EpiBuilder: A Tool for Assembling, Searching, and Classifying B-Cell Epitopes

Bioinformatics and Biology Insights
Volume 16: 1–11
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11779322221095221
SAGE

Renato Simões Moreira[1,2], Vilmar Benetti Filho[3], Nathália Anderson Calomeno[1], Glauber Wagner[3] and Luiz Claudio Miletti[1]

[1]Laboratório de Hemoparasitas e Vetores, Departamento de Produção Animal e Alimentos, Centro de Ciências Agroveterinárias (CAV), Universidade do Estado de Santa Catarina (UDESC), Lages, Brazil. [2]Instituto Federal de Santa Catarina (IFSC), Lages, Brazil. [3]Laboratório de Bioinformática, Universidade Federal de Santa Catarina, Florianópolis, Brazil.

ABSTRACT: Epitopes are portions of a protein that are recognized by antibodies. These small amino acid sequences represent a significant breakthrough in a branch of bioinformatics called immunoinformatics. Various software are available for linear B-cell epitope (BCE) prediction such as *ABCPred*, *SVMTrip*, *EpiDope*, and *EpitopeVec*; a well-known BCE predictor is BepiPred-2.0. However, despite the prediction, there are several essential steps, such as epitope assembly, evaluation, and searching for epitopes in other proteomes. Here, we present EpiBuilder (https://epibuilder.sourceforge.io), a user friendly software that assists in epitope assembly, classifying and searching using input results of BepiPred-2.0. EpiBuilder generates several output results from these data and supports a proteome-wide processing approach. In addition, this software provides the following features: Chou & Fasman beta-turn prediction, Emini surface accessibility prediction, Karplus and Schulz flexibility prediction, Kolaskar and Tongaonkar antigenicity, Parker hydrophilicity prediction, *N*-glycosylation domains, and hydropathy. These information generate a unique topology for each epitope, visually demonstrating its characteristics. The software can search the entire epitope sequence in various FASTA files, and it allows to use BLASTP to identify epitopes that eventually have sequence variations. As an EpiBuilder application, we developed a epitope dataset from the protozoan *Trypanosoma brucei gambiense*, the gram-positive bacterium *Clostridioides difficile*, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

KEYWORDS: Immunoinformatics, BepiPred-2.0, Galaxy

RECEIVED: February 7, 2022. **ACCEPTED:** March 29, 2022.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

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

CORRESPONDING AUTHORS: Glauber Wagner, Laboratório de Bioinformática, Universidade Federal de Santa Catarina, Campus João David Ferreira Lima, Setor F, Bloco A, Sala 318, Caixa postal 476, Trindade, Florianópolis 88040-970, SC, Brazil. Email: glauber.wagner@ufsc.br

Luiz Claudio Miletti, Laboratório de Hemoparasitas e Vetores, Departamento de Produção Animal e Alimentos, Centro de Ciências Agroveterinárias (CAV), Universidade do Estado de Santa Catarina (UDESC), Av. Luiz de Camões, 2090 Bairro Conta Dinheiro, Lages 88520-000, SC, Brazil. Email: luiz.miletti@udesc.br

Introduction

Immune responses are divided into innate and adaptive responses. The adaptive immune response appears as a response to a large number of substances and adapts to them. It is characterized by specificity (ability to distinguish different substances) and memory (ability to respond more vigorously to repeated infection). The components of adaptive immunity are lymphocytes and their secretions, including antibodies or immunoglobulins (secreted by B lymphocytes or B cells).[1]

Secreted antibodies recognize a specific part of the antigen, called an epitope,[2] bind together, neutralize it (preventing it from infecting cells), or mark it for elimination through, for example, phagocytosis, activation of the classical complement pathway, and recognition by eosinophils of immunoglobulin E bound to the pathogen followed by the release of granules harmful to the invading microorganism.

Antibodies are the only components of adaptive immunity that prevent infections. Therefore, the production of potent antibodies is the objective of vaccination.[1] The identification of B-cell epitopes (BCEs) is of great importance for the development of vaccines, immunodiagnostics, and therapeutic antibodies.[2] Experimental methods to identify BCEs in vivo and in vitro are expensive and time-consuming. Therefore, new computational methods must be developed to rapidly identify potential epitopes of B cells.[3]

Currently, immunoinformatics is a promising tool in the study of several organisms, including viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)[4,5]; protozoa such as *Toxoplasma gondii*,[6] *Trypanosoma brucei brucei*,[7] and *Leishmania infantum*[8]; bacteria such as *Leptospira interrogans*[9]; and even cancer cells.[10]

Several immunoinformatics tools have been developed for the identification of BCEs, such as *ABCPred*,[11] *SVMTrip*,[12] *EpiDope*,[13] and *EpitopeVec*,[14] one of the most popular being BepiPred-2.0. In addition, the Epitope Prediction and Analysis Tools (http://tools.iedb.org/bcell/) provide several methods for analyzing individual characteristics, such as Chou & Fasman beta-turn,[15] Emini surface accessibility,[16] Karplus and Schulz flexibility,[17] Kolaskar and Tongaonkar antigenicity,[18] and Parker hydrophilicity.[19] Despite this, such tools do not have integration, generating independent results that need to be generated individually, which makes the task expensive for analysis with large volumes of proteins.

In this regard, we present EpiBuilder, a tool capable of assembling BCEs from data generated by BepiPred-2.0, and from these data, the tool assists the researcher in several types of experiments, including those on a proteome-wide scale.

Materials and Methods

EpiBuilder was developed using the Java programming language (supported by version 1.8) with a graphical user interface (GUI) and command-line options, without any additional prerequisite to perform all functions except BLASTP if this is set. EpiBuilder is prepared to run on any operating system that supports Java technology, such as Windows, MacOS, and Unix-based systems.

In addition to the aforementioned versions, we developed an EpiBuilder version with a GUI for the Galaxy platform.[20] The interfaces were developed and validated using the Planemo (https://planemo.readthedocs.io) program, following the recommendations of good galaxy tool development practices.

Galaxy is an open-source framework that aims to facilitate the reproducibility of bioinformatic analyses and access tools for various types of analysis.[20] The platform allows the user to adapt any program that runs along the command line to a graphical interface. Open servers (https://usegalaxy.org) make analytics more accessible when dealing with large volumes of data, and tools and interfaces are shared with the entire community through the Galaxy ToolShed repository.[21] The repository extends the Galaxy service for developers to create and publish new tools or adaptations of the existing programs.

The GUI and terminal mode executable files, in addition to the source code, are available at https://epibuilder.sourceforge.io. The EpiBuilder galaxy version is available in ToolShed under the name EpiBuilder.

Input files

There are 3 primary forms of system inputs: the first is the CSV file (comma separated values) generated by BepiPred-2.0[22] and the second is the result generated by BepiPred-2.0 through the output of the B-Cell Standalone IEDB program (http://tools.iedb.org/bcell/).[23] The third option allows the user to input a job id (the number used to run BepiPred-2.0 online), and after the process is finished, the file can be downloaded from the BepiPred-2.0 server.

Processing

The file generated by BepiPred-2.0[22] is uploaded to EpiBuilder, the proteins are reassembled from these data, and the scores are calculated for the following features: Emini surface accessibility,[16] Parker hydrophilicity,[19] Chou & Fasman beta-turn,[15] Karplus & Schulz flexibility,[17] and Kolaskar and Tongaonkar.[18] These scores are calculated internally by EpiBuilder, and such calculations have been rewritten in Java based on the IEDB B-Cell Standalone Python script. In addition, the molecular mass (MW), isoelectric point (pI), and hydropathy index of each amino acid are calculated using the BioJava library.[24]

The epitopes are mounted from the threshold of BepiPred-2.0 (default value of 0.6). That is, if the amino acid score is greater than or equal to the cutoff point, it will be concatenated with the following amino acid with a score greater than or equal to the cutoff point. When an amino acid with a score less than the cutoff point is identified, the process reserves the generated epitope. Then, these epitopes are filtered so that only those between the minimum (default value 10) and maximum (default value 30) remain. Next, the presence of the *N*-glycosylation site is verified by searching for the Asn-Xaa-(Ser/Thr) domain, where Xaa is any amino acid except Proline.[25]

Topology

For each identified epitope, an individual analysis of amino acids is performed for the features chosen by the user. If the amino acid has a score equal to or above the threshold chosen for the feature, and the threshold can be calculated (default) or informed by the user, the current amino acid in question receives a representative character "E" when the assigned value is lower.

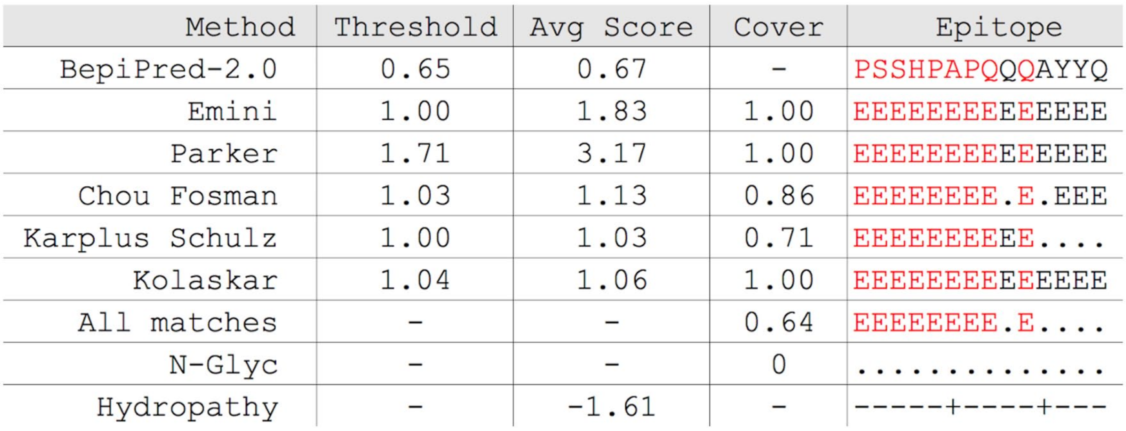
Finally, a structure called All Matches is generated, which includes the amino acids with a score above or equal to the cutoff point for all selected functionalities; thus, it is possible to evaluate the specific stretches in which there is consensus in the analyzed structures. All Matches also receive a coverage value relative to the epitope.

Along with these excerpts of the topology, the *N*-glycosylation sites are also informed through the character "E". If the amino acid has a hydropathy index less than 0, it is assigned the character "–", and if it is greater than or equal to 0, it is assigned the character. The case of epitope topology PSSHPAPQQQQAYYQ extracted from the tbg972.9.8930 protein of *Trypanosoma brucei gambiense* reference proteome (strain TREU927, TriTrypdb version 49) is shown in Figure 1.

For each feature, the number of amino acids above the cutoff point is determined, thus generating the percentage coverage of each feature in relation to the complete epitope. The average of all coverage values is calculated by generating the Avg cover attribute and these values appear in the descending order during the generation of data.

Search for epitopes

The epitopes generated are identified in the proteomes informed (FASTA file) by the user. The proteomes are loaded into the RAM, and the search is performed by searching for the complete epitope sequence in the proteins. Therefore, we recommend adjusting the execution memory of the java parameters settings, -Xms (start memory) and -Xmx (max memory), according to the file size of the proteomes.



Method	Threshold	Avg Score	Cover	Epitope
BepiPred-2.0	0.65	0.67	-	PSSHPAPQQQAYYQ
Emini	1.00	1.83	1.00	EEEEEEEEEEEEEE
Parker	1.71	3.17	1.00	EEEEEEEEEEEEEE
Chou Fosman	1.03	1.13	0.86	EEEEEEEE.E.EEE
Karplus Schulz	1.00	1.03	0.71	EEEEEEEEEE....
Kolaskar	1.04	1.06	1.00	EEEEEEEEEEEEEE
All matches	-	-	0.64	EEEEEEEE.E....
N-Glyc	-	-	0	....
Hydropathy	-	-1.61	-	-----+----+---

Figure 1. Topology generated by EpiBuilder, the epitope is individually analyzed with visual presentation of its features, besides presenting the element All Matches, where the amino acid is above the cutoff point in all selected methods. The topology also presents the sites of *N*-glycosylation and also hydropathy.

The search can also be performed by BLASTP[26] (requires prior installation on the computer), which is necessary to inform the location of the installation in the execution command or in the graphical interface (if the command is already in the system $PATH, one can inform only the blastp and makeblastdb commands, without full path). The identity (default value 90), cover (default value 90), word size (default value 4), and task (blastp-short default value) parameters can also be adjusted. The Blastp results are converted internally and integrated with the EpiBuilder result, and next to the positive hit, the protein accession number is added to the identity and coverage if the user wants to know the hits. For extensive searches, it is suggested that this configuration be removed. As these are small sequences for searching, it is important to evaluate blastp values, as they can eliminate results by exchanging only 1 amino acid.

For EpiBuilder installed via Galaxy ToolShed, if a BLASTP search is chosen, the blastp and makeblastdb commands are required to be on the system $PATH. For the desktop version, there is a test option to verify that the blastp and makeblastdb commands setup is correct.

Output files

The output files are generated with the name <basename>-epibuilder-<file>.<archive>, so the user can perform multiple experiments in the same directory by swapping the <basename> of the experiment in question. The files generated are listed in Table 1.

If the user chooses to run the query via BLASTP, all output files generated by BLASTP follow the <basename>-epibuilder-blast-<proteome alias>.<extension>, and the result of BLASTP is generated in the CSV standard. The full EpiBuilder Stream is shown in Figure 2.

Experiments

For the experiments, we selected 3 human pathogens: the protozoan *T. b. gambiense*, causative agent of human African trypanosomiasis (HAT—sleeping sickness), a fatal neglected tropical disease[27,28]; the gram-positive bacterium *Clostridioides difficile*, formerly called *Clostridium difficile*, which causes severe diarrhea, colitis, toxic megacolon, and potentially death[29,30]; and SARS-CoV-2, also fatal[31] which caused a pandemic in 2020.[32]

The proteomes of SARS-CoV-2 (UP000464024, Uniprot), *Clostridioides difficile* (NCBI: txid1496), and *T. b. gambiense* (strain DAL972, TriTrypdb version 49) were downloaded and manually preprocessed to eliminate characters other than valid amino acids such as "*" and "X", which is a condition for running BepiPred-2.0. The proteomes were submitted to BepiPred-2.0 analysis using the B-Cell Standalone IEDB software, and this result was used as an input for EpiBuilder with the following configurations: BepiPred-2.0 threshold (0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, and 0.9), minimum epitope size (10), and maximum epitope size (30), all parameters were selected with default cutoff points. The searches were performed with the direct search of the sequence, as well as with the BLASTP option with identity parameters = 90, coverage = 90, word size = 4, and search method = blastp-short. In all experiments, the proteome was included in the search for epitopes that were repeated in other proteins. In addition, for each experiment, epitopes were searched in the human proteome (UP000005640, Uniprot).

To search for *T. b. gambiense*, we used the proteomes of *Trypanosoma brucei brucei* (strain TREU927, TriTrypdb version 49), *Trypanosoma evansi* (strain STIB902, TriTrypdb version 49), *Trypanosoma congolense* (strain IL3000, TriTrypdb version 49), and *Trypanosoma vivax* (strain Y486, TriTrypdb version 49). For *C. difficile*, we used the proteomes of *Clostridium*

Table 1. EpiBuilder provides several files generated at the final of the process.

FILE	TYPE	DESCRIPTION
epibuilder-detail	CSV	Individual epitope detail
epibuilder-epitopes	FASTA	epitope FASTA file
epibuilder-parameters	TXT	Parameters of execution
epibuilder-protein-summary	TSV	Summary of the run
epibuilder-scores	TSV	All generated scores
epibuilder-topology	TSV	Epitopes topology
epibuilder	TXT	Full report with all information except the score files
epibuilder	XLSX	XLSX file with the following tabs: • Tab 1 = epibuilder-detail • Tab 2 = epibuilder-protein-summary • Tab 3 = epibuilder-topology
epibuilder	LOG	The run log
epibuilder-blast*	–	The files generated in the BLASTP process

These files are provided in a different type. The main file is epibuilder.xlsx.

botulinum (strain Hall/ATCC 3502/NCTC 13319/Type A) (UP00001986, Uniprot), *Clostridium argentinense* (strain CDC 2741, UP000031366), and *Clostridium perfringens* (strain 13/Type A, UP000000818).

For runs with SARS-CoV-2 in addition to the proteome and human proteome, we also added a database of spike proteins (4 854 709 proteins) and all deposited proteins (133 081 009 proteins) in GISAID to the day (November 9, 2021). Blastp evaluation in this round was suppressed because of the number of sequences.

The last experiment with SARS-CoV-2 used the assembly of epitopes from the proteins S (spike), M (membrane), N (nucleocapsid), and E (envelope) of the Reference of SARS-CoV-2 (UP000464024, Uniprot) and were submitted to EpiBuilder to search for the respective proteins of the VoC (variant of concern) lines obtained from GISAID: alpha (EPI_ISL_6949888), beta (EPI_ISL_5905874), gamma (EPI_ISL_6943989), delta (EPI_ISL_6950127), and omicron (EPI_ISL_6914032). The genomes obtained from GISAID were submitted to NextStrain[33] for assembly, protein prediction, and lineage confirmation (Supplementary Figure 1). For this experiment, the BepiPred-2.0 threshold = 0.6 was explicitly chosen.

Finally, the SARS-CoV-2 proteins sequences of the reference, alpha, beta, gamma, delta, and omicron were aligned with Clustal Omega[34] (https://www.ebi.ac.uk/Tools/msa/clustalo/) with the default parameters values to verify mutations in the epitopes, and the results were organized by Jalview[35] omitting the regions that were not predicted as epitopes.

Results

GUI

Figure 3 shows the graphical interface of the EpiBuilder main screen, where the user can perform all the necessary settings, and the results are displayed in the results tab in text format.

Similar to the desktop graphical interface, it is possible to observe the graphical interface developed for the Galaxy platform (Figure 4). The search settings are presented in Supplementary Figure 2.

Experiments

In all experiments, epitopes were found up to a threshold of 0.75; from threshold = 0.8, no epitopes were identified in any of the experiments (Table 2).

Epitopes of SARS-CoV-2

Table 3 shows that the epitope with the most occurrences of spike protein was CTEVPVAIHADQ, which was present in ~99% of the proteins of the protein spikes bank and ~4% of the complete protein bank (133 081 009). The other epitopes MSLGAENSVAY and YRLFRKSNLKP were found in ~95% and NNLDSKVGGN and DEVRQIAPGQTGK in ~95%, which shows their exemplary conservation. No epitope contained *N*-glycosylation sites. Full results can be seen in Supplementary Data 1.

Considering the S, N, M, and E proteins of SARS-CoV-2, only protein E had no epitopes predicted for the adjusted values. Protein S presented 6 epitopes, some of which were completely identified in VoC sequences only by BLASTP, indicating amino acid exchanges in the epitopes of these variants, which is the case for the epitope QTQTNSPRRARSVA, whose sequence with the search for the complete structure only had an occurrence in the beta and gamma variants. Even with the blastp search, there was no occurrence of these epitopes in the omicron variant, including the epitope NNLDSKVGGN. The complete list of epitopes with their hits is shown in Table 4, and the alignment with the epitopes of the S protein can be seen in Figure 5, which precisely shows the exchange of a single

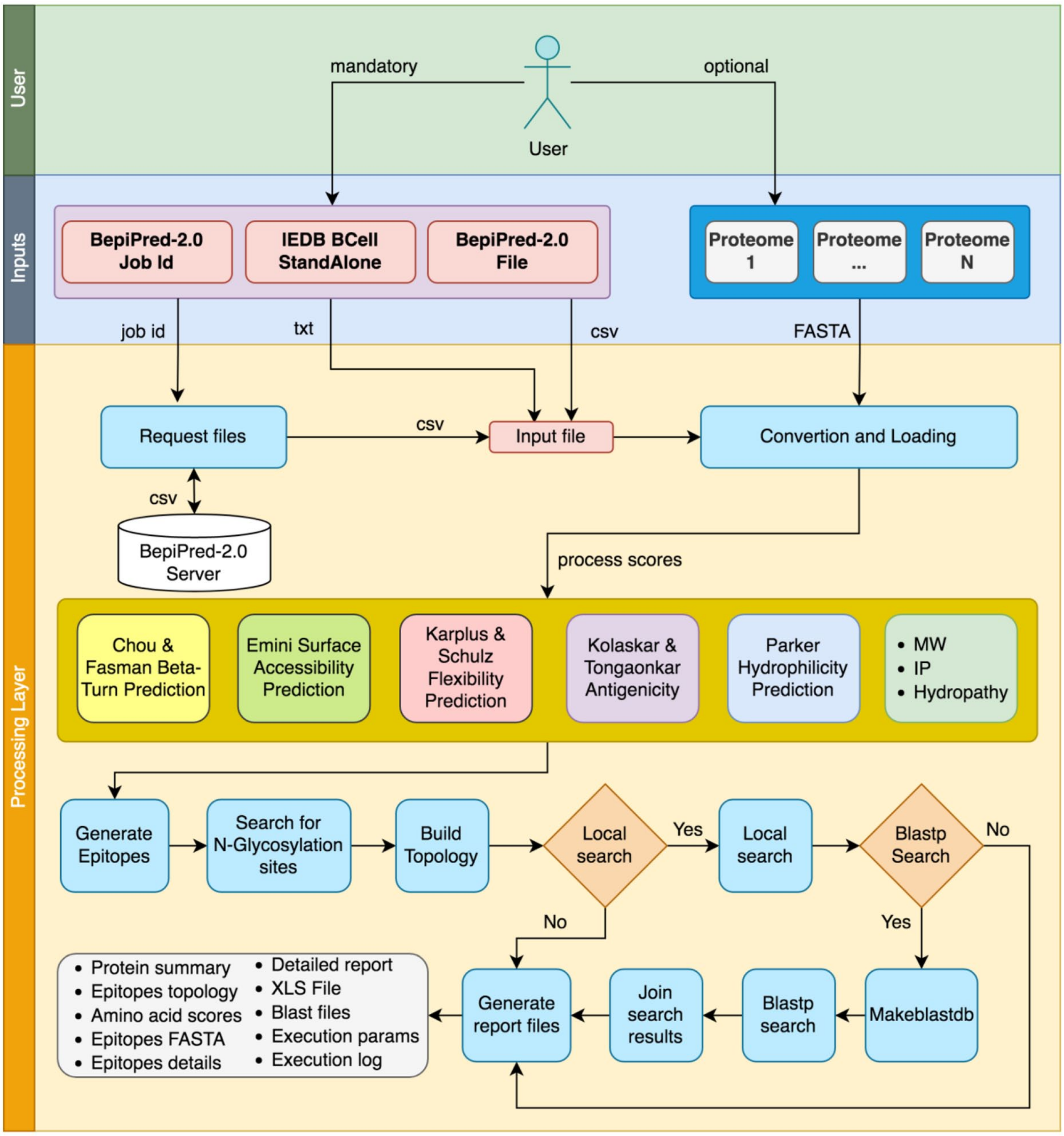
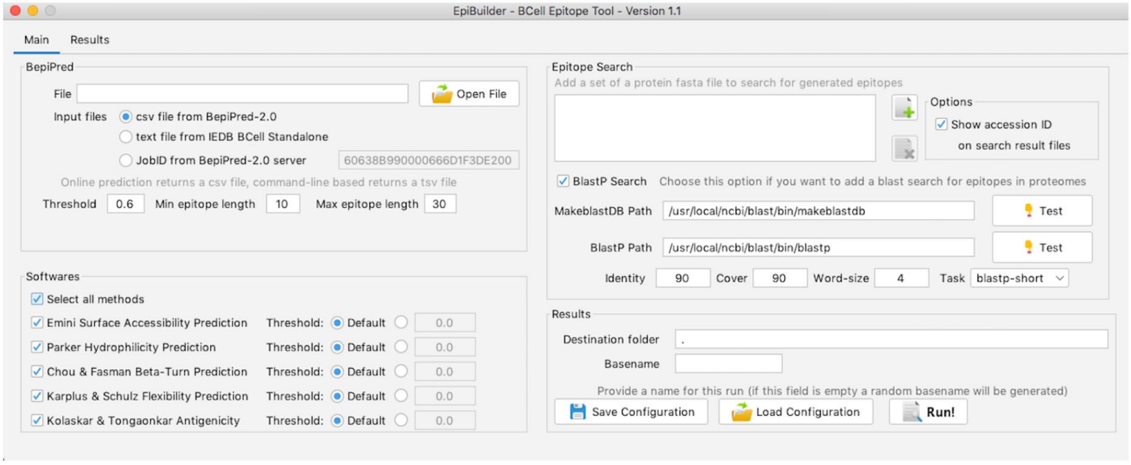


Figure 2. The complete EpiBuilder pipeline, indicating input files (BepiPred-2.0 files and proteomes for query) and the internal process such as input loading and processing of software scores, searching for *N*-glycosylation, assembling of the topology, and the searching in proteomes. After all these steps, the reports are generated in several files. IEDB indicates Immune Epitope Database; MW, molecular mass; IP, Isoleletric Point.


EpiBuilder - BCell Epitope Tool - Version 1.1

Main Results

BepiPred

File [Open File]

Input files ● csv file from BepiPred-2.0
○ text file from IEDB BCell Standalone
○ JobID from BepiPred-2.0 server 60638B990000666D1F3DE200

Online prediction returns a csv file, command-line based returns a tsv file

Threshold 0.6 Min epitope length 10 Max epitope length 30

Softwares

☑ Select all methods
☑ Emini Surface Accessibility Prediction Threshold: ● Default ○ 0.0
☑ Parker Hydrophilicity Prediction Threshold: ● Default ○ 0.0
☑ Chou & Fasman Beta-Turn Prediction Threshold: ● Default ○ 0.0
☑ Karplus & Schulz Flexibility Prediction Threshold: ● Default ○ 0.0
☑ Kolaskar & Tongaonkar Antigenicity Threshold: ● Default ○ 0.0

Epitope Search

Add a set of a protein fasta file to search for generated epitopes

Options
☑ Show accession ID on search result files

☑ BlastP Search Choose this option if you want to add a blast search for epitopes in proteomes

MakeblastDB Path /usr/local/ncbi/blast/bin/makeblastdb [Test]

BlastP Path /usr/local/ncbi/blast/bin/blastp [Test]

Identity 90 Cover 90 Word-size 4 Task blastp-short

Results

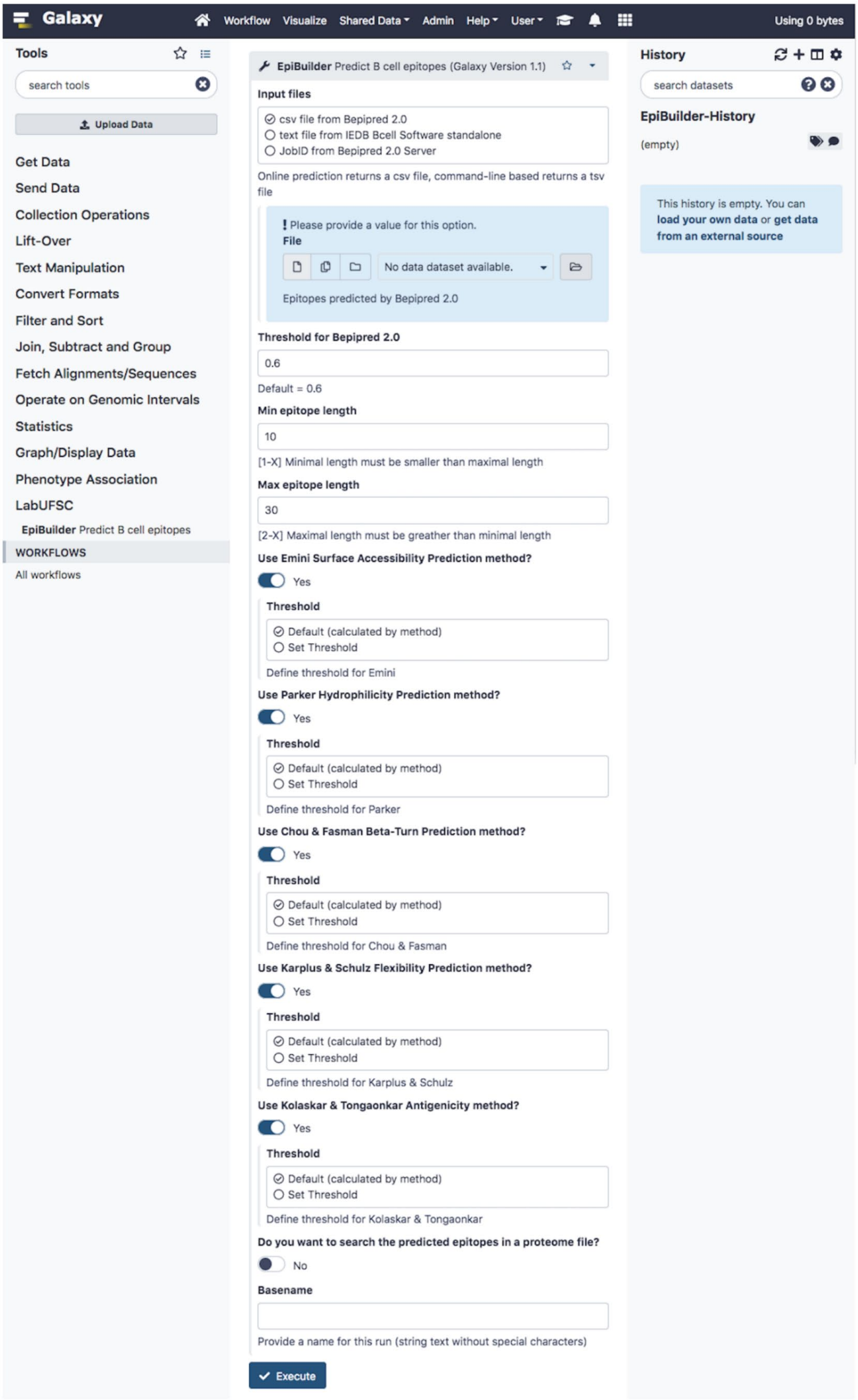
Destination folder .

Basename

Provide a name for this run (if this field is empty a random basename will be generated)

[Save Configuration] [Load Configuration] [Run!]

Figure 3. EpiBuilder desktop main screen. From this screen, the user can configure the input file, threshold, the minimum and maximum size of epitopes, the additional features it intends to analyze, the proteomes for searching the epitopes, and the option to search proteomes by BLASTP.



EpiBuilder Predict B cell epitopes (Galaxy Version 1.1)

Input files

- ● csv file from Bepipred 2.0
- ○ text file from IEDB Bcell Software standalone
- ○ JobID from Bepipred 2.0 Server

Online prediction returns a csv file, command-line based returns a tsv file

! Please provide a value for this option.

File

No data dataset available.

Epitopes predicted by Bepipred 2.0

Threshold for Bepipred 2.0

0.6

Default = 0.6

Min epitope length

10

[1-X] Minimal length must be smaller than maximal length

Max epitope length

30

[2-X] Maximal length must be greather than minimal length

Use Emini Surface Accessibility Prediction method?

Yes

Threshold

- ● Default (calculated by method)
- ○ Set Threshold

Define threshold for Emini

Use Parker Hydrophilicity Prediction method?

Yes

Threshold

- ● Default (calculated by method)
- ○ Set Threshold

Define threshold for Parker

Use Chou & Fasman Beta-Turn Prediction method?

Yes

Threshold

- ● Default (calculated by method)
- ○ Set Threshold

Define threshold for Chou & Fasman

Use Karplus & Schulz Flexibility Prediction method?

Yes

Threshold

- ● Default (calculated by method)
- ○ Set Threshold

Define threshold for Karplus & Schulz

Use Kolaskar & Tongaonkar Antigenicity method?

Yes

Threshold

- ● Default (calculated by method)
- ○ Set Threshold

Define threshold for Kolaskar & Tongaonkar

Do you want to search the predicted epitopes in a proteome file?

No

Basename

Provide a name for this run (string text without special characters)

This history is empty. You can **load your own data** or **get data from an external source**

Figure 4. EpiBuilder main screen on galaxy platform. From this screen, the user can configure the input file, threshold, the minimum and maximum size of epitopes, the additional features it intends to analyze, the proteomes for searching the epitopes, and the option to search proteomes by BLASTP.

Table 2. The total generated epitopes considering the various thresholds for the organisms *C. difficile*, *T. b. gambiense*, and SARS-CoV-2.

BEPIPRED-2.0 THRESHOLD	EPITOPES		
	C. DIFFICILE	*T. B. GAMBIENSE*	SARS-COV-2
0.50	13 395	45 547	182
0.55	5 664	37 946	96
0.60	1 431	19 907	29
0.65	401	5 462	3
0.70	47	329	2
0.75	7	8	0

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 3.SARS-CoV-2 epitopes generated using the threshold of 0.6.

ACCESSION ID	EPITOPE	N-GLYC	LENGTH	AVG COVER	DIRECT PROTEOME SEARCH		
					SCOV	ALL SPIKES	ALL PROTEINS
R1AB_SARS2	DDDSQQTVGQQDG	N	13	0.78	2	0	4 876 200
AP3A_SARS2	GTTSPISEHD	N	10	0.76	1	0	4 802 655
VME1_SARS2	KLNTDHSSSSDNI	N	13	0.75	1	0	4 862 069
R1A_SARS2	EQEEDWLDDDSQQTVGQQD	N	19	0.75	2	0	4 875 778
SPIKE_SARS2	QTQTNSPRRARSVA	N	14	0.74	1	1 127 228	1 130 945
R1A_SARS2	DLGDELGTDPYEDFQENWN	N	19	0.72	2	0	4 774 399
R1AB_SARS2	LHPTQAPTHLS	N	11	0.71	1	0	4 861 900
R1AB_SARS2	LGDELGTDPYED	N	12	0.7	2	0	4 785 788
SPIKE_SARS2	NNLDSKVGGN	N	10	0.7	1	4 607 437	4 743 622
NCAP_SARS2	MSGKGQQQQGQTVTK	N	15	0.69	1	0	3 518 539
R1AB_SARS2	EEVKPFITESKPSVEQRKQDDKK	N	23	0.69	2	0	4 744 396
R1A_SARS2	CEEEEFEPSTQYEYGTEDDYQGKPLEF	N	27	0.68	2	0	4 798 844
NCAP_SARS2	SDSTGSNQNGERSGARSKQRRPQGLPN	N	27	0.68	1	0	4 812 247
R1A_SARS2	EVKPFITESKPSVEQRKQDDKKIKA	N	25	0.67	2	0	4 743 252
R1AB_SARS2	EPSTQYEYGTEDDYQGKPLEFG	N	22	0.66	2	0	4 799 515
NCAP_SARS2	KDKKKKADETQALPQRQKKQQTV	N	23	0.58	1	0	2 253 691
R1A_SARS2	NKGAGGHSYGADL	N	13	0.57	2	0	4781487
SPIKE_SARS2	DEVRQIAPGQTGK	N	13	0.57	1	4 595 459	4733939
NCAP_SARS2	RRIRGGDGKM	N	10	0.52	1	0	4866340
SPIKE_SARS2	YRLFRKSNLKP	N	11	0.51	1	4 661 055	4797597
R1AB_SARS2	VNNLDKSAGF	N	10	0.5	1	0	4854988
R1AB_SARS2	LKKDAPYIVG	N	10	0.48	2	0	4888434
R1AB_SARS2	IDKCSRIIPA	N	10	0.46	1	0	4769110
R1A_SARS2	LEETKFLTENLL	N	12	0.45	2	0	4835320
R1AB_SARS2	PQEHYVRITG	N	10	0.42	1	0	4888859
SPIKE_SARS2	MSLGAENSVAY	N	11	0.42	1	4 681 033	4820746
SPIKE_SARS2	CTEVPVAIHADQ	N	12	0.42	1	4 789 431	4929716
R1A_SARS2	TIQTIVEVQPQLEMELT	N	17	0.34	2	0	3725516
R1AB_SARS2	VEVQPQLEMELT	N	12	0.33	2	0	4875451

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
SCoV indicates SARS-CoV-2. It was identified a total of 29 epitopes. The search was performed using the GISAID database protein for spike and all proteins.

Table 4.SARS-CoV-2 epitopes of the proteins spike (S), membrane (M), and nucleocapsid (N).

PROTEIN	EPITOPE	LENGTH	AVG COVER	DIRECT SEARCH						BLASTP					
				SCOV	α	β	γ	δ	Ο	SCOV	α	β	γ	δ	Ο
S	QTQTNSPRRARSVA	14	0.74	1	0	1	1	0	0	1	1	1	1	1	0
	NNLDSKVGGN	10	0.70	1	1	1	1	1	0	1	1	1	1	1	0
	DEVRQIAPGQTGK	13	0.57	1	1	0	0	1	0	1	1	1	1	1	1
	YRLFRKSNLKP	11	0.51	1	1	1	1	1	1	1	1	1	1	1	1
	MSLGAENSVAY	11	0.42	1	1	0	1	1	1	1	1	1	1	1	1
	CTEVPVAIHADQ	12	0.42	1	1	1	1	1	1	1	1	1	1	1	1
N	MSGKGQQQQGQTVTK	15	0.69	1	0	1	1	1	1	1	1	1	1	1	1
	SDSTGSNQNGERSGARSKQRRPQGLPN	27	0.68	1	1	1	1	1	0	1	1	1	1	1	1
	KDKKKKADETQALPQRQKKQQTV	23	0.58	1	1	1	1	0	1	1	1	1	1	0	1
	RRIRGGDGKM	10	0.52	1	1	1	1	1	1	1	1	1	1	1	1
M	KLNTDHSSSSDNI	13	0.75	1	1	1	1	1	1	1	1	1	1	1	1

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VoC, variant of concern.
SCoVSARS-CoV-2Reference; SARS-CoV-2strain Alpha; SARS-CoV-2strain Beta; SARS-CoV-2strain Gamma; SARS-CoV-2strain Delta; ΟSARS-CoV-2strain Omicron.
The search of these epitopes compares the VoC proteins of SARS-CoV-2.

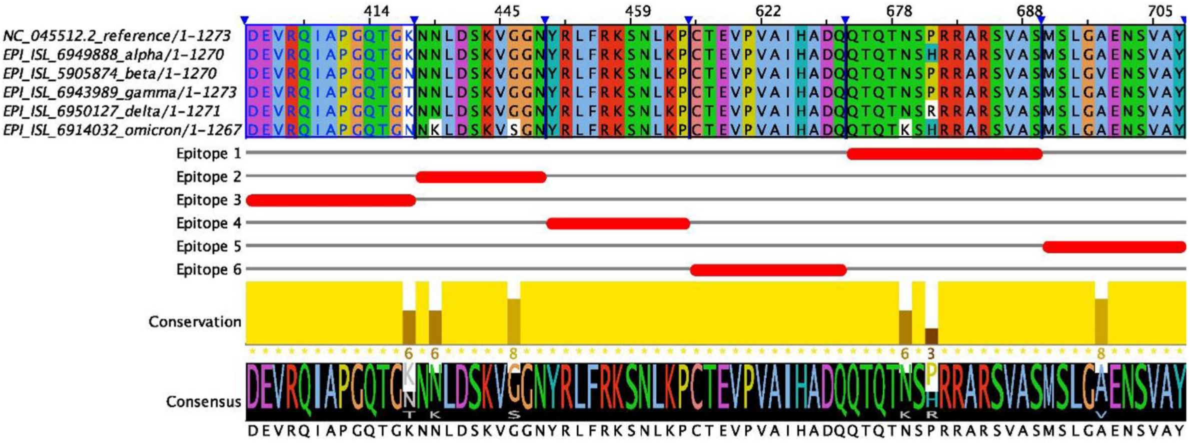

414
445
459
622
678
688
705
NC_045512.2_reference/1-1273
EPI_ISL_6949888_alpha/1-1270
EPI_ISL_5905874_beta/1-1270
EPI_ISL_6943989_gamma/1-1273
EPI_ISL_6950127_delta/1-1271
EPI_ISL_6914032_omicron/1-1267
Epitope 1
Epitope 2
Epitope 3
Epitope 4
Epitope 5
Epitope 6
Conservation
Consensus
DEVRQIAPGQTGKNNLDSKVGGNYRLFRKSNLKPCTEVPVAIHADQQTQTNSPRRARSVASMSLGAENSVAY

Figure 5. Alignment of the spike proteins (S) of SARS-CoV-2, compared with the VoC variants where it is possible to analyze that there are mutations that impact amino acids present in identified areas epitopes, especially in the omicron variant. The alignment was performed using Clustal Omega with default parameter values. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; VoC, variant of concern.

amino acid in the alpha, delta, and omicron variants in the epitope QTQTNSPRRARSVA and the exchange of 2 amino acids in the epitope NNLDSKVGGN only in the omicron variant. Other data are presented in Supplementary Data 2.

There were no occurrences of epitopes of SARS-CoV-2 in the human proteome according to the established criteria. However, by individually analyzing the files generated by BLASTP, it can be seen that if the values of identity and coverage were 50%, there would be several valid hits, such as the epitope QTQTNSPRRARSVA with 100% coverage hit and 50% identity in the genes |Q92902| HPS1_HUMAN (SPRRARS), |J3KPV6| J3KPV6_HUMAN (RRARSVA), sp|P37088| SCNNA_HUMAN (RRARSVA), and |C5HTZ1| C5HTZ1_HUMAN (RRARSVA). The epitope DEVRQIAPGQTGK had 17 hits, the epitope MSLGAE NSVAY had 2 hits, and the epitope CTEVPVAIHADQ had only 1 hit.

Epitopes of C. difficile

Considering the threshold point of 0.75, 7 epitopes of *C. difficile* were identified (Table 5), 5 of which presented *N*-glycosylation sites. Epitopes were not found in the other *Clostridioides* species either directly or by BLASTP. However, similar to SARS-CoV-2, if the identity and coverage values were modified to 60% coverage and identity, it was possible to find several hits. The exciting thing is that despite this, 54 hits were found with BLASTP, including 2 directly from the

Table 5. List of the epitopes from *C. difficile*, with the threshold of 0.75.

ID	EPITOPE	N-GLYC	LENGTH	AVG COVER	PROTEOME SEARCH									
					DIRECT SEARCH					BLASTP				
					CDIF	CPER	CBOT	CARG	HSAP	CDIF	CPER	CBOT	CARG	HSAP
WP_003427432.1	QTNENNTTNNSE	Y	12	0.8	1	0	0	0	0	1	X	X	0	X
WP_003434503.1	TNNNQQNNANTNQQNNNS	Y	18	0.8	1	0	0	0	0	1	X	0	0	0
WP_021368964.1	NNSSNSNSTNN	Y	11	0.8	1	0	0	0	0	X	X	X	0	X
WP_021368964.1	KPNSSSNQNSQPNSNSK	Y	17	0.8	1	0	0	0	0	1	X	0	0	0
WP_021390451.1	NSNSNSNSNSNS	N	12	0.8	3	0	0	0	0	13	0	X	0	0
WP_003438294.1	SNSNSDNSSNSNGNNSSSS	Y	19	0.79	1	0	0	0	0	1	0	0	0	X
WP_021364658.1	ASSSSSSSSSN	N	11	0.73	1	0	0	0	2	2	X	X	X	54

Abbreviations: Carg, *C. argentinense*; Cbot, *C. botulinum*; Cdif, *C. difficile*; Cper, *C. perfringens*; Hsap, *H. sapiens*.
Xno hit identified for the epitope, when the value is 0, that is, there was hit but did not reach the cutoff point of coverage and identity.

Table 6. List of the epitopes from *T. b. gambiense*, with the threshold of 0.75.

ACCESSION ID	EPITOPE	N-GLYC	LENGTH	AVG COVER	PROTEOME SEARCH											
					DIRECT SEARCH						BLASTP					
					TBG	TBB	TCO	TEV	TVI	HSAP	TBG	TBB	TCO	TEV	TVI	HSAP
Tbg972.11.480	QPPQQQQQQA	N	10	0.94	1	1	0	1	0	0	0	0	0	0	0	0
Tbg972.11.11840	QQQPQQQPQQQQQQG	N	15	0.84	1	0	0	1	0	0	1	0	0	1	0	0
Tbg972.9.8810	NNNNNNNNNNNNS	Y	13	0.8	4	1	0	2	0	0	3	3	0	7	0	–
Tbg972.1.2160	NNNKNNNNNN	N	10	0.8	1	0	0	1	0	0	1	1	0	1	0	–
Tbg972.3.1330	NNNSNNNNNNNNNN	Y	14	0.8	1	1	0	1	0	0	0	0	0	0	0	–
Tbg972.1.1570	NNNNNNNNNNNSGNG	Y	15	0.75	1	0	0	0	0	0	1	0	0	0	0	–
Tbg972.3.3000	QQQQQQQQQQQQQL	N	14	0.7	1	1	0	1	0	10	4	0	0	0	27	416
Tbg972.9.4350	QQQHHNTHQTTAQQ	N	14	0.69	1	0	0	1	0	0	1	0	0	1	0	0

Abbreviations: Hsap, *H. sapiens*; Tbb, *T. b. brucei*; Tbg, *T. b. gambiense*; Tev, *T. evansi*; Tvi, *T. vivax*.

epitope ASSSSSSSSN in humans. In addition, the epitopes identified showed almost 100% coverage in the selected features, except for the Kolaskar[18] method, increasing the value of EpiBuilder's Avg cover. Additional information is available in Supplementary Data 3.

Epitopes of T. b. gambiense

Considering the threshold of 0.75, we found 8 epitopes, 3 of them had *N*-glycosylated sites (Table 6). The presence of epitopes with a small variety of amino acids was noted, with the most frequent epitopes being asparagine (N) and glutamine (Q). The higher frequency of occurrence of epitopes in *T. evansi* than that in *T. b. brucei* was also verified. In *T. congolense* and *T. vivax*, epitopes were not identified via direct search, but for *T. vivax*, the occurrence of the epitope QQQQQQQQQQQQQQL1 was observed 27 times via BLASTP, and this same epitope had the occurrence of 416 in humans. Additional information is available in Supplementary Data 4.

Discussion

EpiBuilder is designed to be an intuitive and easy-to-use tool, with no installation required, and it can be rotated via the terminal, on servers for heavier processing, or on a personal computer with a GUI. This tool is also available for the Galaxy platform through ToolShed and can be installed on any server. Because it is an assembly tool based on the results of BepiPred-2.0, we understand that there is no comparative software because this tool is a pipeline tool to generate several results that would be done manually.

After execution, we verified the possibility of running EpiBuilder as a proteome-wide tool for epitope generation, searching, and classification. From the executions, we noticed that finding epitopes with a threshold of BepiPred-2.0 greater than or equal to 0.8 was challenging; there were no results for all 3 evaluated organisms.

An experiment with SARS-CoV-2 did not generate any identifiable epitopes in humans. However, this result can change according to the settings. We also noticed that for *T. b. gambiense* and *C. difficile*, some of the epitopes generated did not occur in humans in general, even with BLASTP consultation, which may indicate potential targets with less possibility of cross-reaction.

We found that the higher the threshold of the established BepiPred-2.0, the greater the coverage of the selected EpiBuilder features, which shows a direct relationship between these features and the threshold definition.

As these are genome-wide experiments, these results need to be validated more cautiously because they may contain data from the assembly and prediction of proteins generated with some type of error.

The next steps of EpiBuilder are will be to allow the use of results from other BCE predictors and integrate them using docker to turn the application more accessible for the non-specialist.

Author Contributions

RSM: Investigation, Writing - original draft, Formal analysis. Data curation. NAC: Investigation. VBF: Formal analysis. Data curation GW: Data curation, Validation. LCM: Conceptualization, Validation, Writing - review & editing, Project administration, Funding acquisition.

ORCID iDs

Vilmar Benetti Filho https://orcid.org/0000-0003-0309-4877

Nathália Anderson Calomeno https://orcid.org/0000-0002-8785-2390

Glauber Wagner https://orcid.org/0000-0001-5003-6595

Luiz Claudio Miletti https://orcid.org/0000-0001-5926-0286

Data Availability and Requirements

All the experimental data, source code, and binaries are available in https://epibuilder.sourceforge.io.

Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Abbas A, Lichtman A, Pillai S. *Cellular and Molecular Immunology*. 9th ed. Amsterdam, The Netherlands: Elsevier; 2017.
2. Shirai H, Prades C, Vita R, et al. Antibody informatics for drug discovery. *Biochim Biophys Acta*. 2014;1844:2002-2015. doi:10.1016/j.bbapap.2014.07.006.
3. Manavalan B, Govindaraj RG, Shin TH, Kim MO, Lee G. iBCE-EL: a new ensemble learning framework for improved linear B-cell epitope prediction. *Front Immunol*. 2018;9:1695.
4. Ahammad I, Lira SS. Designing a novel mRNA vaccine against SARS-CoV-2: an immunoinformatics approach. *Int J Biol Macromol*. 2020;162:820-837.
5. Sadat SM, Aghadadeghi MR, Yousefi M, Khodaei A, Sadat Larijani M, Bahramali G. Bioinformatics analysis of SARS-CoV-2 to approach an effective vaccine candidate against COVID-19. *Mol Biotechnol*. 2021;63:389-409. doi:10.1007/s12033-021-00303-0.
6. Foroutan M, Ghaffarifar F, Sharifi Z, Dalimi A, Pirestani M. Bioinformatics analysis of ROP8 protein to improve vaccine design against Toxoplasma gondii. *Infect Genet Evol*. 2018;62:193-204. doi:10.1016/j.meegid.2018.04.033.
7. Manivel G, Meyyazhagan A, Durairaj DR, Piramanayagam S. Genome-wide analysis of excretory/secretory proteins in Trypanosoma brucei brucei: insights into functional characteristics and identification of potential targets by immunoinformatics approach. *Genomics*. 2019;111:1124-1133. doi:10.1016/j.ygeno.2018.07.007.
8. Ostolin TLVDP, Gusmão MR, Mathias FAS, et al. A chimeric vaccine combined with adjuvant system induces immunogenicity and protection against visceral leishmaniasis in BALB/c mice. *Vaccine*. 2021;39:2755-2763. doi:10.1016/j.vaccine.2021.04.004.
9. Lata KS, Kumar S, Vaghasia V, et al. Exploring Leptospiral proteomes to identify potential candidates for vaccine design against Leptospirosis using an immunoinformatics approach. *Sci Rep*. 2018;8:6935.
10. Kaliamurthi S, Selvaraj G, Junaid M, Khan A, Gu K, Wei D-Q. Cancer immunoinformatics: a promising era in the development of peptide vaccines for human papillomavirus-induced cervical cancer. *Curr Pharm Des*. 2019;24:3791-3817.
11. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*. 2006;65:40-48. http://www.ncbi.nlm.nih.gov/pubmed/16894596
12. Yao B, Zheng D, Liang S, Zhang C. SVMTriP: a method to predict B-cell linear antigenic epitopes. *Methods Mol Biol*. 2020;2131:299-307. http://www.ncbi.nlm.nih.gov/pubmed/32162263
13. Collatz M, Mock F, Barth E, Hölzer M, Sachse K, Marz M. EpiDope: a deep neural network for linear B-cell epitope prediction. *Bioinformatics*. http://www.ncbi.nlm.nih.gov/pubmed/34109374. Published 2021.
14. Bahai A, Asgari E, Mofrad MRK, Kloetgen A, McHardy AC. EpitopeVec: linear epitope prediction using deep protein sequence embeddings. *Bioinformatics*. 2021;37:4517-4525. http://www.ncbi.nlm.nih.gov/pubmed/34180989
15. Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. *Adv Enzymol Relat Areas Mol Biol*. 2006;47:45-148.
16. Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *J Virol*. 1985;55:836-839.
17. Karplus PA, Schulz GE. Prediction of chain flexibility in proteins — a tool for the selection of peptide antigens. *Naturwissenschaften*. 1985;72:212-213.
18. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett*. 1990;276:172-174.
19. Parker JMR, Guo D, Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. *Biochemistry*. 1986;25:5425-5432.
20. Afgan E, Baker D, Batut B, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res*. 2018;46:W537-W544.
21. Blankenberg D, Von Kuster G, Bouvier E, et al. Dissemination of scientific software with Galaxy ToolShed. *Genome Biol*. 2014;15:2-4.
22. Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res*. 2017;45:W24-W29.
23. Vita R, Mahajan S, Overton JA, et al. The Immune Epitope Database (IEDB): 2018 update. *Nucleic Acids Res*. 2019;47:D339-D343.
24. Lafita A, Bliven S, Prlić A, et al. BioJava 5: a community driven open-source bioinformatics library. *PLoS Comput Biol*. 2019;15:e1006791.
25. Pitti T, Chen CT, Lin HN, Choong WK, Hsu WL, Sung TY. N-GlyDE: a two-stage N-linked glycosylation site prediction incorporating gapped dipeptides and pattern-based encoding. *Sci Rep*. 2019;9:15975. doi:10.1038/s41598-019-52341-z.
26. Roesch LFW, Fulthorpe RR, Jaccques RJS, Bento FM, de Oliveira Camargo FA. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *World J Microbiol Biotechnol*. 2006;22:3389-3402.
27. Büscher P, Cecchi G, Jamonneau V, Priotto G. Human African trypanosomiasis. *Lancet*. 2017;390:2397-2409. https://linkinghub.elsevier.com/retrieve/pii/S0140673617315106
28. Kennedy PGE. Update on human African trypanosomiasis (sleeping sickness). *J Neurol*. 2019;266:2334-2337. http://www.ncbi.nlm.nih.gov/pubmed/31209574
29. Czepiel J, Drdóżdż M, Pituch H, et al. *Clostridium difficile* infection: review. *Eur J Clin Microbiol Infect Dis*. 2019;38:1211-1221. http://link.springer.com/10.1007/s10096-019-03539-6

30. Mullish BH, Williams HR. *Clostridium difficile* infection and antibiotic-associated diarrhoea. *Clin Med (Northfield Il)*. 2018;18:237-241. https://www.rcpjournals.org/lookup/doi/10.7861/clinmedicine.18-3-237.

31. Wang M-Y, Zhao R, Gao L-J, Gao XF, Wang DP, Cao JM. SARS-CoV-2: structure, biology, and structure-based therapeutics development. *Front Cell Infect Microbiol*. 2020;10:587269. https://www.frontiersin.org/articles/10.3389/fcimb.2020.587269/full

32. Awadasseid A, Wu Y, Tanaka Y, Zhang W. SARS-CoV-2 variants evolved during the early stage of the pandemic and effects of mutations on adaptation in Wuhan populations. *Int J Biol Sci*. 2021;17:97-106. https://www.ijbs.com/v17p0097.htm

33. Hadfield J, Megill C, Bell SM, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. 2018;34:4121-4123. https://academic.oup.com/bioinformatics/article/34/23/4121/5001388

34. Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol*. 2011;7:539. https://onlinelibrary.wiley.com/doi/10.1038/msb.2011.75.

35. Procter JB, Carstairs GM, Soares B, et al. Alignment of biological sequences with Jalview. In: Katoh, K, ed. *Multiple Sequence Alignment. Methods in Molecular Biology*. New York, NY: Humana; 2021:203-224. http://link.springer.com/10.1007/978-1-0716-1036-7_13