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

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Research Article

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

Posted Date: July 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-553828/v2

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, *Trypanosoma cruzi*, 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 disease[1]. 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 disease[2]. 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 Ro52[8]. 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 cytokines[9]. Knock-out of Ro2 in mice strains showed that they were susceptible to tissue inflammation and systemic autoimmunity after injury induced by skin tagging[10]. 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 macrophages[11]. TRIM21-deficient bone marrow-derived macrophages showed a reduced response to Toll-like receptor agonist Bacillus Calmette-Guerin[12].

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 membrane[13–15]. 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 state[16]. 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 parasites[16]. 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	*Homo sapiens*	41/60	68%
Chimpanzee	*Pan Trogolodytus*	36/60	60%
Dog	*Canis familiiaris*	34/60	57%
Shrew	*Sorex araneus*	30/60	50%
Ferret	*Mustela potorius furo*	27/60	43%
Bat, common Vampire	*Desmodus rotundus*	26/60	43%
Cat, domestic	*Felis cattus*	24/60	40%
Armadillo (9 band)	*Dasypus ovemcinctus*	23/60	38%
Deer, White tailed	*Otocoileus virginiansus*	19/60	32%
Aardvark	*Orycteropus afer*	20/60	30%
Rabbit	*Oryctuolagus cuniculus*	19/60	17%
Rat	*Rattus norwegicus*	0/60	0%
Mouse	*Mus musculus*	0/60	0%
Gerbil	*Meriones unguaiculatus*	0/60	0%
Sheep	*Ovis aires*	0/60	0%
Cow	*Bos taurus*	0/60	0%
Horse	*Equus caballus*	0/60	0%
Goat	*Capra hircus*	0/60	0%
Marmoset	*Callithex jacchus*	0/60	0%
Lynx	*Lynx canadensis*	0/60	0%

The mammalian TRIM21 genes were compared to T. cruzi 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* infection[18–21]. Domesticated cats have been known to manifest the acute stages of Chagas' disease and there is only limited data of CCC in these animals[22]. 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 times[7] would increase the chances of the homology's putative effect[17]. 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* infection[23]. 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 algorithm[27]. 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.

Abbreviations

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

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the http://www.usegalaxy.eu repository at the following persistent link:

https://usegalaxy.eu/u/martinawinklerphd/h/copy-of-copy-of-mammalian-trim-genes-compared-with-antigen-36 . This bioinformatics workflow is available to all, but registration (which is free) is required to view the results.

The Species gene IDs or accession numbers analyzed during the current study with persistent link:

Species Gene IDs at http://www.ncbi.nlm.nih.gov

Chimpanzee *Pan Trogolodytusk 450975*

Dog *Canis familiiaris 485226*

Shrew *Sorex Araneus 101546923, Entry Currently withdrawn by NCBI staff*

Ferret *Mustela potorius furo 101676071*

Bat, common Vampire *Desmodus rotundus 112315984*

Cat, domestic *Felis cattus 10198574*

Armadillo (9 band) *Dasypus ovemcinctus 1001426419*

Deer, White tailed *Otocoileus virginiansus110131867*

Aardvark *Orycteropus afer 103197186*

Rabbit *Oryctuolagus cuniculus 100345560*

Rat *Rattus norwegicus 308901*

Mouse *Mus musculus 20821*

Gerbil *Meriones unguaiculatus 110542163*

Sheep *Ovis aires 101114640*

Cow *Bos taurus 359715*

Horse *Equus caballus 100066782*

Goat *Capra hircus 102186786*

Marmoset *Callithex jacchus 100387049*

Lynx *Lynx canadensis 115525374*

Species' Genbank accession numbers at http://www.ncbi.nlm.nih.gov

Homo sapiens M34551

Trypanosoma cruzi Clone 36 Antigen M21331

Competing interests

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

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)).

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.

Acknowledgements

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

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Figures

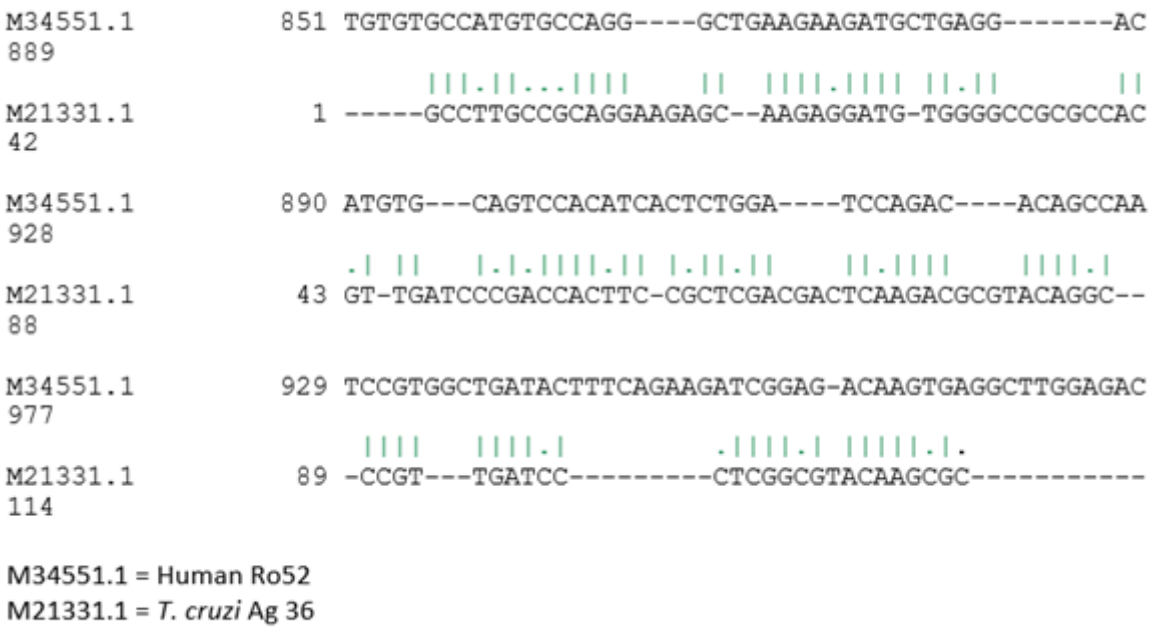


Figure 1

Needleman-Wunsch alignment of T. cruzi Ag 36 and human TRIM21 genes Fig 1 legend. 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.

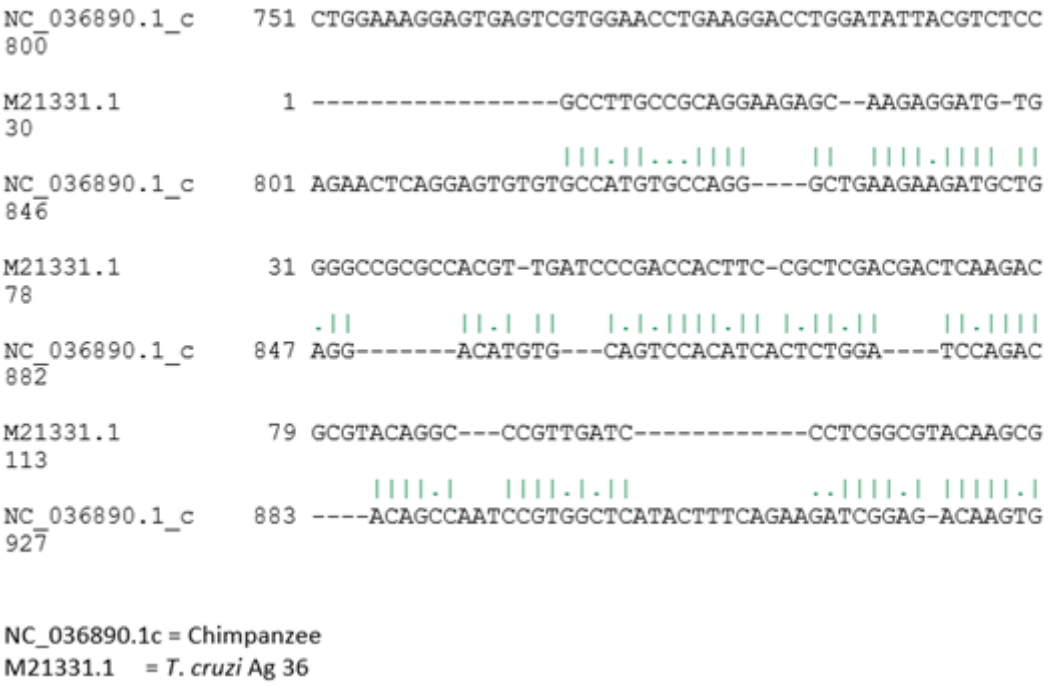


Figure 2

Needleman-Wunsch alignment of T. cruzi Ag 36 and chimpanzee TRIM21 genes Fig 2 legend. 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.

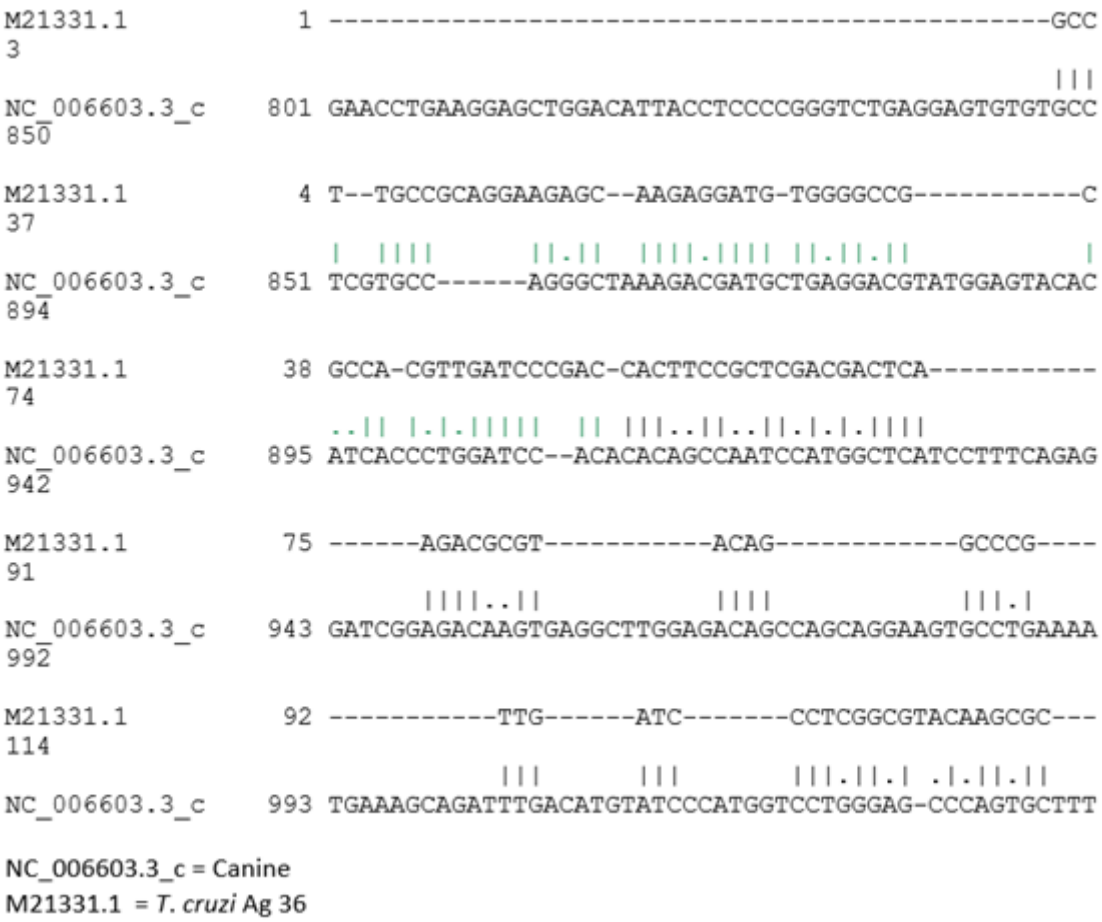
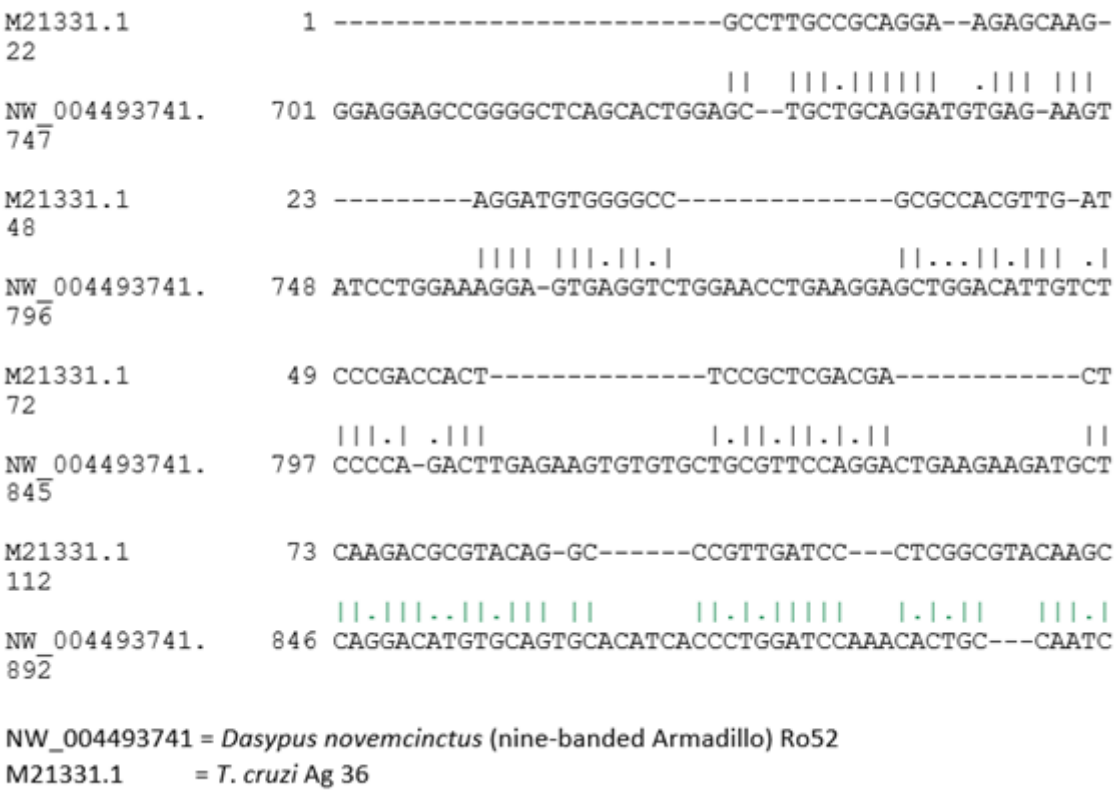


Figure 3

Needleman-Wunsch alignment of T. cruzi Ag 36 and canine TRIM21 genes Fig 3 legend. 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.



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

Figure 4

Needleman-Wunsch alignment of T. cruzi Ag 36 and Nine-banded Armadillo TRIM21 genes Fig 4 legend. 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.