Gene Homologies Identified between *Trypanosoma cruzi* Antigen 36 and Mammalian TRIM21 Genes Using Bioinformatics Analysis

Martin A Winkler (✉ winklma01@gmail.com)

The Biotech Advisor

Alfred A Pan

TNTC, Inc.

Research Article

Keywords: Chagas' disease, Chronic Chagas Cardiomyopathy, *Trypanosoma cruzi*, Ro52, TRIM21, Antigen 36 (Ag 36), Needleman-Wunsch algorithm

Posted Date: June 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-553828/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: We previously reported that a Human Ro52 gene sequence (TRIM21) produced a significant stretch of protein sequence homologous to *T. cruzi* Antigen 36 (Ag 36) protein sequence, when Ag 36 was translated in the second reading frame. Comparison of their respective DNA sequences demonstrated a 114 nucleotide region of both genes having ~70 percent partial homology. After Ro52 was shown to be an E3 Ubiquitin dependent Type I ligase for transcription factors for Interferon genes, we proposed that the Ag 36 gene, which contains a repetitive motif within it, may function to repress Ro52 in the human heart through RNA interference, or other unknown mechanism, giving rise to autoimmunity found in Chronic Chagas Cardiomyopathy (CCC).

Results: To test that hypothesis, we compared various mammalian TRIM genes to the *T. cruzi* Ag 36 DNA sequence using the Needleman-Wunsch algorithm in the <http://usegalaxy.eu> bioinformatics tool base. In addition to human and chimpanzee, TRIM21 comparable gene regions from canine, shrew, ferret, bat, feline, and armadillo, and aardvark showed homology to the gene for Ag 36 ranging from 68 to 30 percent. However, mouse and eight other mammalian species showed no significant homology. Since mice have been shown to have severe cardiac cardiomyopathy after infection, but their TRIM21 was not homologous to Ag 36 in this study, we conclude that the gene homology has no causative link to CCC.

Conclusions: In addition to human TRIM21, eight mammalian species showed partial gene homology to *T. cruzi* Ag 36, and some of these have been demonstrated to have CCC. However, rats and mice TRIM21 showed no partial homology to Ag 36. Since these species have been demonstrated to have CCC, the partial gene homology between Ag36 and TRIM 21 may not be causative or associated with CCC, as was originally hypothesized.

Background

Chagas' disease has become a progressive emerging disease in the United States. In South and Central America, the disease may affect over 18 million people. Typically, the causative agent, *Trypanosomacruzi*, may not immediately kill the host, but by its definition, the parasite keeps the host in check, in order to maximize transmission and therefore, prolong its life cycle. The mammalian host can gradually develop illness from an acute or mild form of the disease to a latent stage, to a chronic stage. There is significant mortality due to the Acute and Chronic stages of this disease¹. In particular, Chronic Chagas Cardiomyopathy (CCC) is thought to be caused by an autoimmune attack upon nerve and/or heart tissue.

Recognition of this disease as a potential threat to the US blood supply was in 1992 when several biopharmaceutical companies developed diagnostic tests to identify this disease². In 1995 the FDA accepted a 510(k) diagnostic test for Chagas' disease^{3,4}. Progressive improvements to identify several highly antigenic *T. cruzi* molecules reactive with human chagasic antiserum were made in subsequent

years (2006-2011); the American Association of Blood Banks (AABB), the FDA, CDC, and WHO have now recognized this disease as one of the 13 donor screening assays for Infectious Agents.

Presently there are no vaccines against Chagas' disease and therapeutic agents (benznidazole) have only recently (2017) been FDA approved for use in children ages 2 to 12 years old with Chagas' disease. Side effects are common, frequent, and severe with increasing age. In this regard, alternative methods for treatment are necessary. Our primary focus has been to search and identify highly antigenic *T. cruzi* molecules reactive with human chagasic antiserum^{2,3,5,6}. To this end, one particular cloned gene from *T. cruzi* amastigotes was sequenced and found to be identical to the repetitive antigen Clone 36 ("Antigen 36")⁷.

Search of GenBank using our DNA sequence translated by the TFASTA program revealed a high degree of homology of Human Ro52 with the translated sequence in the second reading frame of Ag 36. Direct comparison of the Ag 36 DNA sequence with Ro52 DNA sequence revealed a significant homology of the Ag 36 DNA sequence to TRIM21, the gene for human Ro52⁸. The TRIM21 (tripartite motif containing-21) gene protein product Ro52 was subsequently shown to be an E3 Ubiquitin ligase that modifies transcription factors for alpha and beta interferons and other cytokines⁹. Knock-out of Ro2 in mice strains showed that they were susceptible to tissue inflammation and systemic autoimmunity after injury induced by skin tagging¹⁰. However, the TRIM21 protein product Ro52 also can stimulate innate immunity, for example, in macrophages. There it acts in a non-degradation pathway of ubiquitination, as an E3 ligase for IRF-8 that upregulates cytokines such as IL-12/p40, and therefore contributes to innate immunity in macrophages¹¹. TRIM21-deficient bone marrow-derived macrophages showed a reduced response to Toll-like receptor agonist Bacillus Calmette-Guerin¹².

TRIM21 is also an antibody receptor and cytosolic ubiquitin ligase that provides a line of defense against invading viruses by acting as a sensor that intercepts antibody-coated viruses that have evaded extracellular neutralization and breached the cell membrane¹³⁻¹⁵. After interaction with the Fc of antibodies bound to the virus, the TRIM21 receptor triggers a coordinated effector and signaling response that prevents viral replication while at the same time inducing an anti-viral cellular state¹⁶. This dual effector function is tightly regulated by auto-ubiquitination and phosphorylation. In addition, TRIM21 has been studied extensively with antibody receptors bound to viruses but minimally with parasites¹⁶. We proposed that the partial gene homology between TRIM21 and Ag 36 may cause RNA interference, mRNA silencing, or other down-regulation of Ro52, leading to the autoimmunity of Chronic Chagas Cardiomyopathy (CCC)¹⁷. We also suggested that the interference could be beneficial for the parasite, during its invasion of macrophages by blocking TRIM21 stimulation of innate immunity in these phagocytic cells. In this work, we extend the comparison of DNA sequences of Ag 36 to eighteen additional mammalian TRIM21 sequences, using online bioinformatics tools to determine significant partial homologies and to test whether the homology is associated with CCC.

Results

Homology Comparison of TRIM21 (nucleotides 856-916) to Ag 36 in Mammals

The human TRIM21 sequence in nucleotides region 856 to 916 had the greatest homology to Ag 36 and was used for comparison. In the regions of Ag 36 gene homology compared to TRIM21 genes (TRIM21 base numbers 856 to 916), the greatest percentage homology was human (68%), followed by chimpanzee (60%), dog (57%) and shrew (50%). Seven other species showed partial homologies in the compared region of 43 to 17% (Table 1). The nine other mammalian TRIM21 genes shown in the Table 1 had no significant homology in this region.

Table 1 Percent Homology Comparison of TRIM21 (856-916) in Mammals using the Needleman-Wunsch Alignment of *T. cruzi* Ag 36

Mammal	Genus/Species	# Nucleotides Homologous in TRIM21 856-916	Percent Homology
Human	<i>Homo sapiens</i>	41/60	68%
Chimpanzee	<i>Pan Trogolodytus</i>	36/60	60%
Dog	<i>Canis familiaris</i>	34/60	57%
Shrew	<i>Sorex araneus</i>	30/60	50%
Ferret	<i>Mustela potorius furo</i>	27/60	43%
Bat, common Vampire	<i>Desmodus rotundus</i>	26/60	43%
Cat, domestic	<i>Felis cattus</i>	24/60	40%
Armadillo (9 band)	<i>Dasypus ovemcinctus</i>	23/60	38%
Deer, White tailed	<i>Otocoileus virginiansus</i>	19/60	32%
Aardvark	<i>Orycteropus afer</i>	20/60	30%
Rabbit	<i>Oryctuolagus cuniculus</i>	19/60	17%
Rat	<i>Rattus norwegicus</i>	0/60	0%
Mouse	<i>Mus musculus</i>	0/60	0%
Gerbil	<i>Meriones unguiculatus</i>	0/60	0%
Sheep	<i>Ovis aires</i>	0/60	0%
Cow	<i>Bos taurus</i>	0/60	0%
Horse	<i>Equus caballus</i>	0/60	0%
Goat	<i>Capra hircus</i>	0/60	0%
Marmoset	<i>Callithex</i>	0/60	0%

	<i>jacchus</i>		
Lynx	<i>Lynx canadensis</i>	0/60	0%
The mammalian TRIM21 genes were compared to <i>T. cruzi</i> Ag 36 by the Needleman-Wunsch Alignment at www.usegalaxy.org (please see Methods). The number of nucleotides that were homologous in the TRIM21 nucleotide 856 to 916 range were counted and divided by 60, the total nucleotides in this range.			

Discussion

Human TRIM21 gene sequence was shown to have a significant stretch of DNA sequence homologous to *T. cruzi* Ag 36. Comparison of Ag 36 to mammalian TRIM21 DNA sequences demonstrated a 114 nucleotide region ranging from 68 to 38% partial homology in human, chimpanzee, canine, armadillo, and ten other species (Table 1). Of these species, humans and dogs have been shown to have CCC after *T. cruzi* infection¹⁸⁻²¹. Domesticated cats have been known to manifest the acute stages of Chagas' disease and there is only limited data of CCC in these animals²². Ro52 is an E3 Ubiquitin dependent Type I ligase needed for transcription factors for Interferon genes. It was proposed that the Ag 36 sequence motif, repeated at least three times⁷ would increase the chances of the homology's putative effect¹⁷. If this gene homology has an outcome on the infected host, for example, through RNA interference or mRNA silencing, decreased expression of Ro52 may result, giving rise to the autoimmunity found in CCC, and conferring an advantage for the parasite in infecting monocytes. However, mice have been shown to have severe cardiac cardiomyopathy after *T. cruzi* infection²³. Our investigations showed no homology of mouse TRIM21 to Ag 36, implying that there may be no causative or associative link to CCC from the homology of TRIM21 to Ag 36. There is only one isoform and no splice variants of TRIM21 reported in laboratory mice, which are inbred strains. The TRIM 21 gene therefore does not show variability in laboratory mice, ruling out the possibility of the homology being in them. It would be of interest to determine if TRIM21 in wild mice shows splice variants or isoforms, and if so, whether they display CCC when infected with *T. cruzi*. TRIM genes are a large family of mammalian and vertebrate genes, which share homologous domains^{14,24}. Consequently, since TRIM21 is homologous to many genes in this family, it would be worthwhile to examine mammalian TRIM genes for homology to Ag 36 as well. The results demonstrate the power of a comparative bioinformatics analysis of these TRIM 21 genes between the mammalian species.

Conclusions

In this study, we used a comparative bioinformatics approach to determine whether the partial homology observed of Human TRIM21 to *T. cruzi* Ag 36 was observed in 19 other mammalian species, and if observed homologies correlated with CCC in those species. Ten species demonstrated the partial homology by the Needleman-Wunsch Algorithm. Nine species demonstrated no homology. However, rats

and mice lacked the homology, though they have been shown to have CCC. This exception rules out the partial homology as a possible cause or association with CCC.

Methods

Sequences of TRIM21s from 19 mammalian species were retrieved from Genbank or the University of California Santa Barbara databases, and sequences compared to the Ag 36 gene (Genbank M21331) with tools at <https://usegalaxy.org>^{25,26}. The mammalian TRIM21 analyzed were: primates (human, chimpanzee), mouse, rat, gerbil, dog, cat, nine-banded armadillo, shrew, lynx, ferret, marmoset, bat, sheep, cow, deer, horse, and goat. Gene sequences were aligned by the Needleman-Wunsch algorithm²⁷. The Needleman-Wunsch global alignment tool is an algorithm used to find the optimum alignment (including gaps) of two sequences when considering their entire length. The workflow and results are stored at the URL <https://usegalaxy.eu/u/martinawinklerphd/h/copy-of-copy-of-mammalian-trim-genes-compared-with-antigen-36>. The bioinformatics workflow is visible to all, but registration (which is free) is required to view the results.

Declarations

Supplementary Information

The online version contains supplementary material available at <https://>

Acknowledgements

The authors would like to thank Abbott Laboratories and our colleagues where the foundational investigations were performed.

Authors' contributions

MAW and AAP conceptualized the research questions. MAW did the computational data analysis. MAW and AAP and wrote the manuscript, reviewed and supervised the work. All authors read and approved the final manuscript.

Funding

The Galaxy server that was used for some calculations is in part funded by Collaborative Research Centre 992 Medical Epigenetics (DFG grant SFB 992/1 2012) and German Federal Ministry of Education and Research (BMBF grants 031 A538A/A538C RBC, 031L0101B/031L0101C de.NBI-epi, 031L0106 de.STAIR (de.NBI)).

Availability of data and materials

The workflow and results are stored at the URL: <https://usegalaxy.eu/u/martinawinklerphd/h/copy-of-copy-of-mammalian-trim-genes-compared-with-antigen-36> . The bioinformatics workflow is visible to all, but registration (which is free) is required to view the results.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author details

¹The Biotech Advisor, Lawrence, KS, USA. ²TNTC, Inc., Pleasant Hill, CA, USA.

Abbreviations

Ag: Antigen; CCC: Chronic Chagas Cardiomyopathy; TRIM21: Tripartite Motif containing-21

References

1. Aiello VD, de Campos FPF. Chronic Chagas cardiomyopathy. Autopsy Case Rep 2015;5(3):7-9. doi: 10.4322/acr.2015.012.
2. Pan AA, Rosenberg GB, Hurley MK, Schock GJH, Chu VP, Aiyappa AA. Clinical evaluation of an EIA for the sensitive and specific detection of serum antibody to *Trypanosomacruzi* (Chagas' disease). J Infect Dis 1992;165(3):585–588. doi: 10.1093/infdis/165.3.585.
3. Brashear RJ, Winkler MA, Schur JD, Lee H, Burczak JD, Hall HJ, Pan AA. Detection of antibodies to *Trypanosomacruzi* among blood donors in the southwestern and western United States. I. Evaluation of the sensitivity and specificity of an enzyme immunoassay for detecting antibodies to *T. cruzi*. Transfusion 1995;35(3):213-218. doi: 10.1046/j.1537-2995.1995.35395184277.x.
4. Pan AA, Winkler MA. The threat of Chagas' disease in transfusion medicine. The presence of antibodies to *Trypanosomacruzi* in the US blood supply. Lab Med 1995;28:269-274.
5. Pan AA, McMahon-Pratt D. Amastigote and epimastigote stage-specific components of *Trypanosomacruzi* characterized by using monoclonal antibodies. Purification and molecular characterization of an 83-kilodalton amastigote protein. J Immunol 1989;143(3):1001-1008. PMID: 2501384.

6. Winkler MA, Brashear RJ, Hall HJ, Schur JD, Pan AA. Detection of antibodies to *Trypanosomacruzi* among blood donors in the southwestern and western United States. II. Evaluation of a supplemental enzyme immunoassay and radioimmunoprecipitation assay for confirmation of seroreactivity. *Transfusion* 1995;35(3):219-225. doi: 10.1046/j.1537-2995.1995.35395184278.x
7. Ibañez CF, Affranchino JL, Macina RA, Reyes MB, Leguizamon S, Camargo ME, Lena Åslund L, Pettersson U, Frasch, ACC. Multiple *Trypanosomacruzi* antigens containing tandemly repeated amino acid sequence motifs. *Mol Biochem Parasitol* 1988;30(1):27-34. doi: 10.1016/0166-6851(88)90129-6.
8. Winkler MA, Rivera DM, Pan AA, Nowlan SF. Homology of *Trypanosomacruzi* clone 36 repetitive DNA sequence to sequence encoding human Ro/SSA 52 kD autoantigen. *Parasite* 1998;5(1):94-95. PMID: 9754305..
9. Higgs R, Gabhann JN, Larbi NB, Breen EP, Fitzgerald KA, Jefferies C. The E3 ubiquitin ligase Ro52 negatively regulates IFN- production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. *J Immunol* 2008;181(3):1780-1786. doi: 10.4049/jimmunol.181.3.1780.
10. Espinosa A, Dardalho, V, Brauner, Ambrosi A, Higgs R, Quintana FJ, Sjostrand M, Eloranta M-L, Gabhann, JN, Winqvist O, Sundelin, B, Jefferies CA, Rozell B, Kuchroo, VK, Wahren-Herlenius, M. Loss of the lupus autoantigen Ro52/Trim21 induces tissue inflammation and systemic autoimmunity by disregulating the IL-23-Th17 pathway. *J Exp Med* 2009;206(8):1661-1671. doi: 10.1084/jem.20090585.
11. Kong HJ, Anderson DE, Lee CH, Jang MK, Tamura T, Tailor P, Cho HK, Cheong JH, Xiong H, Morse III, HC, et al. Autoantigen Ro52 is an interferon inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. *J Immunol* 2007;179(1):26-30. doi: 10.4049/jimmunol.179.1.26.
12. Sjostrand M, Carrow B, Espinosa A. TRIM21 controls toll-like receptor 2 responses in bone-marrow-derived macrophages. *Immunology* 2020;159(3):335-343. doi: 10.1111/imm.13157.
13. Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of single protein RING finger' E3 ubiquitin ligases. *Bioessays* 2005;27(11):1147-1157. doi: 10.1002/bies.20304.
14. Ozato K, Shin DM, Chang TH, Morse HC III. TRIM family proteins and their emerging roles in innate immunity. *Nat Rev Immunol.* 2008(11);8:849-860. doi: 10.1038/nri2413.
15. Nisole S, Stoye JP, Saib A. TRIM family proteins: retroviral restriction and antiviral defence. *Nat Rev Microbiol* 2005;3(10):799–808. doi: 10.1038/nrmicro1248.
16. Burbelo PD, Teos LY, Herche JL, Iadarola MJ, Aleviizos II. Autoantigens against the immunoglobulin-binding region of Ro2 link its autoantigenicity with pathogen neutralization. *Sci Rep* 2018;8(1):1-11. doi: 10.1038/s41598-018-21522-7.
17. Winkler MA, Pan AA. Is there a link between the human TRIM21 and *Trypanosomacruzi* clone 36 genes in Chagas' disease? *Mol Immunol* 2010;48(1-3):365-367. doi: 10.1016/j.molimm.2010.07.013.
18. Andrade Z. The canine model of Chagas' Disease. *Mem Inst Oswaldo Cruz, Rio de Janeiro, Suppl.* 1984;79:77-83.

19. Gutierrez FRS, Guedes PMM, Gazzinelli RT, Silva JS. The role of parasite persistence in pathogenesis of Chagas heart disease. *Parasite Immunol* 2009;31(11):673-685. doi: 10.1111/j.1365-3024.2009.01108.x
20. Santos FM, Mazzeti AL, Caldas S, Concalves KR, Lime WG, Torres RM, Bahia MT. Chagas cardiomyopathy: The potential effect of benznidazole treatment on diastolic dysfunction and cardiac damage in dogs chronically infected with *Trypanosomacruzi*. *Acta Tropica* 2016;161:44-54. doi: 10.1016/j.actatropica.2016.05.007.
21. Jansen AM, Xavier SCC, Roque ALR. 11-Ecological aspects of *Trypanosoma cruzi*: Wild hosts and reservoirs. *American Trypanosomiasis Chagas' Disease (Second Edition), One Hundred Years of Research* 2017;243-264.
22. Jimenez-Coell M, Acosta-Viana KY, Guzman-Marin E, Gomez-Rios A, Ortega-Pacheco A. Epidemiological survey of *Trypanosoma cruzi* infection in domestic owned cats from the tropical southeast of Mexico. *Zoonoses Public Health* 2012;59(Suppl 2):102–109. doi: 10.1111/j.1863-2378.2012.01463.x
23. Hoffman KA, Reynolds C, Bottazzi ME, Hotez P, Jones K. Improved biomarker and imaging analysis for characterizing progressive cardiac fibrosis in a mouse model of chronic Chagasic cardiomyopathy. *J Am Heart Assoc* 2019;8(22):1-15. doi: 10.1161/JAHA.119.013365.
24. Sardiello M, Cairo S, Fontanella B, Ballabio A, Meroni G. Genomic analysis of the TRIM family reveals two groups of genes with distinct evolutionary properties. *BMC Evol Biol* 2008;8:225-247. doi:10.1186/1471-2148-8-225
25. Afgan E, Baker D, van den Beek M, Blankenberg D, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Eberhard C, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses. *Nucl Acid Res* 2016;44(W1):W3-W10. doi:10.1093/nar/gkw343.
26. Blankenberg D, Taylor J, Schenck I, He J, Zhang Y, Ghent M, Veeraaghavan N, Albert I, Miller W, Makova KD, Hardison RC, Nekrutenko A. A framework for collaborative analysis of ENCODE data: Making large-scale analyses biologist-friendly. *Genome Res* 2007;17(6):960-964. doi.org/10.1101/gr.5578007.
27. Rice P, Longden I, Bleasby A. EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genet* 2000;16(6):276-277. doi.org/10.1016/S0168-9525(00)02024-2.

Figures

```

M34551.1      851 TGTGTGCCATGTGCCAGG----GCTGAAGAAGATGCTGAGG-----AC
889
          |||.||...|||  ||  |||.|||.|||.|||.||  ||
M21331.1      1  -----GCCTTGCCGCAGGAAGAGC--AAGAGGATG-TGGGGCCGCGCCAC
42
M34551.1      890 ATGTG---CAGTCCACATCACTCTGGA----TCCAGAC----ACAGCCAA
928
          .|  ||  |.|.||||.||  |.|||.||  ||.||||  |||.|
M21331.1      43 GT-TGATCCCGACCACTTC-CGCTCGACGACTCAAGACGCGTACAGGC--
88
M34551.1      929 TCCGTGGCTGATACTTTCAGAAGATCGGAG-ACAAGTGAGGCTTGGAGAC
977
          |||.||  |||.||  .|||||.|||.|||.|.
M21331.1      89 -CCGT---TGATCC-----CTCGGCGTACAAGCGC-----
114

M34551.1 = Human Ro52
M21331.1 = T. cruzi Ag 36

```

Figure 1

Comparison of TRIM21 from human with *T. cruzi* Ag 36, using the Needleman-Wunsch Algorithm, which is a global alignment algorithm to find the optimum alignment (including gaps) of two sequences when considering their entire length.

```

NC_036890.1_c  751 CTGGAAAGGAGTGAGTCGTGGAACCTGAAGGACCTGGATATTACGTCTCC
800
M21331.1      1  -----GCCTTGCCGCAGGAAGAGC--AAGAGGATG-TG
30
          |||.||...|||  ||  |||.|||.|||.|||.||
NC_036890.1_c  801 AGAACTCAGGAGTGTGTGCCATGTGCCAGG----GCTGAAGAAGATGCTG
846
M21331.1      31 GGGCCGCGCCACGT-TGATCCCGACCACTTC-CGCTCGACGACTCAAGAC
78
          .||  |||.||  |.|.||||.||  |.|||.||  ||.||||
NC_036890.1_c  847 AGG-----ACATGTG---CAGTCCACATCACTCTGGA----TCCAGAC
882
M21331.1      79 GCGTACAGGC---CCGTTGATC-----CCTCGGCGTACAAGCG
113
          |||.||  |||.||.||  ..|||||.|||.|||.||
NC_036890.1_c  883 ----ACAGCCAATCCGTGGCTCATACTTTCAGAAGATCGGAG-ACAAGTG
927

NC_036890.1c = Chimpanzee
M21331.1 = T. cruzi Ag 36

```

Figure 2

Comparison of TRIM21 from chimpanzee with *T. cruzi* Ag 36, using the Needleman-Wunsch Algorithm, which is a global alignment algorithm to find the optimum alignment (including gaps) of two sequences when considering their entire length.

```
M21331.1      1 -----GCC
3
NC_006603.3_c 801 GAACCTGAAGGAGCTGGACATTACCTCCCCGGGTCTGAGGAGTGTGTGCC
850
M21331.1      4 T--TGCCGCAGGAAGAGC--AAGAGGATG-TGGGGCCG-----C
37
NC_006603.3_c 851 TCGTGCC-----AGGGCTAAAGACGATGCTGAGGACGTATGGAGTACAC
894
M21331.1      38 GCCA-CGTTGATCCCGAC-CACTTCCGCTCGACGACTCA-----
74
NC_006603.3_c 895 ATCACCCTGGATCC--ACACACAGCCAATCCATGGCTCATCCTTTCAGAG
942
M21331.1      75 -----AGACGCGT-----ACAG-----GCCCG----
91
NC_006603.3_c 943 GATCGGAGACAAGTGAGGCTTGGAGACAGCCAGCAGGAAGTGCCTGAAAA
992
M21331.1      92 -----TTG-----ATC-----CCTCGGCGTACAAGCGC---
114
NC_006603.3_c 993 TGAAAGCAGATTTGACATGTATCCCATGGTCCTGGGAG-CCCAGTGCTTT

NC_006603.3_c = Canine
M21331.1 = T. cruzi Ag 36
```

Figure 3

Comparison of TRIM21 from dog with *T. cruzi* Ag 36, using the Needleman-Wunsch Algorithm, which is a global alignment algorithm to find the optimum alignment (including gaps) of two sequences when considering their entire length.

```

M21331.1      1  -----GCCTTGCCGCAGGA--AGAGCAAG-
22
NW_004493741. 701 GGAGGAGCCGGGGCTCAGCACTGGAGC--TGCTGCAGGATGTGAG-AAGT
747
M21331.1      23  -----AGGATGTGGGGCC-----GCGCCACGTTG-AT
48
NW_004493741. 748 ATCCTGGAAAGGA-GTGAGGTCTGGAACCTGAAGGAGCTGGACATTGTCT
796
M21331.1      49  CCCGACCACT-----TCCGCTCGACGA-----CT
72
NW_004493741. 797 CCCCA-GACTTGAGAAGTGTGTGCTGCGTTCAGGACTGAAGAAGATGCT
845
M21331.1      73  CAAGACGCGTACAG-GC-----CCGTTGATCC---CTCGGCGTACAAGC
112
NW_004493741. 846 CAGGACATGTGCAGTGCACATCACCTGGATCCAAACACTGC---CAATC
892

NW_004493741 = Dasypus novemcinctus (nine-banded Armadillo) Ro52
M21331.1      = T. cruzi Ag 36

```

Figure 4

Comparison of TRIM21 from Nine-banded Armadillo with *T. cruzi* Ag 36, using the Needleman-Wunsch Algorithm, which is a global alignment algorithm to find the optimum alignment (including gaps) of two sequences when considering their entire length.