile	Gly	Glu	Phe	Arg	Pro
2360						2365					2370			
Val	Ala	Cys	Gly	Val	Cys	Asp	Ala	Asp	Val	Arg	Cys	Phe	Ala	Ala
2375						2380					2385			
Ala	Thr	Arg	Ala	Met	Ser	Gly	Ser	Cys	Gly	Cys	Arg	Cys	Ala	Glu
2390						2395					2400			
Gly	Gly	His	Gly	Arg	Asp	Cys	Leu	Pro	Val	Tyr	Leu	Pro	His	Val
2405						2410					2415			
Asp	Gly	Cys	Asn	Arg	Thr	Pro	Gly	Met	Pro	Pro	Leu	Ser	His	Thr
2420						2425					2430			
Ala	Thr	Leu	Thr	Glu	Thr	Arg	Ser	Leu	Thr	Pro	Thr	Trp	Thr	Pro
2435						2440					2445			
Ser	Leu	Ser	Ala	Ala	His	Tyr	Ser	Pro	Thr	His	Tyr	Arg	Pro	Thr

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Leu Cys Val Val Cys Leu Ile Ala Ala Val His Pro Pro Val Gly
 2855 2860 2865
 Gly Cys His Val Leu Tyr Phe Cys Met Ala Ala Val Leu Leu Ala
 2870 2875 2880
 Gly Ala Gly Val Val Ala Phe Thr Asn Met Met Arg Ser Ala Phe
 2885 2890 2895
 Leu Thr Val Met Gln Thr Ala Ser Phe Val Leu Leu Ala Ala Leu
 2900 2905 2910
 Cys Val Ile Ser Ala Ala Asn His Leu Ala Pro Ser Asp Gly Gly
 2915 2920 2925
 Ala Arg Ala Tyr Ala Ala Ile Val Leu Leu Leu Thr Thr Val Val
 2930 2935 2940
 Leu Ala Val Thr Val Tyr Ser Val Val Val Trp Tyr Ala Glu Asp
 2945 2950 2955
 His His Trp Gln Glu Leu Arg Glu Pro Arg Arg Gly Gly Leu Glu
 2960 2965 2970
 Ala Leu Leu Arg Asp Asp Glu Glu Ser Asp Glu Glu Thr Gln Lys
 2975 2980 2985
 Pro His Asp Met Ala Ser Ser Ser Tyr Ala Ser Gly Thr Thr Val
 2990 2995 3000
 Ala Ser Ser Tyr Arg Pro Pro Ala Pro Ser Leu Gln Leu Met Ala
 3005 3010 3015
 Val Thr Pro Ala Arg Pro Cys Ile Gln Cys Glu Leu Gln His
 3020 3025 3030

<210> SEQ ID NO 64

<211> LENGTH: 257

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 64

Met Pro Ala Lys Ser Ala Asn Lys Pro Ala Ser Lys Pro Ala Ser Lys
 1 5 10 15
 Pro Ala Ala Lys Pro Ala Ala Lys Pro Ala Ala Lys Ala Pro Ala Pro
 20 25 30
 Lys Ala Glu Lys Lys Gly Ala Ala Lys Ala Pro Ala Pro Lys Ala Ala
 35 40 45
 Ala Pro Ala Pro Lys Ala Ala Ala Ala Ala Pro Lys Pro Ala Val Arg
 50 55 60
 Asp Ala Lys Gln Arg Ser Asp Ala Ala Asn His Asn Gly Leu Tyr Val
 65 70 75 80
 Lys Asn Trp Gly Gln Gly Ser Val Asp Asp Ala Arg Ala Leu Phe Gly
 85 90 95
 Thr Ala Gly Lys Val Val Gly Val Arg Val Arg Arg Arg Arg Tyr Ala
 100 105 110
 Ile Ile Phe Phe Glu Asn Ala Ala Ala Val Lys Lys Ala Ile Asp Leu
 115 120 125
 Phe Asn Gly Lys Glu Phe Met Gly Asn Val Leu Ser Val Val Pro Ala
 130 135 140
 Lys Thr Thr Pro Lys Pro Asp Pro His Ala Asn Ser Ser Val Val Phe
 145 150 155 160
 Val Ser Pro Ile Phe Arg Ala Ser Thr Thr Lys Lys Gln Ile Leu Glu
 165 170 175
 Leu Phe Ser Gly Met Lys Val Leu Arg Leu Arg Thr Tyr Arg Asn Asn
 180 185 190

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Tyr Ala Tyr Val Tyr Leu Asp Thr Pro Ala Ala Ala Gln Arg Ala Val
 195 200 205
 Lys Glu Lys Asn Gly Ala Glu Phe Arg Gly Lys Gln Leu Arg Val Ala
 210 215 220
 Leu Ser Thr Arg Ser Leu Ala Lys Asp Arg Ala Arg Ala Glu Arg Ala
 225 230 235 240
 Arg Leu Leu Met Ala Ala Gln Lys Phe Asn Lys Arg Lys Asn His Thr
 245 250 255

Lys

<210> SEQ ID NO 65
 <211> LENGTH: 134
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 65

Met Ala Asp Asn Ala Met Thr Arg Gly Ser Arg Ala Cys Tyr Asn Cys
 1 5 10 15
 Gly Gln Pro Gly His Leu Ser Arg Glu Cys Pro Thr Arg Pro Pro Gly
 20 25 30
 Val Met Gly Asp Arg Ala Cys Tyr Asn Cys Gly Arg Met Gly His Leu
 35 40 45
 Ser Arg Glu Cys Pro Thr Arg Pro Pro Gly Val Met Gly Asp Arg Ala
 50 55 60
 Cys Tyr Asn Cys Gly Arg Met Gly His Leu Ser Arg Glu Cys Pro Asn
 65 70 75 80
 Arg Pro Ala Gly Gly Phe Arg Gly Val Ala Arg Gly Ala Cys Tyr His
 85 90 95
 Cys Gln Gln Glu Gly His Leu Ala Arg Asp Cys Pro Asn Ala Pro Pro
 100 105 110
 Gly Gly Glu Arg Ala Cys Tyr Asn Cys Gly Gln Thr Gly His Thr Ser
 115 120 125
 Arg Ala Cys Pro Val Lys
 130

<210> SEQ ID NO 66
 <211> LENGTH: 222
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 66

Met Ser Val Lys Asp Val Asn Lys Arg Ser Gly Glu Leu Glu Gly Lys
 1 5 10 15
 Leu Lys Gly Lys Leu Phe Leu Gly Gly Thr Lys Pro Ser Gln Glu Asp
 20 25 30
 Val Lys Leu Phe Asn Asp Leu Leu Gly Ala Glu Asn Thr Ser Leu Tyr
 35 40 45
 Arg Trp Val Lys His Met Ala Ser Phe Thr Glu Ala Glu Arg Lys Ala
 50 55 60
 Trp Gly Ala Pro Val Lys Val Thr Ala Thr Thr Ser Ala Ser Ala Pro
 65 70 75 80
 Ala Lys Gln Ala Pro Lys Lys Ala Ala Ser Ala Pro Ala Lys Gln Ala
 85 90 95
 Asp Glu Asp Glu Asp Ile Asp Leu Phe Gly Glu Ala Thr Glu Glu Glu
 100 105 110
 Thr Ala Ala Leu Glu Ala Lys Lys Lys Lys Asp Ala Asp Ala Lys Lys
 115 120 125

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Ala Lys Lys Glu Val Ile Ala Lys Ser Ser Ile Leu Phe Asp Val Lys
 130 135 140

Pro Trp Asp Asp Thr Val Asp Leu Gln Ala Leu Ala Asp Lys Leu His
 145 150 155 160

Ala Val Lys Arg Asp Gly Leu Leu Trp Gly Asp His Lys Leu Val Pro
 165 170 175

Val Ala Phe Gly Val Lys Lys Leu Gln Gln Leu Ile Val Ile Glu Asp
 180 185 190

Asp Lys Val Ser Ser Asp Asp Leu Glu Glu Leu Ile Met Ser Phe Glu
 195 200 205

Asp Glu Val Gln Ser Met Asp Ile Val Ala Trp Asn Lys Ile
 210 215 220

<210> SEQ ID NO 67
 <211> LENGTH: 675
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (17)..(17)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (32)..(32)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (62)..(62)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (119)..(119)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (143)..(143)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (146)..(146)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (166)..(166)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (209)..(209)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (213)..(214)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (275)..(275)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <400> SEQUENCE: 67

Met Gln Gly Gly Gly Gly Gly Cys Thr Gly Gly Gly Asp Asn Asp Ala
 1 5 10 15

Xaa Cys Arg Trp Gly Gly Ala Glu Ala Cys Glu Gly Arg Glu Asp Xaa
 20 25 30

Gly Trp Lys Trp Thr Gly Ala Val Glu Glu Gly Gly Gly Ala Asp Cys
 35 40 45

Ala Phe Ser Gly Glu Gly Lys Gly Gly Gly Gly Gly Gly Xaa Glu Gly
 50 55 60

Gly Ser Gly Gly Ala Glu Asn Pro Gly Ser Ser Arg Glu Gly Ser Ser

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65		70				75				80					
Gly	Ala	Asp	Leu	Gly	Thr	Arg	Cys	Cys	Arg	Asn	Gly	Gln	Cys	Ala	Ala
				85					90					95	
Ala	Ala	Ala	Gly	Glu	Gly	Gly	Gly	Glu	Ala	Lys	Ser	Glu	Gly	Gln	Gly
			100					105					110		
Glu	Asp	Leu	Arg	Trp	Lys	Xaa	Thr	Arg	Arg	Glu	Cys	His	Glu	Gly	Cys
		115					120					125			
Val	Ala	Gly	Val	Gly	Gly	Val	Leu	Cys	Cys	Gly	Ser	Phe	Gln	Xaa	Arg
		130				135					140				
Asn	Xaa	Ser	Trp	Gln	Trp	His	Phe	Phe	Phe	Leu	Tyr	Phe	Lys	Ile	Asp
145					150					155					160
Tyr	Cys	Tyr	Gly	Asn	Xaa	Asp	Phe	Leu	Cys	Met	Asn	Gly	Asp	Val	Ile
			165						170					175	
Tyr	Leu	Cys	Val	Cys	Gly	Trp	Val	Leu	Phe	Thr	Ser	Thr	Phe	Ile	Cys
			180					185					190		
Thr	Ala	Phe	Cys	Cys	Arg	Phe	Phe	Leu	Ile	Leu	Leu	Lys	Phe	Leu	Lys
		195				200						205			
Xaa	Asn	Ser	Phe	Xaa	Xaa	Ala	Cys	Val	Ser	Val	Met	Tyr	Phe	Phe	Gly
	210					215					220				
Phe	Phe	Phe	Phe	Leu	Leu	Leu	Phe	Leu	Phe	Val	Leu	Phe	Gly	Ser	Cys
225				230						235					240
Thr	Ala	Pro	His	Leu	Asn	Phe	Gly	Gln	Leu	Pro	Leu	Arg	Leu	Leu	Thr
			245						250						255
Phe	Trp	Met	Pro	Leu	Phe	Ile	Val	Phe	Phe	Phe	Ser	Phe	Phe	Leu	Leu
			260					265						270	
Leu	Leu	Xaa	Gly	Lys	Phe	Cys	Val	Ile	Phe	Phe	Glu	Leu	Tyr	Leu	Phe
		275					280					285			
Phe	Phe	Phe	Cys	Phe	Gln	Cys	Val	Phe	Tyr	Val	Leu	Arg	Ser	Ala	Gly
	290					295					300				
Asp	Leu	Thr	Cys	Asp	Pro	Val	Phe	Phe	Leu	Cys	Phe	Phe	Phe	Phe	Phe
305					310					315					320
Arg	Leu	Gln	Glu	Cys	Ala	Ile	Gln	Lys	Thr	Phe	Met	Thr	Arg	Asn	Arg
				325					330						335
Pro	Phe	Phe	Ser	Leu	Leu	Leu	Leu	Leu	Ser	Cys	Ser	Val	Ile	Val	Gly
			340					345						350	
Ala	Asn	Ser	Leu	Glu	Lys	Lys	Ala	Ala	Thr	Pro	Gly	Lys	Ala	Glu	Gly
		355					360					365			
Ala	Gln	Pro	Gln	Ser	Ile	Ser	Pro	Ser	Ser	Ser	Ser	Pro	Gly	Asp	Arg
	370					375						380			
Thr	Gly	Val	Pro	Leu	Lys	Leu	Glu	Leu	Gly	Glu	Leu	Arg	Asp	Lys	Thr
385					390					395					400
Leu	Leu	Thr	Ala	Lys	Asp	Ala	Phe	Gly	Asn	Thr	Thr	Gly	Ala	Ala	Leu
			405						410					415	
Gln	Cys	Met	Gln	Ala	Lys	Thr	Asp	Val	Glu	Glu	Thr	Lys	Lys	Tyr	Ala
			420					425						430	
Glu	Glu	Ala	Lys	Lys	Leu	Phe	Asp	Lys	Ile	Gly	Arg	Asp	Tyr	Val	Ser
		435					440					445			
Lys	Ser	Ala	Ala	Leu	Ala	Asp	Ala	Val	Asn	Ala	Ser	Thr	Asp	Ala	Glu
	450					455						460			
Glu	Ala	Leu	Lys	Ser	Cys	Val	Glu	Ala	Glu	Lys	Ala	Ala	Val	Asp	Ala
465					470					475					480
Asp	Thr	Ala	Val	Leu	Ala	Ala	Leu	Leu	Glu	Val	Leu	Gln	His	Ser	Lys
			485						490						495

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Phe Trp Arg Arg Asp Thr Ala Val Ser Thr Glu Lys Leu Ala Asn Val
 500 505 510

 Ser Lys His Ser Ala Asn Ala Thr Asn Glu Ala Gln Lys Ala Glu Ile
 515 520 525

 Gln Ala Ser Lys Ala Ala Glu Ala Ala Lys Arg Ala Ala Glu Ser Lys
 530 535 540

 Lys Lys Ala Ala Ala Ala Leu Asp Thr Val Lys Glu Val Val Ala Met
 545 550 555 560

 Ala Glu Met Leu Lys Lys Lys Phe Phe Glu Asn Glu Arg Leu Gln Lys
 565 570 575

 Glu Lys His Lys Ala Gln Leu Glu Ala Glu Arg Arg Phe Ile Gln Glu
 580 585 590

 Lys Leu Glu Lys Lys Glu Ala Glu Val Lys Lys Ala Leu Asn Arg Ala
 595 600 605

 Ala Ala Ala Asp Lys Arg Val Ala Glu Leu Glu Arg Ala Arg Gln Lys
 610 615 620

 Gln Ser Lys Glu Gln Gly Asn Glu Gly Ser Gly His Arg Arg Val Arg
 625 630 635 640

 Arg Ser Gly Ser Asp Ser Ser Ser His Tyr Ala Pro Ala Tyr Glu Pro
 645 650 655

 Arg Leu Leu Leu Leu Pro Leu Leu Ser Phe Thr Leu Phe Cys Phe Val
 660 665 670

 Ala Trp Cys
 675

<210> SEQ ID NO 68

<211> LENGTH: 235

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 68

Cys Val Gln Ala Gly Met Ser Ala Lys Asn Ala Arg Glu His Glu Glu
 1 5 10 15

 Gly Ala Lys Asn Ala Leu Arg Lys Leu Gly Ser Glu Ala Thr Arg Met
 20 25 30

 Ser Arg Ala Leu Gln Gln Ala Glu Glu Ala Val Lys Leu Ala Asp Ala
 35 40 45

 Ala Val Ala Glu Cys Lys Ala Ala Glu Glu Ala Ala Gln Ala Ala Gly
 50 55 60

 Ile Met Thr Leu Asp Ala Val Gly Glu Val Leu Lys His Val Lys Asp
 65 70 75 80

 Glu Lys Thr Lys Val Gly Ser Gly Pro Glu Leu Leu Lys Arg Ala Ala
 85 90 95

 Glu Gln Thr Val Leu Ser Leu Glu Lys Ala Lys Glu Ala Glu Ala Glu
 100 105 110

 Ser Glu Lys Ala Ala Ala Ala Ala Arg Lys Thr Leu Glu Ala Ala Glu
 115 120 125

 Lys Ala Ala Ala Ala Arg Thr Leu Ala Gln Asp Val Ala Ala Thr Ala
 130 135 140

 Ser Ala Leu Leu Arg Gln Arg Glu Arg Glu Glu Arg Arg Arg Ala
 145 150 155 160

 Lys Asp Arg Glu Ala Ala Glu Ala Ala Lys Lys Ala Ala Ile Ala Glu
 165 170 175

 Val Met Lys Lys Phe Ala Ala Lys Lys Gly Asn Asp Ala Ala Ser Gly
 180 185 190

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Arg Asn Ser Thr Ala Thr Arg Ile Gln Arg Thr Arg Pro Arg Val Asp
195 200 205

Gly Gly Gly Ile Pro Leu Leu Leu Arg Ala Pro Leu Leu Met Val Ala
210 215 220

Ala Val Ala Ser Val Phe Gly Phe Leu Leu Cys
225 230 235

<210> SEQ ID NO 69

<211> LENGTH: 152

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 69

Met Ser Ile Glu Ser Ala Phe Tyr Ala Phe Ala Ser Phe Gly Gly Ala
1 5 10 15

Pro Thr Lys Glu Met Asp Asn Ala His Phe Ser Lys Met Leu Lys Glu
20 25 30

Thr Lys Val Ile Gly Lys Gln Phe Thr Ser Thr Asp Ala Asp Leu Leu
35 40 45

Phe Asn Lys Val Lys Ala Lys Gly Ala Arg Lys Ile Thr Leu Ser Asp
50 55 60

Phe Val Asp Lys Ala Val Pro Glu Ile Ala Ser Lys Leu Lys Lys Ser
65 70 75 80

Val Glu Glu Leu Ile Ala Asp Ile Ser Ser Cys Ser Pro Glu Ala Arg
85 90 95

Ala Thr Lys Ala Asp Ala Val Lys Phe His Asp Asp Lys Asn Met Tyr
100 105 110

Thr Gly Val Tyr Lys Ala Gly Gly Pro Thr Asn Val Asp Arg Asn Ser
115 120 125

Gly Ser Leu Ser Gly Val Val Asp Arg Arg Val Ala Gln Thr Asp Val
130 135 140

Cys Gly Thr Thr Ala Ser Gln Lys
145 150

<210> SEQ ID NO 70

<211> LENGTH: 211

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 70

Met Gly Ala Cys Gly Ser Lys Gly Ser Thr Ser Asp Lys Gly Leu Ala
1 5 10 15

Ser Asp Lys Asp Gly Lys Asn Ala Lys Asp Arg Lys Glu Ala Trp Glu
20 25 30

Arg Ile Arg Gln Ala Ile Pro Arg Glu Lys Thr Ala Glu Ala Lys Gln
35 40 45

Arg Arg Ile Glu Leu Phe Lys Lys Phe Asp Lys Asn Glu Thr Gly Lys
50 55 60

Leu Cys Tyr Asp Glu Val His Ser Gly Cys Leu Glu Val Leu Lys Leu
65 70 75 80

Asp Glu Phe Thr Pro Arg Val Arg Asp Ile Thr Lys Arg Ala Phe Asp
85 90 95

Lys Ala Arg Ala Leu Gly Ser Lys Leu Glu Asn Lys Gly Ser Glu Asp
100 105 110

Phe Val Glu Phe Leu Glu Phe Arg Leu Met Leu Cys Tyr Ile Tyr Asp
115 120 125

Phe Phe Glu Leu Thr Val Met Phe Asp Glu Ile Asp Ala Ser Gly Asn

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Phe Ala Gly Gln Glu Pro Gly Thr Ala Leu Glu Val Lys Glu Phe Ala
 20 25 30
 Cys Thr Arg Phe Lys Ala Asp Phe Pro Ile Met Ala Lys Ile Asp Val
 35 40 45
 Asn Gly Ser Lys Ala His Pro Leu Tyr Glu Phe Met Lys Ala Thr Ile
 50 55 60
 Pro Gly Leu Phe Gly Thr Lys Ala Ile Lys Trp Asn Phe Thr Ser Phe
 65 70 75 80
 Leu Ile Asp Arg His Gly Val Pro Val Glu Arg Phe Ser Pro Gly Ala
 85 90 95
 Ser Val Glu Asp Ile Glu Lys Lys Leu Leu Pro Leu Leu Gly Gly Ala
 100 105 110

Arg Ile

<210> SEQ ID NO 73
 <211> LENGTH: 556
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 73

Met Asp Ala Thr Glu Leu Lys Asn Arg Gly Asn Gln Glu Phe Ser Ser
 1 5 10 15
 Gly Arg Tyr Lys Glu Ala Ala Glu Phe Phe Ser Gln Ala Ile Asn Leu
 20 25 30
 Asp Pro Ser Asn His Val Leu Tyr Ser Asn Arg Ser Ala Cys His Ala
 35 40 45
 Ala Leu His Gln Tyr Pro Asn Ala Leu Gln Asp Ala Glu Lys Cys Val
 50 55 60
 Ser Ile Lys Pro Asp Trp Val Lys Gly Tyr Val Arg Lys Gly Ala Ala
 65 70 75 80
 Leu His Gly Leu Arg Arg Tyr Glu Glu Ala Ala Ala Tyr Asn Lys
 85 90 95
 Gly Leu Ser Leu Asp Pro Ser Ser Ala Ala Cys Thr Glu Gly Ile Ala
 100 105 110
 Ala Val Glu Lys Asp Lys Val Ala Ser Arg Met Gln Asn Pro Phe Ala
 115 120 125
 Asn Val Phe Gly Pro Asp Ala Ile Gly Lys Ile Gln Ala His Pro Lys
 130 135 140
 Leu Ser Leu Phe Leu Leu Gln Pro Asp Tyr Val Arg Met Ile Asp Glu
 145 150 155 160
 Val Met Lys Asp Pro Ser Ser Val Gln Lys Tyr Leu Lys Asp Gln Arg
 165 170 175
 Phe Met Ala Thr Phe Met Val Leu Ser Gly Leu Glu Leu Pro Glu Asp
 180 185 190
 Glu Asp Glu Glu Glu Lys Val Arg Arg Gln Gln Gln Lys Gln Lys
 195 200 205
 Glu Lys Glu Met Arg Glu Glu Gln Glu Lys Lys Arg Ala Ala Ala Thr
 210 215 220
 Glu Leu Ser Ser Glu Ala Lys Glu Ala Leu Arg Lys Lys Glu Glu Gly
 225 230 235 240
 Asn Ala Leu Tyr Lys Gln Arg Lys Phe Asp Glu Ala Leu Gln Lys Tyr
 245 250 255
 Gln Glu Ala Leu Ala Lys Asp Ser Thr Asn Thr Val Tyr Leu Leu Asn
 260 265 270

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Ile Thr Ala Val Ile Phe Glu Lys Gly Glu Tyr Ala Ala Cys Val Glu
 275 280 285
 Lys Cys Glu Glu Ala Leu Glu His Gly Arg Glu Asn Arg Cys Asp Tyr
 290 295 300
 Thr Val Leu Ala Lys Leu Met Thr Arg Glu Ala Leu Cys Leu Gln Arg
 305 310 315 320
 Leu Lys Arg Phe Asp Glu Ala Ile Ala Leu Phe Lys Lys Ala Leu Val
 325 330 335
 Glu His Arg Asn Ala Asp Thr Leu Ala Lys Leu Thr Ala Cys Glu Lys
 340 345 350
 Glu Lys Glu Lys Phe Glu Ile Glu Ala Tyr Leu Asp Pro Glu Ile Ala
 355 360 365
 Leu Gln Lys Lys Glu Glu Gly Asn Thr Phe Phe Lys Ser Asp Lys Phe
 370 375 380
 Pro Glu Ala Val Glu Ala Tyr Thr Glu Ala Ile Lys Arg Asn Pro Asp
 385 390 395 400
 Glu His Thr Thr Tyr Ser Asn Arg Ala Ala Ala Tyr Leu Lys Leu Gly
 405 410 415
 Ala Tyr Ser Gln Ala Leu Ala Asp Ala Glu Lys Cys Ile Ser Leu Lys
 420 425 430
 Pro Glu Phe Val Lys Ala His Ala Arg Arg Gly His Ala Phe Phe Trp
 435 440 445
 Thr Lys Gln Tyr Asn Lys Ala Leu Gln Ala Tyr Asp Glu Gly Leu Lys
 450 455 460
 His Asp Lys Glu Asn Ala Glu Cys Lys Glu Gly Arg Met Arg Thr Leu
 465 470 475 480
 Met Lys Ile Gln Glu Met Ala Thr Gly Asn Ser Ala Asp Gly Asp Glu
 485 490 495
 Val Ala Lys Arg Ala Met Ala Asp Pro Glu Val Ala Ala Ile Met Gln
 500 505 510
 Asp Ser Tyr Met Gln Leu Val Leu Gly Glu Met Gln Arg Asp Pro Ser
 515 520 525
 Arg Val Gln Glu Tyr Met Arg Asp Pro Thr Ile Ala Ala Lys Ile Asn
 530 535 540
 Thr Leu Ile Ser Ala Gly Ile Ile Arg Phe Gly Lys
 545 550 555

<210> SEQ ID NO 74
 <211> LENGTH: 396
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 74

Met Ala Ala Arg Ser Met Asp His Thr Gln Trp Leu Ala Lys Leu Val
 1 5 10 15
 Ala Phe Asp Thr Thr Ser Arg Asn Ser Asn Leu Glu Leu Ile His Tyr
 20 25 30
 Cys Lys Asp Tyr Leu Glu Gly Leu Gly Val Lys Cys Thr Leu Leu His
 35 40 45
 Asn Ala Glu Arg Asn Lys Ala Asn Leu Trp Ala Thr Leu Pro Gly Asp
 50 55 60
 Gly Gly Val Thr Lys Gly Gly Ile Ile Leu Ser Gly His Thr Asp Val
 65 70 75 80
 Val Pro Val Asp Gly Gln Lys Trp Asp Ser Asp Pro Phe Thr Leu Thr
 85 90 95

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Glu Arg Asp Gly Lys Leu Tyr Gly Arg Gly Thr Ser Asp Met Lys Gly
 100 105 110

Phe Val Ala Val Cys Met Ser Leu Ala Pro Glu Leu Leu Lys Met Lys
 115 120 125

Arg Ala Lys Pro Ile His Phe Ala Trp Ser Tyr Asp Glu Glu Val Ser
 130 135 140

Cys Leu Gly Gly Met Glu Leu Ala Glu Phe Ala Arg Asp His Asp Val
 145 150 155

Arg Ala Glu Gly Cys Ile Ile Gly Glu Pro Thr Gly Met Thr Val Val
 165 170 175

Ile Ala His Lys Gly Thr Ser His Phe Trp Val Arg Val Arg Gly Lys
 180 185 190

Ala Ala His Ser Ser Leu Ala Leu Thr Gly Glu Ser Cys Asn Ala Ile
 195 200 205

Asp Tyr Ala Thr Lys Leu Ile Thr Lys Leu Arg Glu Ile Ala Glu Glu
 210 215 220

Tyr Arg Arg Asn Gly Thr Arg His Asp Phe Gln Val Pro Phe Ser Thr
 225 230 235 240

Leu Ser Thr Asn Leu Ile Ser Gly Gly Asn Ala Ser Asn Thr Val Pro
 245 250 255

Ala Glu Cys Glu Phe Leu Phe Glu Phe Arg Ala Leu Pro Asn Glu Thr
 260 265 270

Val Ser Lys Met Met Gln Gln Val Arg Ser Tyr Val Glu Thr Gln Leu
 275 280 285

Leu Pro Ala Met Lys Ala Glu Phe Glu Asp Ala Glu Ile Val Ile Thr
 290 295 300

Pro Arg Asp Glu Thr Pro Ser Phe Glu Gly Ser Glu Glu Ala Pro Ile
 305 310 315 320

Thr Lys Leu Ala Cys Ala Ile Ile Asn Asp Tyr Lys Val Trp Lys Lys
 325 330 335

Asn Tyr Cys Thr Glu Ala Gly His Tyr Ser Gly Ile Ala Gly Ala Pro
 340 345 350

Thr Val Ile Cys Gly Pro His Gly Gly Ala Ile His Cys Ala Asn Glu
 355 360 365

Tyr Val Thr Pro Ala Gln Leu Asp Lys Cys Arg Glu Phe Val Leu Arg
 370 375 380

Val Ala Glu Ser Leu Lys Ala Ser Pro Ala His Leu
 385 390 395

<210> SEQ ID NO 75
 <211> LENGTH: 1125
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 75

Met Lys Arg Val Leu Val Leu Asn Val Asp Ser Phe Val Gly Arg Glu
 1 5 10 15

Val Cys Arg Arg Phe Phe Asp Ala Lys Glu Tyr Leu Val Asp Gly Thr
 20 25 30

Leu Tyr His Ser Gln Ser Thr Arg Gly Asn Pro Glu Ala Lys Glu Ser
 35 40 45

Leu Arg Thr Asp Gly Leu Asp Ser Ala Gly Thr Met Lys Pro His Cys
 50 55 60

Ala Thr Ala Ser Thr Ser Ser Val Arg Leu Asp Val Pro Ala Asp Met
 65 70 75 80

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Arg	Pro	Phe	Leu	Asn	Ser	Val	Met	Pro	Arg	His	Gly	Asp	Ile	Ser	Asp	85	90	95
Glu	Gln	Phe	Arg	Ala	Arg	Val	Leu	Thr	Tyr	Asp	Ile	Ile	Ile	Ala	Ile	100	105	110
Leu	Glu	Glu	Asp	Ala	Phe	Glu	Ala	Asp	Cys	Thr	Ile	Lys	Val	Leu	Arg	115	120	125
Gly	Thr	His	Tyr	Glu	Val	Glu	Lys	Thr	Phe	Val	Leu	Val	Ser	Asn	Val	130	135	140
Phe	Thr	Trp	Thr	Gln	Thr	Asp	Ala	His	Glu	Arg	Glu	Leu	Arg	Arg	Ala	145	150	155
Ala	Arg	Arg	Ala	Glu	Arg	Glu	Ala	Ala	Arg	Ala	Gln	Arg	Tyr	Glu	Asp	165	170	175
Asp	Val	Ile	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	180	185	190
Glu	Met	Glu	Gln	Lys	Val	Trp	Thr	Glu	Glu	Asp	Tyr	Arg	Gln	Arg	Tyr	195	200	205
Ser	Asp	Leu	Arg	Tyr	His	Leu	Trp	Lys	Asp	Leu	Glu	His	Ala	Val	Lys	210	215	220
His	Ala	Asn	Ser	Glu	Thr	Leu	His	Thr	Tyr	Val	Leu	Trp	Ala	Gly	Leu	225	230	235
Pro	Tyr	Gly	Arg	Gly	Glu	Asp	Leu	Leu	Ala	Pro	His	Phe	Asn	Ala	Ala	245	250	255
Trp	Arg	His	Gln	Glu	Met	Leu	Gln	Tyr	Gly	Asp	Gly	Ser	Asn	Tyr	Ile	260	265	270
Pro	Thr	Ile	His	Val	Lys	Asp	Leu	Ala	Arg	Ile	Ile	Tyr	Leu	Val	Gly	275	280	285
Ser	Ser	Tyr	Asp	Thr	Leu	Glu	Asp	Arg	Tyr	Met	Phe	Ala	Val	Asp	Gln	290	295	300
Gly	Asn	Asn	Thr	Gln	Ser	Asp	Ile	Leu	Gln	Gly	Ile	Lys	Asp	Phe	Ile	305	310	315
Gly	Gly	Val	Val	Ser	Val	Thr	Ala	Pro	Glu	Ile	Asp	Arg	Lys	Asn	Gln	325	330	335
Val	Val	Ile	Pro	Gly	Asn	Gln	Lys	Lys	Asp	Ile	Arg	Glu	Ala	Asp	Leu	340	345	350
Lys	Glu	Asn	Glu	Ser	Ala	Glu	Glu	Ile	Gln	His	Glu	His	Asp	Val	Leu	355	360	365
Val	Glu	Leu	Gly	Met	Arg	Glu	Ala	Arg	Val	Leu	Asp	Glu	Ala	Lys	Glu	370	375	380
Glu	Leu	Ser	Arg	Ile	Arg	Glu	Ile	Ser	Gln	Arg	Glu	Lys	Leu	Leu	Arg	385	390	395
Leu	Gly	Leu	Gly	Val	Gln	Glu	Ser	Gly	Asn	Asp	Asp	Asp	Thr	Asn	His	405	410	415
Ala	Leu	Met	Pro	Glu	Ala	Ala	Ser	Lys	Val	Gly	Ala	Ser	Leu	Thr	Ile	420	425	430
Ala	Pro	Pro	His	Gly	Leu	Ala	Asn	Trp	Phe	Ser	Ala	Val	Asp	Met	Arg	435	440	445
Cys	Glu	Pro	Gly	Ala	Val	Leu	Ala	Leu	Leu	Glu	Glu	Glu	Glu	Trp	Val	450	455	460
Ser	Leu	Gly	Gly	Phe	Phe	Ala	Asn	Val	Asp	Lys	Ile	Val	Gln	Glu	Phe	465	470	475
Lys	Ala	Ala	Arg	Gln	Leu	Arg	Pro	Met	Arg	Leu	Val	Leu	Ser	Gly	Pro	485	490	495
Pro	Leu	Ser	Lys	Val	Gly	Glu	Val	Ala	Gly	Val	Leu	Ala	Glu	Leu	Phe	500	505	510

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Asp Ile Pro His Leu Thr Leu Ala Asn Val Thr Ala Ala Tyr Glu Glu
 515 520 525
 His Val Arg Ser Leu Arg Glu Glu Leu Ile Gly Ile Leu Val Ser Arg
 530 535 540
 Gln Leu Arg Arg His Thr Ala Arg Glu Glu Arg Arg Arg Arg Glu Lys
 545 550 555 560
 Glu Arg Ala Lys Glu Ala Arg Arg Lys Ala Arg Glu Glu Glu Gly Glu
 565 570 575
 Glu Gly Glu Glu Glu Glu Glu Glu Glu Glu Gly Glu Glu Glu Gly
 580 585 590
 Glu Glu Glu Gly Glu Glu Gly Ala Glu Glu Glu Glu Glu Lys Gly Glu
 595 600 605
 Glu Gly Ser Pro Ala Ala Gln Asp Leu Asp Pro Ala Glu Arg Ile Arg
 610 615 620
 Ala Ala Ile Thr Glu Ala Met Leu Leu His Leu Pro Glu Asp Glu Glu
 625 630 635 640
 Glu Glu Glu Glu Asp Asp Ser His Tyr Asn Ala Asp Asp Asp Glu Glu
 645 650 655
 Val Asp Glu Lys Ala Ala Ala Met Ala Gln Gln Val Asp Glu Ala Glu
 660 665 670
 Arg Arg Arg Ile Ala Thr Leu Arg Glu Glu Tyr Arg Phe Ala Thr Gln
 675 680 685
 Leu Leu Ser Leu Arg Leu Leu Asn Gly Glu Phe Pro Arg Arg Pro Lys
 690 695 700
 Ser Ala Asp Glu Glu Glu Glu Gly Glu Glu Glu Glu Glu Tyr Asp Glu
 705 710 715 720
 Tyr Asp Asp Glu Glu Glu Tyr Met Asn Arg Lys Lys Arg Arg Glu Gln
 725 730 735
 Lys Lys Asp Val Lys Lys Asp Val Thr Glu Glu Pro Pro Pro Thr Ile
 740 745 750
 Arg Tyr Phe Asp Glu Val Met Ala Val Met Val Arg Trp Arg Leu Arg
 755 760 765
 Gln Glu Asp Cys Lys Asn Gln Gly Tyr Ile Leu Glu Ala Phe Pro Glu
 770 775 780
 Thr Val Arg Gln Ala Trp Leu Thr Phe Leu Ala Asp Thr Glu Glu Gln
 785 790 795 800
 Lys Glu Met Gln Arg Pro Lys Leu Gln Lys Val Arg Asp Glu Glu Ala
 805 810 815
 Val Pro Gln Pro Leu Leu Asp Ala Asn Phe Pro Leu Pro Val Leu Ala
 820 825 830
 Asp Leu Glu Glu Asp Arg Lys Gln Glu Ile Met Glu Leu Met Thr Gly
 835 840 845
 Arg Arg Phe Ala Ser Ala Glu Glu Val Leu Val Pro Thr His Asp Thr
 850 855 860
 Phe Phe Pro Asp Cys Phe Ile Ala Leu Thr Gly Asn Glu Val Thr Leu
 865 870 875 880
 Arg Ser Gln Ile Tyr Ala Ala Ile Ala Thr Ala Thr Pro Gly Asp Thr
 885 890 895
 Thr Pro Asp Phe Ser Glu Gln Phe Thr Arg Tyr Met Thr His Asn Arg
 900 905 910
 Pro Gly Ala Pro Pro Thr Glu Ser Leu Ile Cys Trp Phe Gln Thr Val
 915 920 925
 Val Ala Glu Arg Val Asp Glu Asp Gly Asp Val Ala Val Ala Thr Glu

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180

<210> SEQ ID NO 77
 <211> LENGTH: 734
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi
 <400> SEQUENCE: 77

Met Tyr Pro Arg Gln Asp Asp Val Ser Leu Leu Gln Arg Tyr Ile Asn
 1 5 10 15
 Asp Asn Lys Leu Glu Glu Lys Gly Val Pro Leu Thr Glu Leu Arg Arg
 20 25 30
 Leu Glu Gly Thr Gly Gln Val Met Leu Gly Val Asp Gly Thr Lys Ile
 35 40 45
 Ile Asp Leu Ile Thr Gln Ala Val Arg Glu Lys Glu Lys Met Ala Ile
 50 55 60
 Tyr Thr Tyr Thr Thr Pro Phe Thr Val Tyr Asp Lys Ile Thr Asp Val
 65 70 75 80
 Cys Lys Thr Leu Arg Ser Leu Lys Asn Cys Thr Pro Val Leu Val Phe
 85 90 95
 Asn Gly Ile Pro Phe Tyr Pro Asp Pro Ser Asp Asp Asn Cys Arg Glu
 100 105 110
 Lys Asn Met Val Pro Pro Asp Val Ala Ala Leu Asn Gly Thr Asp Ser
 115 120 125
 Ser Arg Leu Cys Asn Met His Ser Met Arg Ala Val Asp Ile Gln Lys
 130 135 140
 Lys Ser Ser Ser Arg Phe Phe Val Glu Glu Asp Val Glu Asn Gln Ile
 145 150 155 160
 Val Lys Leu Phe Arg Ser Glu Phe Lys Asp Thr Met Arg Ala Pro Tyr
 165 170 175
 Leu Ala Trp Ala Gln Leu Ser Ala Phe Cys His Pro Lys Asn Arg His
 180 185 190
 Ile Ser Glu Val Tyr Gly Cys Leu Glu Leu Leu Ala Phe Pro Gly Ile
 195 200 205
 Asp Arg Val Ile Thr Asn Ile Asn Val Met Lys Gly Thr Val Asp Met
 210 215 220
 Val Arg Lys Ser Arg Leu Leu Glu Leu Leu Arg Ile Ser Glu Asp Asp
 225 230 235 240
 Leu Gly Ser Leu Ile Val Val Asp Ser Arg Asn Arg Val Met Arg Thr
 245 250 255
 Val Gly Pro Lys Phe Ser Ser Phe Asp Asp Met Cys Lys Lys Ile Thr
 260 265 270
 Arg Val Asn Asp Leu Ser Leu Gly Ala Ser Tyr Val His Gln Leu His
 275 280 285
 Gln Glu Ala Met His Leu Ser Glu Gln Ala Arg Asn Arg Thr Leu Asn
 290 295 300
 Leu Lys Ser Ala Val Leu Arg Asn Leu Ala Ala Leu Gly Cys Pro Val
 305 310 315 320
 Leu Thr Leu Val Pro Pro Tyr Cys Thr Leu Leu Thr Arg Leu Tyr Glu
 325 330 335
 Ser Arg Arg Gln Leu Pro Ile Asp Pro Lys Met Val Met Gly Cys Pro
 340 345 350
 Leu Pro Pro Val Met Tyr Tyr Met Phe Thr Ala Gly Leu Leu Ser Pro
 355 360 365
 Ser Leu Phe Gly Ala Leu Cys Gln Gly Ser Leu Val Asp Asp Trp Pro

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20					25					30					
Ser	Asp	Gln	Glu	Ser	Thr	Ser	Cys	Ile	Leu	Phe	His	Arg	Gly	Trp	Arg
	35						40					45			
Ala	Asp	Lys	Leu	Tyr	Leu	Leu	Asp	Gly	Ala	Asp	Val	His	Cys	Leu	Ser
	50					55					60				
His	Leu	Asn	Lys	Ser	Val	Arg	Met	Ser	Val	Leu	Glu	Lys	Ser	Glu	Trp
	65					70					75				80
Glu	Arg	Asp	Gln	His	Arg	Gln	Lys	Arg	Glu	Gln	Ile	Lys	Glu	Arg	Met
			85						90					95	
Lys	Glu	Lys	Glu	Met	Arg	Tyr	Val	Leu	Thr	Lys	Tyr	Ser	Gly	Val	Phe
			100						105					110	
Ser	Ala	Cys	Val	Ala	Val	Leu	Gly	Val	Val	Ser	Val	Phe	Gly	Trp	Asn
		115					120					125			
Phe	Lys	Asn	Tyr	Lys	Lys	Gln	Gln	Arg	Ser	Tyr	Gln	Leu	Ala	Ile	Ala
	130					135					140				
Ala	Ser	Ala	Leu	Ser	Gln	Pro	His	Ser	Lys	Arg	Pro	Ile	Lys	Asp	Tyr
	145					150					155				160
Val	His	Arg	Glu	Asp	Glu	Glu	Gln	Arg	Leu	Arg	Gln	Thr	Leu	Arg	Gln
			165						170					175	
Gln	Asp	Leu	Ser	His	Pro	Arg	Ile	Leu	Val	Phe	Ala	Gly	Phe	Tyr	Gly
		180							185					190	
Cys	Gly	Lys	Ser	Ile	Leu	Phe	Arg	Ser	Ala	Ile	Arg	Lys	Glu	Lys	Met
		195					200					205			
Ala	Ala	Ala	Phe	Val	Asp	Ile	Arg	Pro	Asn	Glu	Asp	Pro	Leu	Arg	Ser
		210					215					220			
Ile	Val	Lys	Ser	Leu	Asn	Val	Gln	Asn	Ile	Asp	Ala	Cys	Gly	Asp	Leu
		225				230					235				240
Leu	Asn	Phe	Ile	Gly	Glu	Ala	Ser	Asp	Arg	Ala	Arg	Lys	Ala	Met	His
			245						250					255	
Gly	Val	Thr	Pro	Leu	Phe	Val	Leu	Lys	Ile	Arg	Asp	Gly	Ser	Ser	Leu
			260						265					270	
Leu	Arg	Ile	Tyr	Asn	Glu	Val	Val	Ala	Leu	Ala	Cys	Asp	Arg	Arg	Leu
		275							280				285		
Cys	His	Val	Ile	Ile	Glu	Val	Pro	Ile	Glu	Ser	Leu	Thr	Ile	Ala	Met
		290					295					300			
Thr	Ala	Leu	Pro	Arg	Leu	Asp	Phe	His	Leu	Val	Pro	Asn	Phe	Ser	Val
		305				310					315				320
Ser	Glu	Ala	Phe	Arg	Tyr	Thr	His	His	Leu	Ile	Asp	Pro	Leu	Glu	Leu
			325						330					335	
Thr	His	Phe	Val	Glu	Val	Val	Gly	Thr	Asn	Ser	Asn	Asp	Leu	Asp	Glu
			340						345				350		
Leu	Leu	Ala	Ala	Val	Arg	His	Ala	His	Met	Ser	Ala	Thr	Thr	Tyr	Thr
		355					360					365			
Asn	Gln	Lys	Leu	Val	Lys	Ala	Met	Arg	Gln	Leu	Gln	Ala	Ala	Trp	Ala
		370					375					380			
Lys	Asp	Pro	Ser	Leu	Arg	Glu	Ala	Val	Ile	Lys	Leu	Ala	Arg	Phe	Pro
		385				390					395				400
Phe	Glu	Glu	Gly	Gln	Ser	Glu	Gly	Tyr	Asp	Tyr	Ser	Ser	Leu	Arg	Asn
			405						410					415	
Glu	Ala	Leu	Arg	Asp	Ile	Val	Met	Tyr	Asn	Ala	Val	Ala	Asp	Val	Trp
			420						425					430	
Met	Phe	Gln	Gln	Lys	Val	Phe	His	Thr	Ala	Ala	Cys	Cys	Trp	Gln	
		435					440						445		

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<210> SEQ ID NO 79
<211> LENGTH: 565
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 79

Met His Ser Arg Val Ala Ala Val Lys Ala Pro Arg Thr His Asn Arg
1          5          10          15
Arg Arg Val Thr Gly Ser Ser Gly Arg Arg Gly Glu Gly Gly Glu Ser
20          25          30
Glu Pro Pro Arg Pro Asn Met Ser Arg Arg Val Phe Thr Ser Ala Val
35          40          45
Leu Leu Leu Leu Phe Val Leu Met Cys Gly Ala Ala Gly Pro Val Gln
50          55          60
Ala Gln Ser Phe Gly Ala Gly Val Asp Asp Ser Tyr Gly Arg Gln Gly
65          70          75          80
Ser Arg Gln Leu Pro Arg Glu His Gln Ala Ala Asn Pro Pro Ile Pro
85          90          95
Gly His Ile Phe Arg Asn Pro His Leu Val Asn Val Asn Gly Met Leu
100         105         110
Leu Ala Ile Ala Gly Ala Gln Phe Asn Arg Thr Val Gly Ser Gly Ser
115         120         125
Ala Ser Met Gln Leu Thr Ala Gln Leu Ser Val Asn Arg Gly Val Asn
130         135         140
Trp Ser Pro Tyr Pro Arg Pro Gly Asp Ile Asp Cys Phe Ala Ala His
145         150         155         160
Pro Tyr Trp Met Ser Phe Pro Ser Ser Phe Gly Pro Phe Gly Ser Leu
165         170         175
Phe Ala Phe Val Glu Gly Tyr Asp Leu Arg Lys Gly Val Arg Pro His
180         185         190
Tyr Ala Asn Arg Trp Gly Gly Ser Gly Val Glu Pro Val Ile His Phe
195         200         205
Leu Asp Thr Arg Pro Gly Gln Ser Gly Gly Leu Ser Met Ser Ser Val
210         215         220
Ser Met Ser Ile Leu Leu Pro Tyr Pro His Lys Ser Gly Asp Met Ile
225         230         235         240
Gly Phe Leu Asn Asp Ala Ser Thr Pro Ile Thr Lys Met Thr Asp Gly
245         250         255
Thr Leu Val Phe Pro Val Gln Phe Leu Thr Met Gly Gly Asp Thr Ala
260         265         270
Ser Thr Ile Met Tyr Met Asn Phe Thr Gln Gln His Trp Thr Phe Ala
275         280         285
Lys Ser Ala Thr His Ala Gly Cys Thr Asn Pro Ser Ile Leu Glu Trp
290         295         300
Glu Asp Gly Lys Ile Ile Met Ile Thr Ser Cys Glu Tyr Gly Arg Arg
305         310         315         320
Arg Val Tyr Glu Ser Thr Asp Lys Gly Asn Thr Trp Thr Glu Ala Leu
325         330         335
Gly Thr Leu Ser Arg Val Trp Ser Asn Pro Leu Ala Arg Ser Gly Leu
340         345         350
His Ile Gln Gly Gly Phe Ile Thr Ala Thr Ile Asp Gly Lys Lys Val
355         360         365
Ile Leu Leu Thr Gln Leu Glu Tyr Phe Gly Asp Lys Glu Arg Ser Glu
370         375         380

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Ile His Leu Trp Leu Thr Asp Thr Asn Arg Ile Tyr His Val Gly Leu
 385 390 395 400

Leu Pro Thr Gly Tyr Gly Ala Thr Ser Ser Ser Leu Leu Tyr Ala Asn
 405 410 415

Asp Lys Leu Tyr Cys Leu Tyr Glu Ala Gly Val Gly Ser Ser Ser Gly
 420 425 430

Ala Phe Phe Leu Asp Leu Thr His Glu Leu Gln Arg Ile Arg His Ala
 435 440 445

Leu Ser Thr Trp Ala Val Lys Asp Asn Ala Leu Arg Arg Cys Ser Thr
 450 455 460

Gly Ala Ala Asp Ala Ala Leu Ser Arg Trp Asp Cys Ser Val Pro Ile
 465 470 475 480

Pro Thr Ala Gly Leu Val Gly His Leu Ala Asp Thr Leu Arg Gly Asp
 485 490 495

Lys Trp Glu Asp Glu Tyr Leu Gly Val Asn Ala Val Val Arg Gly Ala
 500 505 510

Thr Lys Lys Val Pro Ser Gly Leu Thr Phe Glu Gly Arg Ser Ala Gly
 515 520 525

Ala Glu Trp Pro Val Cys Lys Gln Trp Pro Lys Met Pro Phe His Phe
 530 535 540

Ala Asn Tyr Gly Phe Thr Leu Ala Ala Thr Val Ser Ile Gln Glu Val
 545 550 555 560

Pro Thr Gly Ile Thr
 565

<210> SEQ ID NO 80
 <211> LENGTH: 467
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 80

Met Ser Gly Trp Ala Arg Ala Leu Leu Leu Ala Ala Val Leu Val Val
 1 5 10 15

Met Ala Cys Leu Val Pro Ala Ala Thr Ala Ser Leu His Ala Glu Glu
 20 25 30

Thr Leu Thr Ser Gln Phe Ala Glu Phe Lys Gln Lys His Gly Arg Val
 35 40 45

Tyr Glu Ser Ala Ala Glu Glu Ala Phe Arg Leu Ser Val Phe Arg Glu
 50 55 60

Asn Leu Phe Leu Ala Arg Leu His Ala Ala Ala Asn Pro His Ala Thr
 65 70 75 80

Phe Gly Val Thr Pro Phe Ser Asp Leu Thr Arg Glu Glu Phe Arg Ser
 85 90 95

Arg Tyr His Asn Gly Ala Ala His Phe Ala Ala Ala Gln Glu Arg Ala
 100 105 110

Arg Val Pro Val Lys Val Glu Val Val Gly Ala Pro Ala Ala Val Asp
 115 120 125

Trp Arg Ala Arg Gly Ala Val Thr Ala Val Lys Asp Gln Gly Gln Cys
 130 135 140

Gly Ser Cys Trp Ala Phe Ser Ala Ile Gly Asn Val Glu Cys Gln Trp
 145 150 155 160

Phe Leu Ala Gly His Pro Leu Thr Asn Leu Ser Glu Gln Met Leu Val
 165 170 175

Ser Cys Asp Lys Thr Asp Phe Gly Cys Ser Gly Gly Leu Met Asn Asn
 180 185 190

-continued

Ala Phe Glu Trp Ile Val Gln Glu Asn Asn Gly Ala Val Tyr Thr Glu
 195 200 205

Asp Ser Tyr Pro Tyr Ala Ser Gly Glu Gly Ile Ser Pro Pro Cys Thr
 210 215 220

Thr Ser Gly His Thr Val Gly Ala Thr Ile Thr Gly His Val Glu Leu
 225 230 235 240

Pro Gln Asp Glu Ala Gln Ile Ala Ala Trp Leu Ala Val Asn Gly Pro
 245 250 255

Val Ala Val Ala Val Asp Ala Ser Ser Trp Met Thr Tyr Thr Gly Gly
 260 265 270

Val Met Thr Ser Cys Val Ser Glu Gln Leu Asp His Gly Val Leu Leu
 275 280 285

Val Gly Tyr Asn Asp Ser Ala Ala Val Pro Tyr Trp Ile Ile Lys Asn
 290 295 300

Ser Trp Thr Thr Gln Trp Gly Glu Glu Gly Tyr Ile Arg Ile Ala Lys
 305 310 315 320

Gly Ser Asn Gln Cys Leu Val Lys Glu Glu Ala Ser Ser Ala Val Val
 325 330 335

Gly Gly Pro Gly Pro Thr Pro Glu Pro Thr Thr Thr Thr Thr Ser
 340 345 350

Ala Pro Gly Pro Ser Pro Ser Tyr Phe Val Gln Met Ser Cys Thr Asp
 355 360 365

Ala Ala Cys Ile Val Gly Cys Glu Asn Val Thr Leu Pro Thr Gly Gln
 370 375 380

Cys Leu Leu Thr Thr Ser Gly Val Ser Ala Ile Val Thr Cys Gly Ala
 385 390 395 400

Glu Thr Leu Thr Glu Glu Val Phe Leu Thr Ser Thr His Cys Ser Gly
 405 410 415

Pro Ser Val Arg Ser Ser Val Pro Leu Asn Lys Cys Asn Arg Leu Leu
 420 425 430

Arg Gly Ser Val Glu Phe Phe Cys Gly Ser Ser Ser Ser Gly Arg Leu
 435 440 445

Ala Asp Val Asp Arg Gln Arg Arg His Gln Pro Tyr His Ser Arg His
 450 455 460

Arg Arg Leu
 465

<210> SEQ ID NO 81
 <211> LENGTH: 704
 <212> TYPE: PRT
 <213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 81

Met Thr Glu Thr Phe Ala Phe Gln Ala Glu Ile Asn Gln Leu Met Ser
 1 5 10 15

Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe Leu Arg Glu
 20 25 30

Leu Ile Ser Asn Ser Ser Asp Ala Cys Asp Lys Ile Arg Tyr Gln Ser
 35 40 45

Leu Thr Asn Gln Ala Val Leu Gly Asp Glu Ser His Leu Arg Ile Arg
 50 55 60

Val Ile Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp Thr Gly
 65 70 75 80

Ile Gly Met Thr Lys Ala Glu Leu Val Asn Asn Leu Gly Thr Ile Ala
 85 90 95

-continued

Thr Glu Glu Glu Lys Lys Gln Arg Glu Glu Glu Lys Thr Ala Tyr Glu
 530 535 540
 Arg Leu Cys Lys Ala Met Lys Asp Val Leu Gly Asp Lys Val Glu Lys
 545 550 555 560
 Val Val Val Ser Glu Arg Leu Ala Thr Ser Pro Cys Ile Leu Val Thr
 565 570 575
 Ser Glu Phe Gly Trp Ser Ala His Met Glu Gln Ile Met Arg Asn Gln
 580 585 590
 Ala Leu Arg Asp Ser Ser Met Ser Ala Tyr Met Met Ser Lys Lys Thr
 595 600 605
 Met Glu Ile Asn Pro Ala His Pro Ile Val Lys Glu Leu Lys Arg Arg
 610 615 620
 Val Glu Ala Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Tyr Leu
 625 630 635 640
 Leu Phe Asp Thr Ala Leu Leu Thr Ser Gly Phe Thr Leu Asp Asp Pro
 645 650 655
 Thr Ser Tyr Ala Glu Arg Ile His Arg Met Ile Lys Leu Gly Leu Ser
 660 665 670
 Leu Asp Asp Glu Asp Asn Gly Asn Glu Glu Ala Glu Pro Ala Ala Ala
 675 680 685
 Val Pro Ala Glu Pro Val Ala Gly Thr Ser Ser Met Glu Gln Val Asp
 690 695 700

<210> SEQ ID NO 82

<211> LENGTH: 677

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma cruzi

<400> SEQUENCE: 82

Met Thr Tyr Glu Gly Ala Ile Gly Ile Asp Leu Gly Thr Thr Tyr Ser
 1 5 10 15
 Cys Val Gly Val Trp Gln Asn Glu Arg Val Glu Ile Ile Ala Asn Asp
 20 25 30
 Gln Gly Ser Arg Thr Thr Pro Ser Tyr Val Ala Phe Thr Asp Thr Glu
 35 40 45
 Arg Leu Ile Gly Asp Ala Ala Lys Asn Gln Val Ala Met Asn Pro Thr
 50 55 60
 Asn Thr Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Lys Phe Ser Asp
 65 70 75 80
 Pro Val Val Gln Ser Asp Met Lys His Trp Pro Phe Lys Val Ile Thr
 85 90 95
 Lys Gly Asp Asp Lys Pro Val Ile Gln Val Gln Phe Arg Gly Glu Thr
 100 105 110
 Lys Thr Phe Asn Pro Glu Glu Val Ser Ser Met Val Leu Ser Lys Met
 115 120 125
 Lys Glu Ile Ala Glu Ser Tyr Leu Gly Lys Gln Val Lys Lys Ala Val
 130 135 140
 Val Thr Val Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys
 145 150 155 160
 Asp Ala Gly Thr Ile Ala Gly Met Glu Val Leu Arg Ile Ile Asn Glu
 165 170 175
 Pro Thr Ala Ala Ala Ile Ala Tyr Gly Leu Asp Lys Val Glu Asp Gly
 180 185 190
 Lys Glu Arg Asn Val Leu Ile Phe Asp Leu Gly Gly Gly Thr Phe Asp
 195 200 205

-continued

Val Thr Leu Leu Thr Ile Asp Gly Gly Ile Phe Glu Val Lys Ala Thr
 210 215 220
 Asn Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg Leu Val
 225 230 235 240
 Ser His Phe Thr Asp Glu Phe Lys Arg Lys Asn Lys Gly Lys Asp Leu
 245 250 255
 Thr Thr Ser Gln Arg Ala Leu Arg Arg Leu Arg Thr Ala Cys Glu Arg
 260 265 270
 Ala Lys Arg Thr Leu Ser Ser Ala Ala Gln Ala Thr Ile Glu Ile Asp
 275 280 285
 Ala Leu Phe Asp Asn Val Asp Phe Gln Ala Thr Ile Thr Arg Ala Arg
 290 295 300
 Phe Glu Glu Leu Cys Gly Asp Leu Phe Arg Gly Thr Leu Gln Pro Val
 305 310 315 320
 Glu Arg Val Leu Gln Asp Ala Lys Met Asp Lys Arg Ala Val His Asp
 325 330 335
 Val Val Leu Val Gly Gly Ser Thr Arg Ile Pro Lys Val Met Gln Leu
 340 345 350
 Val Ser Asp Phe Phe Gly Gly Lys Glu Leu Asn Lys Ser Ile Asn Pro
 355 360 365
 Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Phe Ile Leu Thr
 370 375 380
 Gly Gly Lys Ser Lys Gln Thr Glu Gly Leu Leu Leu Leu Asp Val Thr
 385 390 395 400
 Pro Leu Thr Leu Gly Ile Glu Thr Ala Gly Gly Val Met Thr Ser Leu
 405 410 415
 Ile Lys Arg Asn Thr Thr Ile Pro Thr Lys Lys Ser Gln Ile Phe Ser
 420 425 430
 Thr Tyr Ala Asp Asn Gln Pro Gly Val His Ile Gln Val Phe Glu Gly
 435 440 445
 Glu Arg Ala Met Thr Lys Asp Cys His Leu Leu Gly Thr Phe Asp Leu
 450 455 460
 Ser Gly Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr
 465 470 475 480
 Phe Asp Leu Asp Ala Asn Gly Ile Leu Asn Val Ser Ala Glu Glu Lys
 485 490 495
 Gly Thr Gly Lys Arg Asn Gln Ile Val Ile Thr Asn Asp Lys Gly Arg
 500 505 510
 Leu Ser Lys Ala Asp Ile Glu Arg Met Val Ser Glu Ala Ala Lys Tyr
 515 520 525
 Glu Ala Gln Asp Lys Glu Gln Arg Glu Arg Ile Asp Ala Lys Asn Gly
 530 535 540
 Leu Glu Asn Tyr Ala Phe Ser Met Lys Asn Thr Val Asn Glu Pro Asn
 545 550 555 560
 Val Ala Gly Lys Ile Glu Glu Ala Asp Lys Asn Thr Ile Thr Ser Ala
 565 570 575
 Val Glu Glu Ala Leu Gln Trp Leu Asn Asn Asn Gln Glu Ala Ser Lys
 580 585 590
 Glu Glu Tyr Glu His Arg Gln Lys Glu Leu Glu Asn Leu Cys Thr Pro
 595 600 605
 Ile Met Thr Lys Met Tyr Gln Gly Met Gly Ala Gly Gly Gly Met Pro
 610 615 620
 Gly Gly Met Pro Gly Gly Met Pro Gly Gly Met Pro Gly Gly Met Pro

What is claimed is:

1. An article comprising:
a substrate comprising a surface; and
a plurality of different individually addressable antigenic *T. cruzi* polypeptides immobilized onto a surface on said substrate surface;
wherein at least one polypeptide comprises the amino acid sequence of at least one of SEQ ID NO:24-26, 28-34, or 68-70.
2. The article of claim 1 wherein the polypeptides are immobilized on the substrate surface to form a microarray.
3. The article of claim 1 wherein the substrate comprises at least one nanoparticle, and wherein the polypeptides are immobilized on the surface of the nanoparticle.
4. A kit for diagnosis of *T. cruzi* infection comprising:
an article according to claim 1; and
packaging material and instructions for use.
5. A method for obtaining information about a known or suspected *T. cruzi* infection in a mammal, or for determining whether a mammal is or has been infected by *T. cruzi*, said method comprising:
obtaining a biological sample from the mammal;
contacting the biological sample with the article of claim 1; and
evaluating the presence, absence, intensity or pattern of interaction of components of the biological sample with the antigenic *T. cruzi* polypeptides to determine the presence or absence of *T. cruzi* infection, the identity of the infective strain, the length of the infection, the stage of the infection, whether the infection is still present or the mammal has been cured, the vaccination status of the mammal, the success of treatment, or any combination thereof.
6. The method of claim 5 wherein the mammal is a human or a dog.
7. The method of claim 5 wherein the biological sample comprises a body fluid comprising an antibody.
8. The method of claim 7 wherein the body fluid comprises blood, plasma or serum.
9. The method of claim 5 wherein the biological sample comprises a mononuclear blood cell.
10. The method of claim 5 wherein the biological sample comprises a peripheral blood mononuclear cell (PBMC) fraction of blood from the mammal.

11. The method of claim 5 wherein the method is a serodiagnostic method, and wherein the biological sample component that interacts with the antigenic *T. cruzi* polypeptide is an antibody from the mammal.
12. The method of claim 5 wherein the method is a cellular assay method, and wherein the biological sample component that interacts with the antigenic *T. cruzi* polypeptide is T cell from the mammal.
13. The method of claim 5 wherein the method comprises a multiplexed assay wherein the biological sample is contacted simultaneously with the plurality of antigenic *T. cruzi* polypeptides.
14. The method of claim 5 wherein the biological sample is obtained from a blood donor, a potential blood donor, or a transplant donor.
15. The method of claim 5 wherein the biological sample is obtained from a pooled blood product supply intended for use in transfusions or research.
16. A method for detecting contamination of a blood product supply with *T. cruzi*, the method comprising:
selecting a sample from the blood supply;
contacting the sample with the article of claim 1; and
evaluating the presence, absence, intensity or pattern of interaction of components of the sample with the antigenic *T. cruzi* polypeptides to determine whether the blood supply is contaminated with *T. cruzi*.
17. The method of claim 16 wherein the blood product supply comprises whole blood, a blood product, or a blood fraction.
18. The method of claim 16 wherein the blood product supply comprises a cellular blood component, a liquid blood component, a blood protein, or mixtures thereof.
19. The method of claim 16 wherein the blood product supply comprises a red blood cell concentrate, a leukocyte concentrate, a platelet concentrate, plasma, serum, a clotting factor, an enzymes, albumin, plasminogen, or a immunoglobulin, or mixtures of thereof.
20. The method of claim 16 wherein the method is a serodiagnostic method, and wherein the sample component that interacts with the antigenic *T. cruzi* polypeptide is an antibody.

* * * * *

专利名称(译)	克氏锥虫感染的诊断分析		
公开(公告)号	US8329411	公开(公告)日	2012-12-11
申请号	US12/670993	申请日	2008-07-30
申请(专利权)人(译)	乔治亚研究的基础上, Inc.的大学.		
当前申请(专利权)人(译)	乔治亚研究的基础上, Inc.的大学.		
[标]发明人	TARLETON RICK L ETHERIDGE JR RONALD D		
发明人	TARLETON, RICK L. ETHERIDGE, JR., RONALD D.		
IPC分类号	G01N33/53		
CPC分类号	G01N33/569 C12N2799/023 G01N2333/44		
优先权	60/962498 2007-07-30 US 60/564804 2004-04-23 US PCT/US2005/013777 2005-04-22 WO 60/623299 2004-10-29 US		
其他公开文献	US20100323909A1		
外部链接	Espacenet USPTO		

摘要(译)

一种敏感的多组分诊断试验, 用于感染查格斯病的致病因子克氏锥虫, 包括制作方法和使用方法。还提供了筛选克氏锥虫多肽以鉴定抗原多肽作为诊断试验的组分的方法, 以及含有抗原性克氏锥虫多肽的组合物。

		Protein																#>4					
Sera	Serology	HA1	ELISA	DT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	lysize	SD	
Pooled sera from strongly reactive																							
		9.70	19.26	24.42	7.17	-0.12	nd	2.54	23.83	-0.37	5.48	5.08	17.55	8.79	15.94	25.91	42.37	12.58					
		7.65	18.83	18.08	5.48	0.06	nd	2.19	31.80	-0.51	6.64	4.41	23.57	33.21	7.02	16.64	57.60	15.97					
		1.47	16.74	16.65	5.46	0.04	nd	5.91	31.83	8.79	nd	4.00	3.63	20.01	nd	18.47	31.23	38.01	12.91				
		7.33	17.46	15.47	3.63	-0.39	nd	8.21	1.83	27.18	-0.49	4.30	4.35	15.60	20.38	13.70	17.22	44.23	11.83				
		7.23	18.87	14.06	11.59	0.38	nd	7.67	1.80	29.61	6.93	4.16	3.32	20.81	28.22	13.41	26.33	45.36	11.11				
		17.64	46.65	34.56	7.84	nd	nd	7.01	2.91	14.71	-0.05	12.30	9.57	45.06	10.81	22.94	22.01	95.05	20.28				
		13.14	39.82	28.91	4.10	nd	nd	6.35	2.13	22.56	-0.39	10.07	5.03	21.23	6.60	16.63	30.29	91.38	13.71				
		9.03	31.23	20.79	3.92	nd	nd	12.59	2.68	39.70	-0.08	4.31	3.51	27.05	6.67	10.11	70.18	27.91	19.22				
Non-infected based upon conventional serology																							
no	Fc383	neg	tr	tr	tr	0.41	-0.71	-0.16	-1.50	0.43	0.90	-1.22	0.47	-0.35	0.11	-0.45	0.73	0.65	1.61	0.78	-0.51	-0.80	0
other	Fc323	neg	tr	tr	tr	-1.07	-0.50	0.42	-1.94	-1.81	-1.37	-1.26	-0.40	0.16	-0.50	-0.84	-0.71	-1.32	-0.58	4.36	2.89	-0.73	0
hard	Fc438	neg	tr	tr	tr	-1.13	-0.32	0.41	0.52	-0.61	0.93	-0.12	-0.08	-1.15	0.15	-0.79	-0.88	-1.13	0.51	0.32	-0.77	-0.33	0
easy	Fc410	neg	tr	tr	tr	0.45	-0.11	0.66	-1.81	1.02	-0.68	-1.16	1.21	-0.25	-0.37	-0.57	-0.43	-0.66	0.97	0.99	1.08	-1.16	0
disc	Fc411	neg	tr	tr	tr	0.03	0.40	0.42	-0.21	0.05	2.88	0.13	0.44	1.84	-0.29	1.01	-0.07	2.96	0.67	1.65	0.55	3.19	0
	Fc453	neg	tr	tr	tr	1.53	0.59	-0.68	1.51	1.24	-0.22	0.58	0.00	0.45	0.34	0.85	0.61	-0.24	-0.48	-0.66	2.09	0.10	0
	Fc408	discard	32	tr	tr	0.55	-0.11	-0.18	0.39	0.94	-0.38	0.40	1.72	-0.04	0.02	-0.45	0.12	0.99	0.46	2.08	1.16	1.93	0
	Fc409	discard	32	tr	tr	-0.39	1.42	1.61	0.65	-0.64	2.35	0.09	1.59	0.55	0.37	2.36	1.47	3.14	0.20	1.96	2.81	0.35	0
	Fc407	neg	tr	tr	tr	-0.09	2.96	1.90	11.59	0.55	nd	0.07	1.73	-0.78	-1.02	1.13	1.11	4.05	2.29	2.73	0.91	-0.24	2
	Fc416	neg	tr	tr	tr	1.26	3.11	1.41	-0.10	0.28	2.57	0.07	1.40	0.59	2.05	-0.81	4.30	3.05	1.30	1.40	3.10	0.58	2
	Fc415	neg	tr	tr	tr	1.71	4.14	4.12	0.22	1.20	0.61	0.17	1.92	2.27	2.01	1.69	1.24	1.42	2.71	2.34	2.38	-0.27	2
	Fc324	neg	tr	tr	tr	-0.50	0.03	3.62	0.18	-0.69	0.50	-0.11	-0.84	-1.66	2.55	-0.79	-0.35	-0.64	5.33	0.76	-0.54	-0.24	2
	Fc426	discard	tr	tr	tr	1.39	2.45	2.01	0.77	2.06	-0.09	0.08	1.24	-0.81	-1.42	-1.15	-0.98	2.19	4.52	4.45	0.07	-0.06	2
	Fc320	neg	tr	tr	tr	-0.79	0.76	3.19	1.44	0.92	1.56	-1.67	-0.16	2.41	-0.49	3.86	1.28	4.53	0.63	3.65	1.12	3.63	4
	Fc404	neg	tr	tr	tr	0.68	1.78	6.73	-0.45	17.00	2.04	-0.73	2.82	3.18	3.67	1.82	1.58	2.69	0.48	1.91	3.56	0.82	4
	Fc409	neg	tr	tr	tr	-1.31	-0.84	6.22	4.55	11.18	0.81	0.95	0.88	-1.09	0.17	-0.44	-0.97	0.80	4.76	1.23	1.18	0.48	4