VAPPER: High-throughput Variant Antigen Profiling in African trypanosomes

Sara Silva Pereira[1¶*], John Heap[2], Andrew R. Jones[3], Andrew P. Jackson[1*]

1.
Department of Infection Biology
Institute of Infection and Global Health
University of Liverpool
Liverpool Science Park Ic2
146 Brownlow Hill
Liverpool L3 5RF
United Kingdom

2.
Computational Biology Facility
University of Liverpool
Liverpool L69 7ZB
United Kingdom

3.
Institute of Integrative Biology
University of Liverpool
Liverpool L69 7ZB
United Kingdom

¶ Current Address:
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

*Corresponding authors

Email: ssilvapereira@medicina.ulisboa.pt

Email: a.p.jackson@liverpool.ac.uk

Abstract

Background: Analysing variant antigen gene families on a population scale is a difficult challenge for conventional methods of read mapping and variant calling due to the great variability in sequence, copy number and genomic loci. In African trypanosomes, hemoparasites of humans and animals, this is complicated by variant antigen repertoires containing hundreds of genes subject to various degrees of sequence recombination. **Findings:** We introduce Variant Antigen Profiler (VAPPER), a tool that allows automated analysis of variant antigen repertoires of African trypanosomes. VAPPER produces variant antigen profiles for any isolate of the veterinary pathogens *Trypanosoma congolense* and *Trypanosoma vivax* from genomic and transcriptomic sequencing data and delivers publication-ready figures that show how the queried isolate compares with a database of existing strains. VAPPER is implemented in Python. It can be installed to a local Galaxy instance from the ToolShed (https://toolshed.g2.bx.psu.edu/) or locally on a Linux platform via the command line (https://github.com/PGB-LIV/VAPPER). The documentation, requirements, examples, and test data are provided in the Github repository. **Conclusion**: Our approach is the first to allow large-scale analysis of trypanosome variant antigens and establishes two different methodologies that may be applicable to other multi-copy gene families that are otherwise refractory to high-throughput analysis.

Keywords: VAPPER; variant antigen profiling; African trypanosomes; variant surface glycoproteins

Background

Advances in next-generation sequencing have enabled researchers to produce high-throughput genomic data for diverse pathogens. However, analysing multi-copy, contingency gene families remains challenging due to their abundance, high mutation and recombination rates, and unstable gene loci [1]. Yet, these gene families are often involved in many processes of pathogenesis, including antigenic variation, virulence, host use, and immune modulation in a multitude of pathogens [2–4]. A prime example of a crucial gene family lacking the necessary analytic tools for high-throughput analysis is the Variant Surface Glycoprotein (*VSG*) superfamily in African trypanosomes [5].

African trypanosomes are extracellular hemoparasites that cause human sleeping sicknessand animal African trypanosomiasis (AAT). Their genomes contain up to 2500 *VSG* genes [6] dispersed through specialized, hemizygous chromosomal regions called subtelomeres, smaller chromosomes, and less frequently in the core of megabase-sized diploid chromosomes. The *VSG* genes encode variant surface glycoproteins, GPI-anchored proteins that coat the entire surface of the parasite in the bloodstream of the mammal host, which function mostly in antigenic variation and immune-modulation [7]. Sporadically, specific *VSG* genes have been shown to evolve other functions, not related to antigenic variation, such as conferring human infectivity to *T. brucei gambiense* (*TgsGP* gene) [8,9] and *T. brucei rhodesiense* (*SRA* gene) [10,11], resistance to the drug suramin (VSG^{sur} gene) [12], and mediating the transport of transferrin (*TfR* genes) [13,14].

As they are key players in host-trypanosome interaction, understanding *VSG* diversity and its impact in pathology, disease phenotype and virulence is of foremost importance in trypanosome research [4]. However, the *VSG* repertoire cannot be accurately analysed using conventional approaches of read mapping and variant calling. Attempts to bypass this challenge have resulted in alternative approaches using manually-curated *VSG* gene databases for specific *T. brucei* strains [6,15–17], but to the best of our knowledge there is no automated tool for the systematic analysis of *VSG* from any trypanosome genome. Thus, we have developed Variant Antigen Profiler (VAPPER), a tool that examines *VSG* repertoires in DNA/RNA sequence data of the main livestock trypanosomes, *Trypanosoma congolense* and *T. vivax*, and quantifies antigenic diversity. This results in a variant antigen profile (VAP) that can be compared between isolates, locations, and experimental conditions [18]. In this paper we briefly present how VAPPER can be used to further our knowledge of antigenic diversity and variation.

Findings

The service

VAPPER is primarily intended for producing and comparing VAPs of livestock trypanosomes, without the need for complex bioinformatic processes. It is available online through the Galaxy ToolShed [19] for a local Galaxy server [20], and as a Linux package for local installation. The program has three pipelines, specific for each organism (*T. congolense* or *T. vivax*) and input data type (genome or transcriptome). VAPPER requires quality-filtered, trimmed, paired sequencing reads in FASTQ format [21] or assembled contigs in FASTA format [22]. Results are presented in tables of frequencies, heatmaps, and Principal Component Analysis (PCA) plots, visualized as HTML files or exported to PDF or PNG format. A typical workflow is shown in Fig. 1.

For *T. congolense* genomic VAPs (gVAP), VAPPER starts with genome assembly of raw, short reads using Velvet 1.2.10 [23]. Assembled contigs are screened for pre-defined protein motifs described by a hidden Markov model using HMMER 3.1b2 [24] after 6-frame translation. A detailed description of the universal protein motifs and their biological significance in a recent manuscript [18], but, in summary, each protein motif or motif combination is diagnostic of a specific phylotype [18]; therefore, phylotype frequency can be calculated from the HMMER output. The proportions of each phylotype represent the gVAP and are recorded in a table of frequencies. The gVAP produced is also placed in the context of a *T. congolense* genome database supplied with VAPPER (N=97, [18,25]), which is regularly updated. This is achieved through a Euclidean distance-based clustering analysis. Results are presented as two heatmaps with corresponding dendrograms, one showing phylotype frequency, and the other showing frequency deviation from the population mean. They are also shown as a PCA plot and a table of frequencies.

For *T. congolense* transcriptomic analyses (tVAP), VAPPER performs read mapping using Bowtie 2 2.2.6 [26], reference-based transcript assembly and abundance calculation using Cufflinks 2.2.1 [27], and *VSG* transcript screening and phylotype assigning as described for gVAP. The proportions of each phylotype are then adjusted for transcript abundance based on the Cufflinks output (Fig. 1). The tVAP is presented as a weighted bar chart and compared to the gVAP of the reference (Fig. 2c). Ideally, the user would provide their own reference genome for the mapping step. As that is not always possible, especially for field isolate analysis, we provide two reference genomes, the IL3000 Kenyan isolate [14,28], and the Tc1/148 Nigerian isolate [29,30]. Choosing the most adequate reference for the sample being analysed may potentially improve the VAPPER results by increasing mapping sensitivity. However, we have previously shown that closely related *T. congolense* strains (i.e.

with short genetic distances) do not always have equally related *VSG* repertoires [18].

For *T. vivax*, the gVAP is based on presence or absence of pre-defined *VSG* genes, rather than phylotype frequencies as described for *T. congolense*. The *T. vivax VSG* repertoire is composed of distantly related lineages with no evidence for recombination [14]. Therefore, unlike *T. congolense* and *T. brucei*, *VSG* genes are often conserved across multiple strains, allowing us to build a *VSG* database for the entire species. No *T. vivax* tVAP is currently offered due to the lack of enough transcriptomic data available for benchmarking, but work is on-going to add this function. *VSG*-containing contigs are identified using BLAST 2.7.1 to detect sequence homology with a *T. vivax VSG* database. This information is added to a regularly updated presence/absence binary matrix of *T. vivax* genomes (N=29) and applied to a Euclidean distance-based clustering analysis. The results are presented as a heatmap and dendrogram, putting the sample in the context of the remaining *T. vivax* genomes and their known countries of origin (Fig. 3).

In its Linux version, VAPPER can process multiple samples concurrently, providing that the input files are compiled in a single directory. Results are shown for all samples simultaneously, allowing direct comparison of variant antigen profiles across multiple isolates, conditions, or replicates. The tabular output can be incorporated in downstream statistical analysis, whilst the graphical outputs provide figures for the visualization of antigen repertoire variability.

Linux Package Installation

To facilitate usage, the installation of VAPPER and its dependencies is automated. Upon first download of the software, a single script will ensure the system has all the

required dependencies and install them in a local directory if necessary. In naïve environments and for users without administrator rights to install the necessary libraries, a Python virtual environment can be set upon each new session.

The Galaxy Tool

VAPPER is available for installation in local Galaxy servers from the Galaxy ToolShed (https://toolshed.g2.bx.psu.edu/repository?repository_id=08b5616f1d3df20c). The purpose of the incorporation of VAPPER in Galaxy is to provide a simple front-end component for non-experienced users (Fig. 2). Results can be visualised directly in Galaxy, or can be downloaded as a compressed folder containing an HTML file with combined results, individual PNG and PDF files of the heatmaps, PCA plots, and bar charts produced, and the CSV files containing the raw values of phylotype proportions and deviation from the mean.

Benchmarking

The performance of the *T. congolense* gVAP pipeline was compared to the manually annotated VAP of the IL3000 reference genome (Fig. 3A) and to the BLAST-based VAPs of 41 isolates (Fig. 3B) [18]. There is a very good correlation between profiles produced by VAPPER and the known IL3000 VAP (R^2 = 0.88, t(13) = 9.7321, P < 0.001) and a good correlation with the BLAST-based method (R^2=0.67, Pearson's product moment correlation, $t_{(566)=}$34.4, p < 0.001). Minor differences were further investigated and found to be due to BLAST's difficulty in either analysing small contigs or quantifying multiple VSGs in the same contig sequence. Therefore, in general, more VSGs were recovered with VAPPER than with BLAST (Mean ± σ=721±277 vs. 669 ± 292, paired *t*-test, *p*-value = 0.005). A further strength of VAPPER is the ability to deal with poor, fragmented, genome assemblies. As

194 described in our previous paper [18], when a single *VSG* gene is located in two
195 distinct contig fragments, BLAST counts them incorrectly as separate genes,
196 whereas VAPPER will not because the diagnostic motif is only present once.
197 Therefore, we can now accurately calculate antigen profiles from incomplete
198 genome assemblies (up to 30%), and with a *VSG* fragmentation level up to 40% of
199 the original gene length (223 nucleotides) (Fig. 3C).
200

201 ## Validation by example

202 ### *T. congolense* gVAP

203 We have used the VAPPER to analyse the genomic repertoire of 98 *T. congolense*
204 samples of savannah and forest-subtypes, collected from 12 countries across Africa,
205 and previously described by us [18] and others [25]. In Fig. 4, two heatmaps and
206 corresponding dendrograms show how the VSG repertoires of each strain relate to
207 each other. On the left, the heatmap represents phylotype proportion, i.e. how many
208 genes a specific phylotype contains in the context of the complete *VSG* repertoire for
209 a given strain (Fig. 4A). This heatmap shows that P4, 8, 9, 10, and 14 have few
210 genes in all strains, whereas other phylotypes (e.g. P1, 2, 15) are more variable,
211 being quite abundant in some strains and rare in others. The heatmap on the right
212 shows phylotype deviation from the mean (Fig. 4B), which is calculated as the
213 difference between the phylotype proportion shown in panel A and the arithmetic
214 mean of phylotype proportions. The latter is calculated from the current database,
215 thus it will change as new samples are added.
216

217 The phylotype proportion variation patterns are perhaps better detected in the
218 normalised heatmap (Fig. 4B). For example, it is possible to detect a signature of
219 underrepresented P15 characteristic of all forest-subtype samples (denoted by “a”),
220 abundant P15 in all Kenyan isolates (in purple), as well as a distinct pattern

221 characteristic of strains IL3578 to IL2326, characterised by the combination of low P1
222 to 3 and high P7 (denoted by "b"). The latter does not seem to be related to
223 geography, as it encompasses isolates from Kenya, Uganda, and Burkina Faso. The
224 PCA plot further indicates that VSG repertoires and geography are only weakly
225 correlated (Fig. 4C), which agrees with our previous observation that *T. congolense*
226 VSG repertoires do not mimic either population structure or geography [18].
227
228 *T. congolense* tVAPs
229 We have used VAPPER to analyse the expressed VSG repertoire of the metacyclic
230 (infective) life stage of *T. congolense.* For that, we have produced a tVAP for the
231 strain TC13, whose transcriptome was published by Awuoche *et al.* (2018) [31]. We
232 have compared its metacyclic tVAP to the metacyclic tVAP of the 1/148 strain
233 (MBOI/NG/60/1-148) that we have previously described [29]. Furthermore, we have
234 compared them to the genomic *VSG* repertoires of the same strain, or a related one
235 (Fig. 5). As we do not have a genome sequence for the TC13 isolate, we compared it
236 to IL3000, which was isolated in the same region (Transmara, Kenya) [32].
237
238 When we compare the gVAPs of 1/148 and IL3000, we see that they are distinct, and
239 so are the tVAPs (e.g. P4 is more represented in TC13, whereas P10 is more
240 represented in 1/148 than in TC13). However, P8 is overrepresented in both isolates
241 compared to the genomic repertoires (Fig. 5). This agrees with our previous
242 observation that the pattern of metacyclic *VSG* expression is significantly different
243 from the genome repertoires, and that the metacyclic *VSG* repertoire is particularly
244 enriched for P8 genes [18]. With the analysis of the TC13 transcriptome, we can now
245 add that this enrichment does not seem to be strain-specific, but rather equally
246 applicable to *T. congolense* strains of distinct backgrounds.
247

T. vivax gVAP

The *T. vivax* gVAP shows the VAPs in the context of the sample cohort (N=29), which currently includes samples from Nigeria, Uganda, Gambia, Ivory Coast, Brazil, Burkina Faso, and Togo. The dendrogram represents the relationships between the multiple strains, whereas the heatmap shows whether *VSG* genes are present or absent in each strain (Fig. 6A). The VAP relationship shows a separation between Nigerian (in dark blue) and the remaining samples, as well as a clear difference between Brazilian and Ugandan isolates. The geographical signature is diminished slightly in the non-Nigerian West African strains, although this is may reflect the smaller number of samples per country and perhaps the geographical closeness between Togo, Burkina Faso, and Ivory Coast. Despite the lack of a transcriptomic pipeline for *T. vivax*, we can use the gVAP to understand the geographical distribution of expressed *VSGs*. As an example, we took the two most abundant *VSGs* in the transcriptomes of three strains (i.e. LIEM-176 from Venezuela [33], IL1392 from Nigeria [34], and Lins from Brazil [35]) and compared them to the VAP database (Fig. 6B). We observe that there are five different *VSGs*, which represent three different geographical patterns (Fig. 6C). Specifically, the first LIEM-176 *VSG* transcript has been found in strains from Venezuela, Nigeria and Gambia, but not in Brazil, Uganda, or Ivory Coast (map 1 in Fig. 6C). The second LIEM-176 *VSG* is present in Brazil, Venezuela, Nigeria, and Uganda, yet not in Ivory Coast, a pattern that is shared with the top two most abundant *VSGs* in Lins (map 2 in Fig. 6C). Finally, the top two most abundant *VSGs* in IL1392 have been found in strains from Brazil, Venezuela, Gambia, Nigeria, but not in Uganda nor Ivory Coast (map 3 in Fig. 6C). It is possible that strain or location-specific *VSGs* might be epidemiologically relevant, perhaps contributing to the considerable phenotypic variation observed in *T. vivax* AAT.

Conclusion

VAPPER is the first tool for the systematic analysis of *VSG* gene and expression diversity across strains and during infections. It establishes a practical approach for measuring antigenic diversity in these important pathogens based on universal protein motifs and/or gene mapping. Despite being often seen as a veterinary extension of HAT, AAT is a spectrum of diseases, dependent on the multiple species and strains of African trypanosomes and their multiple mammal hosts [36]. This predicament results in large variability in pathogenesis, epidemiology, and clinical outcome that remains poorly understood. For example, in East Africa, *T. vivax*, usually causes mild, chronic disease, but has occasionally resulted in acute haemorrhagic syndromes [37] without apparent reason. Likewise, in Brazil, related strains of *T. vivax* can cause both chronic disease of low parasitaemia and localized epidemics of up to 70% mortality rates, even in the same host species (although perhaps not the same genetic background) [38–40]. VAPPER allows us to identify and characterise differences in antigenic repertoires between strains, hosts, and conditions, which may be the starting point to build a real understanding of the association between disease genotypes and phenotypes. Importantly, with time this approach may be extended to the analysis of similar multi-copy, contingency gene families, particularly those involved in antigenic variation, in diverse pathogens.

Availability and requirements

Project name: VAPPER – High-throughput Variant Antigen Profiling in African trypanosomes

Project home page: https://github.com/PGB-LIV/VAPPER

Operating System: Platform independent

Programming language: Python

Installation Requirements: Velvet 1.2.10; HMMER 3.1b2; Bowtie 2 2.2.6; SAMtools 1.6; Cufflinks 2.2.1; BLAST 2.7.1; EMBOSS

License: Apache v.2.0

Figure Legends

Figure 1 Methodological workflow according to species (*T. congolense* or *T. vivax*) and input data [genomic (gVAP) or transcriptomic (tVAP)].

Figure 2 Screenshot of VAPPER on the Galaxy interface. This interface is available after installation of VAPPER from the Galaxy ToolShed [19] into a local Galaxy server. In this case, VAPPER was installed on the University of Liverpool Galaxy server. The blue panel on the right shows how to search and select VAPPER after installation. The white panel at the centre shows the options available for the user, including the prefix name of the sample to appear on the output figures, the species, and the type of input data. If any genomic pipeline is selected, further options for genome assembly parameters are available. Finally, the user can choose whether to get the graphs in PDF format (default is PNG only).

Figure 3 VAPPER performance (*T. congolense* genomic pipeline). (A) Correlation of phylotype frequencies produced by VAPPER and those manually curated in the *T. congolense* IL3000 reference genome sequence [14]. Pearson's product moment correlation statistics: $R^2 = 0.88$, $t(13) = 9.7321$, $P < 0.001$. (B) Correlation of phylotype frequencies produced by VAPPER and BLAST-based [41] phylotype frequencies in a panel of 41 *T. congolense* strains. Pearson's product moment correlation: $R^2 = 0.64$, $t(566) = 34.39$, $P < 0.001$. Phylotypes are color-coded according to the key. (C) VAPPER accuracy in fragmented (red) or incomplete (blue) genomes. Line graphs show correlations of the expected antigen profiles of a known

set of VSGs sequences from the IL3000 genome sequence with antigen profiles produced from fragmented VSGs or incomplete VSG repertoires. Fragmentation and genome incompletion were simulated from random sampling. Gene fragmentation was calculated as a proportion of the mean length of the original VSG sequences (Mean±σ=1163±129 nucleotides). Figure adapted from [18].

Figure 4 VAPPER output for *T. congolense* genomic pipeline. (A) Heatmap and corresponding dendrogram showing the variant antigen profiles (VAP) of the current genomic database expressed as phylotype frequencies [18,25]. (B) Heatmap and corresponding dendrogram showing the variant antigen profiles (VAP) of the current genomic database expressed as deviation from the mean phylotype frequency [18,25]. Labels “a” and “b” are referred to in the text. (C) PCA plot representing variation in *VSG* repertoire across the *T. congolense* genomic database [18,25] (N=97).

Figure 5 VAPPER output for *T. congolense* transcriptomic pipeline. Stacked bar charts showing expressed variant antigen profiles (VAPs) of metacyclic-stage *T. congolense* from strain 1/148 [18] and TC13 [31] compared to the genomic repertoires of the same strain (1/148) or a closely related one (IL3000) [28]. Phylotypes are colour-coded according to key. Size of each stack represents proportion of the phylotype relative to the total repertoire of expressed *VSGs*.

Figure 6 VAPPER output for *T. vivax* and its uses. (A) Heatmap and corresponding dendrogram showing the *T. vivax* variant antigen profiles (VAPs) in the context of the current genomic database (N=29). Strains are colour-coded according to key. (B) Two most abundant *VSG* genes in the three transcriptomes previously published for *T. vivax* (strains LIEM-176 [33], IL1392 [34], and Lins [35]. Numbers 1) to 3) relate to the *VSG* type in C. (C) Geographical distribution of the 6

VSG transcripts described in B. This is information can be obtained from the analysis of the VAP heatmap presented in (A).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by a Grand Challenges (Round 11) award from the Bill and Melinda Gates Foundation, a BBSRC New investigator Award (BB/M022811/1), and the Technology Directorate of the University of Liverpool to APJ.

Authors' contributions

SSP wrote the original code in Perl and tested the software. JH and ARJ wrote the final code in Python. SSP and APJ conceptualized the software and wrote the manuscript. All authors contributed to and approved the final manuscript.

References

1. Barry JD, Ginger ML, Burton P, McCulloch R. Why are parasite contingency genes often associated with telomeres? Int. J. Parasitol. 2003;33:29–45.

2. de la Fuente J, Lew A, Lutz H, Meli ML, Hofmann-Lehmann R, Shkap V, et al. Genetic diversity of anaplasma species major surface proteins and implications for

anaplasmosis serodiagnosis and vaccine development. Anim. Health Res. Rev. 2005;6:75–89.

3. Kyes SA, Kraemer SM, Smith JD. Antigenic variation in Plasmodium falciparum: Gene organization and regulation of the var multigene family. Eukaryot. Cell. 2007;6:1511–20.

4. McCulloch R, Cobbold CA, Figueiredo L, Jackson A, Morrison LJ, Mugnier MR, et al. Emerging challenges in understanding trypanosome antigenic variation. Emerg. Top. Life Sci. 2017;1:585–92.

5. Pays E. The variant surface glycoprotein as a tool for adaptation in African trypanosomes. Microbes Infect. 2006;8:930–7.

6. Cross G a M, Kim HS, Wickstead B. Capturing the variant surface glycoprotein repertoire (the VSGnome) of Trypanosoma brucei Lister 427. Mol. Biochem. Parasitol. Elsevier B.V.; 2014;195:59–73.

7. Matthews KR, McCulloch R, Morrison LJ. The within-host dynamics of African trypanosome infections. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 2015;370:20140288-.

8. Capewell P, Clucas C, DeJesus E, Kieft R, Hajduk S, Veitch N, et al. The TgsGP gene is essential for resistance to human serum in Trypanosoma brucei gambiense. PLoS Pathog. 2013;9:e1003686.

9. Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, et al. Mechanism of Trypanosoma brucei gambiense resistance to human serum. Nature. 2013;501:430–4.

10. De Greef C, Hamers R. The serum resistance-associated (SRA) gene of Trypanosoma brucei rhodesiense encodes a variant surface glycoprotein-like protein. Mol. Biochem. Parasitol. 1994;68:277–84.

11. Van Xong H, Vanhamme L, Chamekh M, Chimfwembe CE, Van Den Abbeele J, Pays A, et al. A VSG expression site-associated gene confers resistance to human serum in Trypanosoma rhodesiense. Cell. 1998;95:839–46.

12. Wiedemar N, Graf FE, Zwyer M, Ndomba E, Kunz Renggli C, Cal M, et al. Beyond immune escape: a variant surface glycoprotein causes suramin resistance in Trypanosoma brucei. Mol. Microbiol. 2018;107:57–67.

13. Salmon D, Geuskens M, Hanocq F, Hanocq-Quertier J, Nolan D, Ruben L, et al. A novel heterodimeric transferrin receptor encoded by a pair of VSG expression site-associated genes in T. brucei. Cell. 1994;78:75–86.

14. Jackson AP, Berry A, Aslett M, Allison HC, Burton P, Vavrova-Anderson J, et al. Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species. Proc. Natl. Acad. Sci. U. S. A. 2012;109:3416–21.

15. Marcello L, Menon S, Ward P, Wilkes JM, Jones NG, Carrington M, et al. VSGdb: A database for trypanosome variant surface glycoproteins, a large and diverse family of coiled coil proteins. BMC Bioinformatics. 2007;8:1–8.

16. Weirather JL, Wilson ME, Donelson JE. Mapping of VSG similarities in Trypanosoma brucei. Mol. Biochem. Parasitol. 2012;181:141–52.

17. Mugnier MR, Cross GAM, Papavasiliou FN. The in vivo dynamics of antigenic variation in Trypanosoma brucei. Science (80-.). 2015;347:1470–3.

18. Silva Pereira S, Casas-Sanchez A, Haines LR, Absolomon K, Ogugo M, Sanders M, et al. Variant antigen repertoires in Trypanosoma congolense populations and experimental infections can be profiled from deep sequence data with a set of universal protein motifs. Genome Res. 2018;28:1383–94.

19. Blankenberg D, Von Kuster G, Bouvier E, Baker D, Afgan E, Stoler N, et al. Dissemination of scientific software with Galaxy ToolShed. Genome Biol. 2014.

20. Afgan E, Baker D, van den Beek M, Blankenberg D, Bouvier D, Čech M, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 2016;44:W3–10.

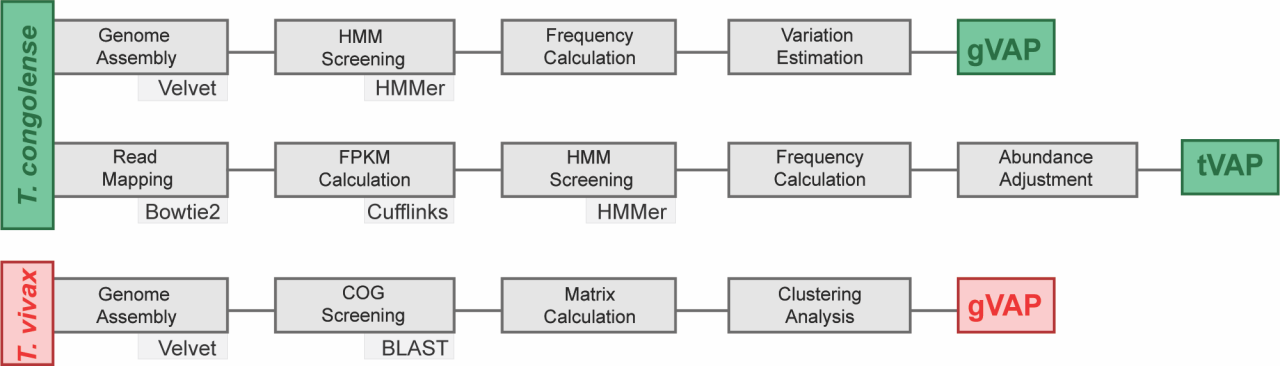
21. Cock PJA, Fields CJ, Goto N, Heuer ML, Rice PM. The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. Nucleic Acids Res. 2009;38:1767–71.

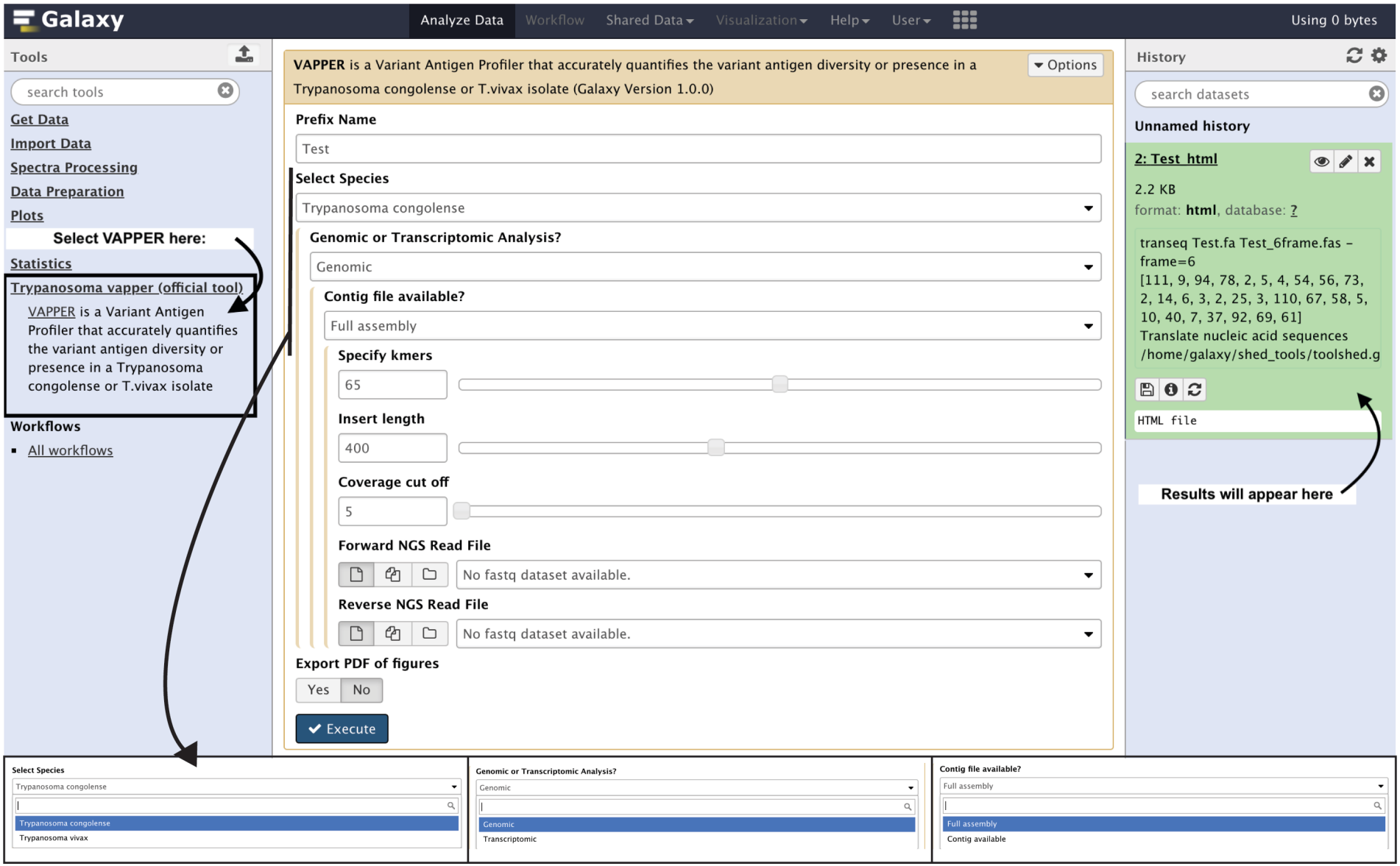
440 22. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison.
441 Proc. Natl. Acad. Sci. 1988;85:2444–8.
442 23. Zerbino DR. Using the Velvet de novo assembler for short-read sequencing
443 technologies. Curr. Protoc. Bioinforma. 2010.
444 24. Eddy SR. A new generation of homology search tools based on probabilistic
445 inference. Genome Inform. 2009;23:205–11.
446 25. Tihon E, Imamura H, Dujardin J-C, Van Den Abbeele J, Van den Broeck F.
447 Discovery and genomic analyses of hybridization between divergent lineages of
448 *Trypanosoma congolense* , causative agent of Animal African Trypanosomiasis. Mol.
449 Ecol. 2017;
450 26. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat
451 Methods. 2012;9:357–9.
452 27. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene
453 and transcript expression analysis of RNA-seq experiments with TopHat and
454 Cufflinks. Nat. Protoc. 2012;7:562–78.
455 28. Gibson W. The origins of the trypanosome genome strains Trypanosoma brucei
456 brucei TREU 927, T. b. gambiense DAL 972, T. vivax Y486 and T. congolense
457 IL3000. Parasit. Vectors. BioMed Central Ltd; 2012;5:71.
458 29. Young CJ, Godfrey DG. Enzyme polymorphism and the distribution of
459 Trypanosoma congolense isolates. Ann. Trop. Med. Parasitol. 1983;77:467–81.
460 30. Abbas AH, Pereira SS, D'Archivio S, Wickstead B, Morrison LJ, Hall N, et al. The
461 structure of a conserved telomeric region associated with variant antigen loci in the
462 blood parasite Trypanosoma congolense. Genome Biol. Evol. 2018;evy186.
463 31. Awuoche EO, Weiss BL, Mireji PO, Vigneron A, Nyambega B, Murilla G, et al.
464 Expression profiling of Trypanosoma congolense genes during development in the
465 tsetse fly vector Glossina morsitans morsitans. Parasit. Vectors. Parasites & Vectors;
466 2018;11:1–18.
467 32. Ferrante A, Allison AC. Alternative pathway activation of complement by African

468 trypanosomes lacking a glycoprotein coat. Parasite Immunol. 1983;5:491–8.
469 33. Greif G, Ponce de Leon M, Lamolle G, Rodriguez M, Piñeyro D, Tavares-
470 Marques LM, et al. Transcriptome analysis of the bloodstream stage from the
471 parasite Trypanosoma vivax. BMC Genomics. 2013;14:149.
472 34. Jackson AP, Goyard S, Xia D, Foth BJ, Sanders M, Wastling JM, et al. Global
473 Gene Expression Profiling through the Complete Life Cycle of Trypanosoma vivax.
474 PLoS Negl. Trop. Dis. 2015;9:e0003975.
475 35. Guedes RLM, Rodrigues CMF, Coatnoan N, Cosson A, Cadioli FA, Garcia HA, et
476 al. A comparative in silico linear B-cell epitope prediction and characterization for
477 South American and African Trypanosoma vivax strains. Genomics. Elsevier;
478 2018;0–1.
479 36. Morrison LJ, Vezza L, Rowan T, Hope JC. Animal African Trypanosomiasis: Time
480 to Increase Focus on Clinically Relevant Parasite and Host Species. Trends
481 Parasitol. 2016;32:599–607.
482 37. Wellde BT, Chumo DA, Adoyo M, Kovatch RM, Mwongela GN, Opiyo EA.
483 Haemorrhagic syndrome in cattle associated with trypanosoma vivax infection. Trop.
484 Anim. Health Prod. 1983;15:95–102.
485 38. Paiva F, Lemos R a a De, Nakazato L, Mori a E, Brum KB, Bernardo KC, et al.
486 Trypanosoma Vivax Em Bovinos No Pantanal Do Estado Do Mato Grosso Do Sul ,
487 Brasil□: I – Acompanhamento Clínico ,. Rev. Bras. Parasitol. Veterinária.
488 2000;9:135–41.
489 39. Cadioli FA, de Athayde Barnabé P, Zacarias Machado R, Alves Teixeira MC,
490 André MR, Sampaio PH, et al. First report of Trypanosoma vivax outbreak in dairy
491 cattle in São Paulo state, Brazil. Rev. Bras. Parasitol. Vet., Jaboticabal.
492 2012;21:118–24.
493 40. Fidelis Jr OL, Sampaio PH, Machado RZ, Andre MR, Marques LC, Cadioli FA.
494 Evaluation of clinical signs , parasitemia , hematologic and biochemical changes in
495 cattle experimentally infected with Trypanosoma vivax. Brazilian J. Vet. Parasitol.

2016;2961:69–81.

41. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. 1990;215:403–10.


T. congolense
Genome Assembly
Velvet
HMM Screening
HMMer
Frequency Calculation
Variation Estimation
gVAP
Read Mapping
Bowtie2
FPKM Calculation
Cufflinks
HMM Screening
HMMer
Frequency Calculation
Abundance Adjustment
tVAP
T. vivax
Genome Assembly
Velvet
COG Screening
BLAST
Matrix Calculation
Clustering Analysis
gVAP


Galaxy
Analyze Data
Workflow
Shared Data
Visualization
Help
User
Using 0 bytes
Tools
search tools
Get Data
Import Data
Spectra Processing
Data Preparation
Plots
Select VAPPER here:
Statistics
Trypanosoma vapper (official tool)
VAPPER is a Variant Antigen Profiler that accurately quantifies the variant antigen diversity or presence in a Trypanosoma congolense or T.vivax isolate
Workflows
All workflows
VAPPER is a Variant Antigen Profiler that accurately quantifies the variant antigen diversity or presence in a Trypanosoma congolense or T.vivax isolate (Galaxy Version 1.0.0)
Options
Prefix Name
Test
Select Species
Trypanosoma congolense
Genomic or Transcriptomic Analysis?
Genomic
Contig file available?
Full assembly
Specify kmers
65
Insert length
400
Coverage cut off
5
Forward NGS Read File
No fastq dataset available.
Reverse NGS Read File
No fastq dataset available.
Export PDF of figures
Yes
No
Execute
History
search datasets
Unnamed history
2: Test_html
2.2 KB
format: html, database: ?
transeq Test.fa Test_6frame.fas -frame=6
[111, 9, 94, 78, 2, 5, 4, 54, 56, 73, 2, 14, 6, 3, 2, 25, 3, 110, 67, 58, 5, 10, 40, 7, 37, 92, 69, 61]
Translate nucleic acid sequences
/home/galaxy/shed_tools/toolshed.g
HTML file
Results will appear here
Select Species
Trypanosoma congolense
Trypanosoma congolense
Trypanosoma vivax
Genomic or Transcriptomic Analysis?
Genomic
Genomic
Transcriptomic
Contig file available?
Full assembly
Full assembly
Contig available