T-Scanner Manual

Content:

What is T-Scanner?

T-Scanner was the first program of its type, that was developed to range CTL epitopes according to their predicted immunodominance. The immunodominance of epitopes is defined as their relative strength in functional assays related to target kill or release of respective cytokines. At the end of the day, it is the functionality of the T-epitope that is of real interest.

The program was originally designed to analyse and sort according to their immunodominance the CTL peptide epitopes eluted from target cells. It may also be used to arrange according to the strength of any other selections of epitopes, i.e. identified by other programs for their potential binding to MHCI (not directly related to their functionality). Only a very low number of peptide epitopes that bind to MHCI have actual functional activity [1].

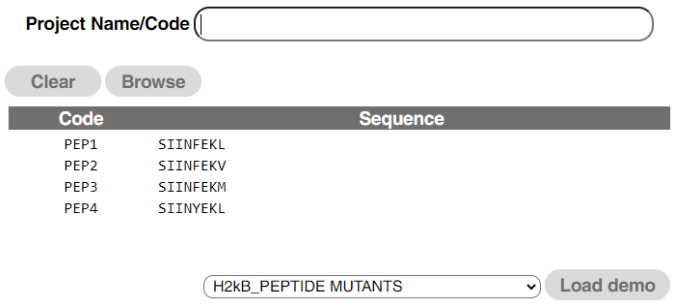
Our data shows that the relative strength of a CTL (T) epitope is defined by the *strength* of its binding to MHCI and to TCR (in the context of MHCI). The algorithm detects the structural and compositional features of peptide epitope that allow it to elicit a high-affinity TCR (many sequences are incapable of this). Identical to the one used in EpiQuest-T, the algorithm defines the sequences with enhanced functionality potential.

The analysis is haplotype-specific; at the moment we have developed the matrix for analysis of HLA-A2 and H2kB haplotypes-binding peptides.

Species specificity

Right now, the program works for Human HLA-A2 haplotype and mice H2kB haplotype. Other haplotypes are coming, please inquire for their availability. We may have a beta -version for them.

Entering the Data



To test the program, select demo sequences of the chosen haplotype and click ***Load demo***. Two columns will appear in the window for sequences : ***Code*** and ***Sequence***.

To empty the window, click ***Clear***.

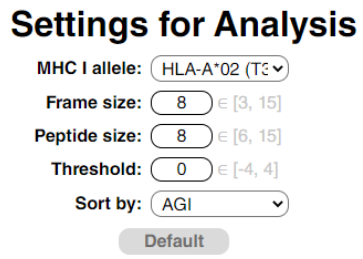
To load your own data, it should be presented in ***XLS*** or ***XLSL*** format (MS-***Excel***) and must contain 2 columns in the datasheet, only one datasheet with data per file can be input at a time. The columns should be named ***Code*** (provide your sequences for identification with numerical or other type of code; we do not recommend going above 15 signs, letters, numbers and

[1] 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*.

dash signs allowed) and ***Sequence*** (peptide sequences, we do not recommend going above 100 amino acids each, single letter code).

You may ***Browse*** for the location of the file and click on it to load.

Settings for CTL epitope analysis



Choose the ***MHCI allele*** for your peptide sequences. For H2kB we present two Matrices for analysis, a more stringent (H1) and less stringent (HI2). The latter allows you to analyse weaker epitopes. You can also adjust your cut-off level for weak/functionally negative epitopes by changing the ***Threshold*** (positive numbers to decrease the sensitivity, negative – to increase). We do not recommend going beyond (-2) or (+2). ***Frame size*** defines the size of the context analysed for every position in the peptide, we recommend keeping it at 8 (the default value). Peptide size defines the size of epitope you are looking in your peptides. If the tested sequences are of different length (i.e. your data set includes 8-mers, 10-mers, and 12-mers), we recommend keeping it at 8; the values of report will be presented to each 8-mer that is present in your sequence. If you are comparing a set of sequence of equal length say, a set of mutated variants of the original 9-mer CTL, you may choose ***Peptide size*** 9.

Sort by: you can select the data to be reported in several ways:

Start: the results will be sorted according to the order of the analysed peptides in your data file. If you analyse 10-mers and defined the Peptide size as 8, your results will contain 3 8-mers for every original peptide with AGI (antigenicity) defined for every 8-mer. In Results all the data will be sorted according to the order of the peptides in the original sequence.

View Save **Report for "Analysis of CTL Epitopes":**

Code	Sequence	Start	End	Peptide	AGI	APB
A2-HH1	AIMDKNIIL	1	8	AIMDKNII	28	3
A2-HH1	AIMDKNIIL	2	9	IMDKNIIL	24	3
A2-HH2	AITTILAAV	1	8	AITTILAA	48	6
A2-HH2	AITTILAAV	2	9	ITTILAAV	42	5
A2-HH3	ALISAFSGS	1	8	ALISAFSG	-26	-3
A2-HH3	ALISAFSGS	2	9	LISAFSGS	-34	-4

AGI: all results will be sorted according to the order of the analysed peptides in data file, for every analysed sequence they will be presented form the best to the lowest.

View Save **Report for "Analysis of CTL Epitopes":**

Code	Sequence	Start	End	Peptide	AGI	APB
A2-HH17	ILDAHSLYLLQFSRV	1	8	ILDAHSLY	68	8
A2-HH17	ILDAHSLYLLQFSRV	2	9	LDAHSLYL	58	7
A2-HH17	ILDAHSLYLLQFSRV	3	10	DAHSLYLL	40	5
A2-HH17	ILDAHSLYLLQFSRV	4	11	AHSLYLLQ	24	3
A2-HH17	ILDAHSLYLLQFSRV	5	12	HSLYLLQF	13	1
A2-HH17	ILDAHSLYLLQFSRV	6	13	SLYLLQFS	-1	0
A2-HH17	ILDAHSLYLLQFSRV	7	14	LYLLQFSR	-16	-2
A2-HH17	ILDAHSLYLLQFSRV	8	15	YLLQFSRV	-25	-3

AGI ALL: the results will be sorted according to the AGI values of detected peptides of the defined peptide length (say, 8) irrespective of their origin from different original sequences, from the best to the lowest.

View Save **Report for "Analysis of CTL Epitopes":**

Code	Sequence	Start	End	Peptide	AGI	APB
A2-HH47	VILLSLIAV	1	8	VILLSLIA	103	12
A2-HH45	TLACFVLAAV	1	8	TLACFVLA	101	12
A2-HH34	MLMIIIVIAI	1	8	MLMIIIVI	87	10
A2-HH57	YLRAILDAHSLYLLQ	5	12	ILDAHSLY	84	10
A2-HH47	VILLSLIAV	2	9	ILLSLIAV	83	10
A2-HH45	TLACFVLAAV	2	9	LACFVLAA	81	10
A2-HH57	YLRAILDAHSLYLLQ	6	13	LDAHSLYL	79	9

AGI BEST: the data will be presented by only the best peptide from every sequence in order of the highest to the lowest (notice that additional peptides for HH47 and HH57 are not shown any longer.)

View Save **Report for "Analysis of CTL Epitopes":**

Code	Sequence	Start	End	Peptide	AGI	APB
A2-HH47	VILLSLIAV	1	8	VILLSLIA	103	12
A2-HH45	TLACFVLAAV	1	8	TLACFVLA	101	12
A2-HH34	MLMIIIVIAI	1	8	MLMIIIVI	87	10
A2-HH57	YLRAILDAHSLYLLQ	5	12	ILDAHSLY	84	10
A2-HH48	VLFGLGFAI	1	8	VLFGLGFA	72	9
A2-HH11	GLCTLVAML	1	8	GLCTLVAM	69	8
A2-HH16	ILDAHSLYL	1	8	ILDAHSLY	68	8

Viewing and saving the Results

You may ***View*** all results in a new window or ***Save*** them for your records as an HTML file. You can always import such files into your spreadsheet program or simply copy the selected areas of interest in other file formats.

Report: Relative strength of CTL epitopes (T-Scanner)

Date & Time: 29.10.2020 15:46:10
Project name:
Program: EpiQuest T-Scanner v1.0.0.1
Haplotype: HLA-A*02 (Human)
Matrix: T3.1
Peptide size: 8
Frame size: 8
Threshold: 0
Sorted by: AGI BEST

Code	Sequence	Start	End	Peptide	AGI	APB
A2-HH47	VILLSLIAV	1	8	VILLSLIA	103	12
A2-HH45	TLACFVLAAV	1	8	TLACFVLA	101	12
A2-HH34	MLMIIIVIAI	1	8	MLMIIIVI	87	10
A2-HH57	YLRAILDAHSLYLLQ	5	12	ILDAHSLY	84	10
A2-HH48	VLFGLGFAI	1	8	VLFGLGFA	72	9
A2-HH11	GLCTLVAML	1	8	GLCTLVAM	69	8
A2-HH16	ILDAHSLYL	1	8	ILDAHSLY	68	8
A2-HH17	ILDAHSLYLLQFSRV	1	8	ILDAHSLY	68	8
A2-HH20	ILSVSSFLFV	1	8	ILSVSSFL	67	8
A2-HH30	LALLLLDRL	1	8	LALLLLDR	66	8
A2-HH25	KLQVFLIVL	1	8	KLQVFLIV	62	7
A2-HH44	SVGNTLYYV	1	8	SVGNTLYY	60	7
A2-HH23	KINQSLAFI	1	8	KINQSLAF	52	6
A2-HH36	QALVVLSEWLPVTGT	1	8	QALVVLSE	49	6
A2-HH2	AITTILAAV	1	8	AITTILAA	48	6
A2-HH43	SVGGVFTSV	1	8	SVGGVFTS	48	6
A2-HH7	FIAGLIAIV	1	8	FIAGLIAI	48	6
A2-HH28	KVDDTFYYV	2	9	VDDTFYYV	47	5

Demo Sequences

We supply several files with epitopes with known properties for Demo and training purposes (or just a random selection of epitopes with known functionality)for every haplotype.