EpiQuest-T Manual

Content:

What is EpiQuest-T?

For all defined MHC-I binding peptide epitopes, only a tiny fraction has functional activity and even less are highly functional[i]. During sequence analysis of real practical interest it's not the finding of the precise MHC – binding epitope(it is most likely non-functional), but rather the approximate location of the *highly functional* CTL-epitopes in the analysed sequence[ii].

This is exactly what EpiQuest-T does. It enables you to locate the presence of potentially dominant and functional CTL epitopes that can effectively trigger the response of CTLs in the presence of a target.

EpiQuest-B is using a ***unique algorithm*** developed by Aptum Biologics Ltd. that evaluates the potential of the peptide sequence to strongly bind MHC class I and the TCR.

The algorithm is haplotype-specific and is currently only available for the most frequent haplotypes of MHCI. The same matrix and algorithm are also used in the T-Scanner program that allows comparing/ranging the large collection of CTL epitopes according to functionality.

Species specificity

Currently the matrixes are developed for human HLA-A2 haplotype (all subtypes) and for murine H2kB.

Entering the Data

To analyse the protein sequence, please simply ***Paste*** it (in a single-letter code) 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. This data will be present in all **Results** you save after the analysis.

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 number of the first and the last amino acids of the fragment you wish to analyse. Please be aware that the numeration of the sequence starts from the first amino acid of the chosen sequence, not of the original one, and the order of numbering will be kept in all results even when you analyse a fragment.

Settings for CTL-Epitope analysis

We recommend the *Default* settings (can be reset by the respective button) for most cases.

As matrixes for new haplotypes of human, mice and other species are developed, the setting was built to accommodate them in a near future.

Depending on your needs, choose *Host*: Human or Mouse. This allows you to select a specific haplotype of this host species in the next drop-down list (MHC allele)

There are two Matrixes for H2kB haplotype. H1 and H12. H12 is more sensitive but may see some intermediate or weak epitopes as well.

Frame size of 9 so far was found to be the most precise setting.

Gap size we recommend keeping it 0 unless you are analysing some artificially designed sequences containing overlapping or subsequent epitopes.

Threshold The strong functional CTL epitopes are visible when Threshold is set to 0. If you do not see any epitopes at this setting, you may lower the threshold to -1 or -2 to see if they appear. It should be said that we used the term "strong epitope" in the interpretation, it is used in the IMDB collection of CTL epitopes, which may often label the epitopes with quite different functional activities. Therefore, your desired epitope may become visible only when the analysis threshold is lowered.

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 N-mers (depending on the length of the fragments). 8 is approximately the shortest CTL-epitope, so we would not recommend going below this length.

You should remember that you are not getting the exact position of epitopes (from first to the last amino acid), but rather detecting the areas that are likely to contain such epitopes. Therefore, we suggest that, to identified epitopes of say 8aa, you add at least 2-3 aa before it and 2-3 after to ensure that it will be cut out correctly in the MHCI processing system.

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 longest goes first), cumulative antigenicity (strength) of the fragment *AGI* (antigenicity index) or according to the 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 (of length rqual or above the set Peptide size)

Viewing and saving the Results

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

Graphical output will show full *diagrams* for Antigenicity and *bar* showing antigenic fragments according to the selected threshold level. Graphics of bars for other parameters will also be shown, if the parameters were selected earlier.

HLA-A2: CTL-recognized antigen on melanoma (CAMEL) [Homo sapiens]; CAA10197.1
Detailed view for EpiQuest-T: CTL epitope presence probability (Matrix A2-T3.1, F09T00G00)
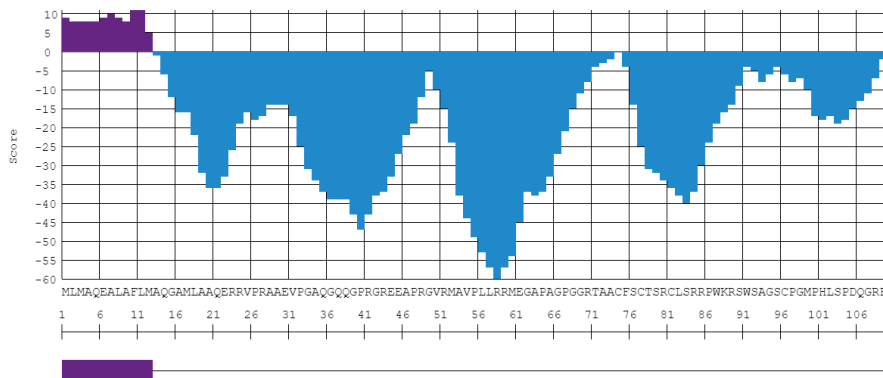
The output contains the name of the sequence (name of the project), acc. number of the sequence, Matrix used, and the parameters you have chosen for the analysis of Antigenicity, where F-Frame, T-Threshold and G – are the selected sizes of allowed gaps).

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 high resolution of 96 ppi.

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

Report for "T-cell epitope":

Matrix = A2-T3.1 / F = 9 / G = 0 / T = 0 / Peptide size = 8+

Start	End	Length	AGI	AGR	Sequence
1	12	12	104.00	8.67	MLMAQEALAFLM

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).

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 you records as a standalone HTML document that may be further converted into other formats.

Report: Analysis of CTL epitope presence probability (EpiQuest-T)

Date & Time: 20.10.2020 15:15:11

Protein Name: HLA-A2: CTL-recognized antigen on melanoma (CAMEL) [Homo sapiens]

Accession Number: CAA10197.1

Sequence: MLMAQEALAFLMAQGAMLAAQERRVPRAAEVPGAQGQQGPRGREEAPRGVRMAVPLLRRMEG
APAGPGGRTAACFSCTSRCLSRRPWKRSWSAGSCPGMPHLSPDQGRF

Fragment: 1 - 109

Matrix: A2-T3.1

Frame size: 9

Gap: 0

Threshold: 0

Peptide size: 8 higher

Sorted by: AGI

Start	End	Length	AGI	AGR	Sequence
1	12	12	*104.00*	*8.67*	MLMAQEALAFLM

Demo Sequences

For Demo and training purposes, we supply several sequences with known **Human** or **Mouse** immunodominant epitopes for the respective haplotypes.

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

They contain **previously identified** strong CTL epitopes.

Table of epitopes for them

Examples of the analysis

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

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[i] Zvi, A., Rotem, S., Bar-Haim, E., Cohen, O., and Shafferman, A. (2011). Whole-Genome Immunoinformatic Analysis of F. tularensis: Predicted CTL Epitopes Clustered in Hotspots Are Prone to Elicit a T-Cell Response. PLoS One *6 (5) e20050*.

[ii] Bijker, M.S., Eeden, S.J.F. van den, Franken, K.L., Melief, C.J.M., Offringa, R., and Burg, S.H. van der (2007). CD8+ CTL Priming by Exact Peptide Epitopes in Incomplete Freund's Adjuvant Induces a Vanishing CTL Response, whereas Long Peptides Induce Sustained CTL Reactivity. J Immunol *179*, 5033–5040.