EpiQuest-B – Operation Manual

Content:

What is EpiQuest-B?

EpiQuest-B builds an antigenicity profile for a protein sequence, allowing the discovery of areas saturated with linear epitopes capable of eliciting high humoral response with IgGs of high affinity. In other words, it allows the identification of areas of immunodominant **linear B-epitopes**.

EpiQuest-B is using a unique algorithm developed by Aptum Biologics Ltd. that evaluates the "score" for an amino acid or cluster of amino acids based on its (their) immediate context. The algorithm, and the matrix B7.1 it uses, were developed and retrospectively analysed on over 16.000 individual immunodominant, positive, and negative B-epitopes.

It should be considered that in vivo **antigenicity** of sequences will manifest itself only when peptide sequences are accessible to the immune system (B-cell receptor), and only **accessible** sequences in folded molecules will elicit the response in vivo. To identify the accessible and non-accessible sequences, please refer to EpiQuest-A or use EpiQuest-IM (new for version 4.0) that evaluated the relative immunogenicity of the linear sequences in folded molecules.

Species specificity

The Matrix B7.1 was primarily developed to access the antigenicity of a sequence for *the human humoral immune system*. It is also applicable to access the antigenicity of a sequence in mice, rabbits, and swine. When it concerns defining the immunodominant sequences, it seems to be applicable to most high mammals, but the data for retrospective analysis is quite limited.

Entering the Data

To analyse the sequence, please simply **Paste** it into the window for sequence. We recommend also entering the accession number of the sequence you use and the name of your sequence or project. These data will be present in all **Results** you save after the analysis.

Protein Name	
Accession Number	
	Clear ---Select a demo sequence---
Sequence in FASTA	Enter the aminoacid sequence in 1-letter code (FASTA)
	☐ Filter ☑ Filter Start: 0 End: 0

If your sequence contains gaps (may appear due to formatting) or contains abnormal signs (punctuation, letters that are not amino acid code) the analysis will be aborted. To avoid it, keep the "Filter" option selected.

To analyse only a fragment of the sequence, please enter the position number of the first and the last amino acids of the fragment you want to select. Please be aware that the original numeration of the amino acid positions will be preserved. Thus, if the first aa of the fragment was 101, it will be indicated as 101 in all further analysis and Results of the fragment as well.

However, if you paste into the sequence window a fragment of some sequence, its first amino acid will be referred as in position 1, not the position in the original sequence- the source of the fragment.

Settings for B-Epitope (Antigenicity) analysis

☑ B-epitopes
Frame size: (9) ∈ [3, 15]
Gap size: (0) ∈ [0, 5]
Threshold: (0) ∈ [-3, 3]
Peptide size: (8) ☑ higher
Sort by: (AGI ▾)
Chart type: (Graph ▾)
[Default]

The antigenicity analysis for B-epitopes allows you to customise the settings. We recommend keeping the *Default* settings for most situations (can be reset by the respective button).

Frame size defines the size of the context sequence that will be reviewed for every position. The most precise mapping of the immunodominant epitopes is achieved at frame 6 or 9. Only when you want to access immunogenicity of large fragments (50 or more aa) do we recommend increasing it to 12 or 15.

Gap size. Every position is scored as positive or negative. Sometimes the negative amino acid may be rare or unique in an otherwise continuously positive (antigenic) region. You may want to allow gaps of 1 to 5 negative amino acids for the fragment to still be considered evenly positive. Then in your results you will get undivided fragments that otherwise would be divided by a few negative amino acids. It will have no effect on how the results will be presented in the Graphics.

Threshold. The sequence may have too many fragments of average antigenicity or, to the contrary, have few or no strongly positive ones. In these cases, you may want to change the threshold at which antigenicity of a particular AA cluster is viewed as negative. You may decrease (entering the positive Threshold values) or increase (entering the negative Threshold values) the sensitivity of your analysis. The graph below depicts analysis of the same sequence with Th=0 (top) and Th=3 (bottom)
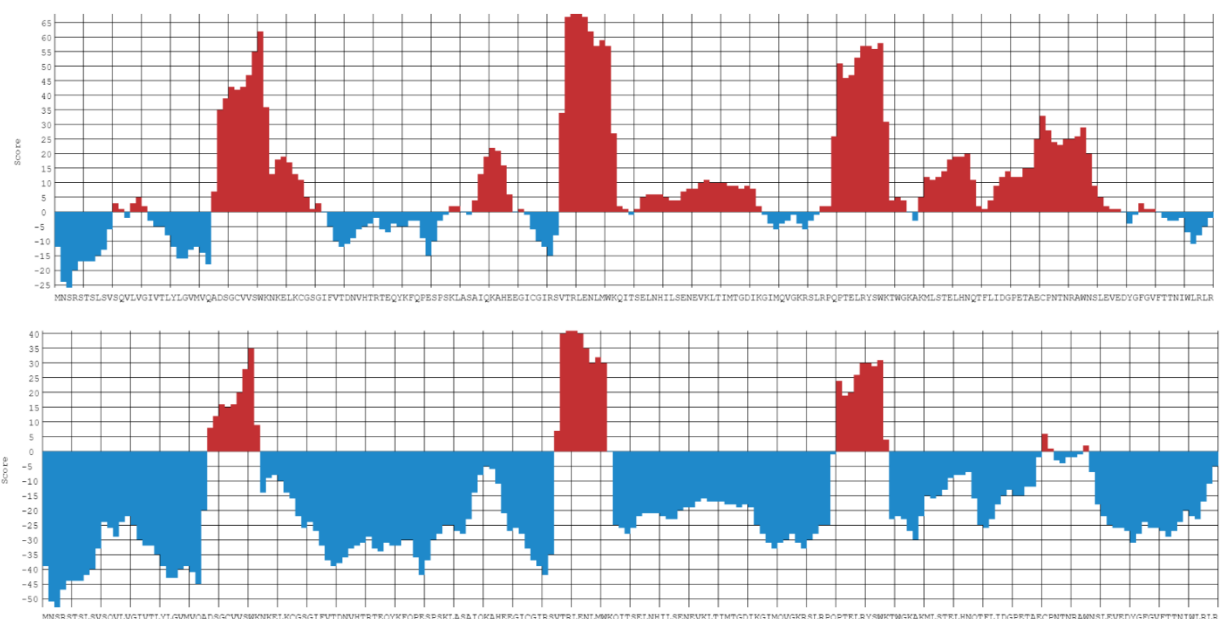
This option allows you to obtain a clearer picture of what the true immunodominant sequences are, or to detect the best epitope in a low-immunogenic sequence.

Peptide size defines whether your non-graphical results contain the set of whole positive fragments (irrespective of their length, i.e. 8 and above) or will be presented as a set of positive 8-mers (or 12, 15 -mers peptides depending on the entered length of the peptide).

8 is approximately the shortest B-epitope, so we would not recommend going below this length. On the other hand, immunodominant linear epitopes of 8-9 AA were observed, and we do not recommend going above 8 (12-15 etc.) in a first run.

Sort by defines how the results for positive fragments will be presented in the report tables: sorted according to the order of the fragments in the original sequence (*Start*), according to their *Length* (the longer go first), cumulative antigenicity of the fragment *AGI* (antigenicity index) or according to average antigenicity per amino-acid residue of the fragment (*AGR*)

Chart Type the Chart for analysis will be presented as a Graph or just Bars, indicating positions of antigenic fragments (the Bar shows only the fragments of size equal or longer than the one selected in **Peptide Size**)
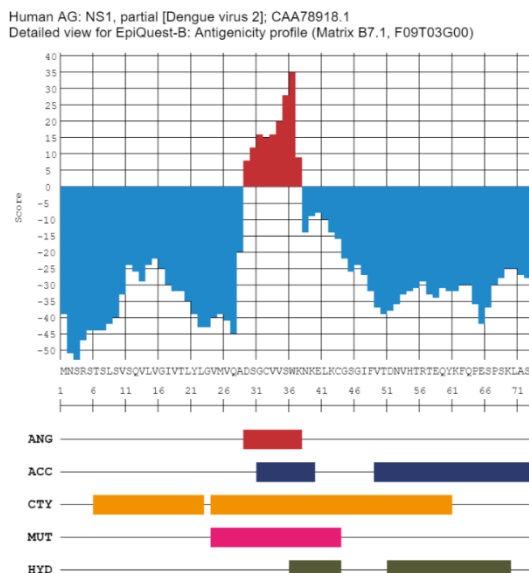
Settings for other tools

In the new version of EpiQuest you may perform multiple parameter analyses along with B-epitopes in one run. The program allows you to also access the surface **Accessibility**, immunological **Complexity** (uniqueness), **Hydrophobicity** (using an algorithm based on Kyle-Doolittle hydrophobicity scores) and **Stability** (potential Mutability) for various regions of the sequence.

We recommend that you keep the Default settings for all. For descriptions of settings, please check the manuals for these individual programs

Viewing and saving the Results

The results of the analysis are presented in 2 ways, graphical and tabular.

Human AG: NS1, partial [Dengue virus 2]; CAA78918.1
Detailed view for EpiQuest-B: Antigenicity profile (Matrix B7.1, F09T03G00)

Graphical output will show full diagrams for Antigenicity and *bar* showing antigenic fragments according to the selected threshold level, as well as graphics of bars for other parameters, if these were selected.

The output contains the name of the sequence (name of the project), acc. number of the sequence, Matrix used and parameters you have chosen for the analysis of Antigenicity, where F-Frame, T- Threshold and G (selected size of allowed gaps)

You may save the entire diagram by clicking the **Save diagram** button above it. The image will be saved in a PNG format with a resolution of 96 ppi.

[Save diagram]

Human AG: NS1, partial [Dengue virus 2]; CAA78918.1
Detailed view for EpiQuest-B: Antigenicity profile (Matrix B7.1, F09T03G00)

The **Tables** of results show the sequences for the detected antigenic fragments:

| View | Save | Copy | **Report for "B-epitopes":** | | | |

Matrix = B7.1 / F = 9 / G = 0 / T = 3 / Peptide size = 8+

Start	End	Length	AGI	AGR	Sequence
88	97	10	296.00	29.60	VTRLENLMWK
136	144	9	213.00	23.67	PTELRYSWK
29	37	9	159.00	17.67	DSGCVVSWK

It contains the settings of the analysis, position of the fragment (Start-End), its length, cumulative Antigenicity index (AGI) and an index per amino-acid (AGR).

You may **Copy** the Table and just Paste it into an Excel sheet or Text (Word, .RTF etc).

	A	B	C	D	E	F	G
1	Matrix = B7.1 / F = 9 / G = 0 / T = 3 / Peptide size = 8+						
2	Start	End	Length	AGI	AGR	Sequence	
3	88	97	10	296	29.6	VTRLENLMWK	
4	136	144	9	213	23.67	PTELRYSWK	
5	29	37	9	159	17.67	DSGCVVSWK	
6							
7							
8							

You may **View** the Report in complete form, with the exact analysed sequence and print it into PDF using your browser Print function or **Save** it for your records as a standalone HTML document that may be further converted into other formats.

Report: Analysis of Antigenicity profile (EpiQuest-B)

Date & Time: 18.10.2020 13:06:37
Protein Name: Human AG: NS1, partial [Dengue virus 2]
Accession Number: CAA78918.1
Sequence:
MNSRSTSLSVSQVLVGIVTLYLGVMVQADSGCVVSWKNKELKCGSGIFVTDNVHTRTEQYKF
QPESPSKLASAIQKAHEEGICGIRSVTRLENLMWKQITSELNHILSENEVKLTIMTGDIKGI
MQVGKRSLRPQPTELRYSWKTWGKAKMLSTELHNQTFLIDGPETAECPNTNRAWNSLEVEDY
GFGVFTTNIWLRLREKQDAFCDSKLMSAAIKDNRAVHADMGYWIESALNDTWKIEKASFIEV
KSCHWPKSHTLWSNGVLESEMVIPKNFAGPKSQHNNRPGYHTQTAGPWHLGKLEMDFDFCEG
TTVVVTEDCGNRGPSLRTTTASGKLITEWCCRSCTLPPLRYRGEDGCWYGMEIRPLKEKEEN LVSSLVTA
Fragment: 1 - 200
Matrix: B7.1
Frame size: 9
Gap: 0
Threshold: 3
Peptide size: 8 higher
Sorted by: AGI

Start	End	Length	AGI	AGR	Sequence
88	97	10	296.00	29.60	VTRLENLMWK
136	144	9	213.00	23.67	PTELRYSWK
29	37	9	159.00	17.67	DSGCVVSWK

Demo Sequences

For Demo and training purposes, we supply several sequences with known **Human** or **Mouse** immunodominant epitopes (indicated in the description for a demo sequence)

You may choose them by opening the drop-down list **-Select a demo sequence-**.

Some of them can be seen with the details of analysis on our site

In our blog we further show how to use the program in combination with other ones to address different research tasks.