B-Scanner - Operation Manual

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What is B-Scanner?

Imagine that, after running EpiQuest-B, you have identified several regions of potentially high antigenicity, or have identified the domains that are accessible at the surface of a mature molecule (using Epiquest-A). You could also select several domains of interest from several different proteins using other means.

What you need now is to select the most prominent epitopes that are present in these selected domains. You may want to either create a peptide antigen to detect the immune response, or prepare high affinity antibodies to a protein (or selection of proteins). In both cases you need to select the most immunodominant epitopes in the selected protein sequences. You may also want to compare the relative immunogenicity, as defined by the best immunodominant epitopes, of several protein molecules by choosing the most prominent ones (i.e. analysing which of viral proteins would most likely to be immunogenic in all/most of infected subjects).

B-Scanner allows you to identify the best epitopes (linear peptide sequences) of a predefined length (we suggest using 9-mers) *that will be ranged according to their antigenicity*.

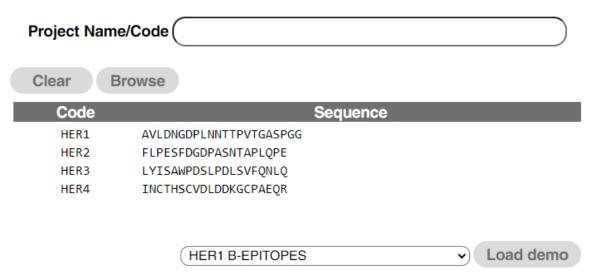
You may later join the overlapping strong epitopes into one peptide to obtain the sequence covering the whole immunodominant area of the sequence.

Species specificity

B-Scanner utilises the same Matrix B7.1 as EpiQuest-B does, which is optimised to recognise sequences that perform as antigenic in humans. Concerning the immunodominant epitopes (relative antigenicity), it performs equally well for mice, rabbits, swine, and chickens.

Entering the Data

To test the program, select demo sequences of the chosen haplotype and click *Load Demo*. There will be two columns in the table: *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**), only one dataset with data per file.

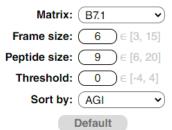
The datasheet should contain 2 columns, named *Code* –your own unique identifier for individual peptide sequence (we do not recommend going above 15 sings, letters, numbers and dash signs allowed) and *Sequence* (peptide sequences in single letter code; we do not recommend going above 100 amino).

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

Settings for B-epitope analysis

Currently only one Matrix is available, B7.1.

Settings for Analysis



Frame size defines the size of the context analysed for every position in the sequence, we recommend keeping it at 6 (the default value).

Peptide size defines the size of epitope you are looking for in your peptides. If the tested sequences are of different length (i.e. your data set includes 10-mers, 20-mers, other), we recommend keeping it at 9, since very few epitopes and practically none of the specific ones are shorter than 9 amino acids. In case you wish to compare a set of peptides of different lengths and select the best of them, set the size

of the shortest one as the reported peptide size.

You can 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).

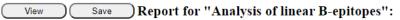
Sort by. You can select the data to be reported in several ways:

Start: the results will be sorted according to the order of the analysed peptides in your data file. If you analyse 19-20-mer peptides/sequences, and have defined the *Peptide size* as 9, your results will contain multiple 9-mers for every original peptide with AGI (antigenicity) defined for every 9-mer. In Results, all the data will be sorted according to the order of this peptide in the original sequence.

View Save Report for "Analysis of linear B-epitopes":

Code	Sequence	Start	End	Peptide	AGI	APB	
HER1	AVLDNGDPLNNTTPVTGASPGG	1	9	AVLDNGDPL	23	2	_
HER1	AVLDNGDPLNNTTPVTGASPGG	2	10	VLDNGDPLN	28	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	3	11	LDNGDPLNN	32	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	4	12	DNGDPLNNT	32	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	5	13	NGDPLNNTT	29	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	6	14	GDPLNNTTP	27	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	7	15	DPLNNTTPV	24	2	
UED1	AVI DNEDDI NNTTDVTGA CDGG	۰	16	DI MMTTDVT	10	2	•

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



Code	Sequence	Start	End	Peptide	AGI	APB	
HER1	AVLDNGDPLNNTTPVTGASPGG	3	11	LDNGDPLNN	32	3	_
HER1	AVLDNGDPLNNTTPVTGASPGG	4	12	DNGDPLNNT	32	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	5	13	NGDPLNNTT	29	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	2	10	VLDNGDPLN	28	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	6	14	GDPLNNTTP	27	3	
HER1	AVLDNGDPLNNTTPVTGASPGG	7	15	DPLNNTTPV	24	2	
HER1	AVLDNGDPLNNTTPVTGASPGG	1	9	AVLDNGDPL	23	2	
HFR1	AVI DNGDPI NNTTPVTGASPGG	8	16	PINNTTPVT	18	2	•

AGI ALL: the results will be sorted according to the AGI values of the detected peptides of the defined peptide length (say, 9) irrespective of their origin from different original sequences, from the best to the worst.

View Save Report for "Analysis of linear B-epitopes":

Code	Sequence	Start	End	Peptide	AGI	APB	
HER4	INCTHSCVDLDDKGCPAEQR	9	17	DLDDKGCPA	82	9	_
HER4	INCTHSCVDLDDKGCPAEQR	8	16	VDLDDKGCP	81	9	
HER4	INCTHSCVDLDDKGCPAEQR	10	18	LDDKGCPAE	79	8	
HER4	INCTHSCVDLDDKGCPAEQR	7	15	CVDLDDKGC	75	8	
HER4	INCTHSCVDLDDKGCPAEQR	11	19	DDKGCPAEQ	71	7	
HER4	INCTHSCVDLDDKGCPAEQR	6	14	SCVDLDDKG	65	7	
HER4	INCTHSCVDLDDKGCPAEQR	12	20	DKGCPAEQR	61	6	
HFR4	TNCTHSCVDI DDKGCPAFOR	5	13	HSCVDI DDK	57	6	•

AGI BEST: the data will be presented by *only* the best peptide from every sequence in the order from the highest to the lowest (here only 1 9-mer is shown for the original 4 peptides of various lengths.

View Save Report for "Analysis of linear B-epitopes":

Code	Sequence	Start	End	Peptide	AGI	APB
HER4	INCTHSCVDLDDKGCPAEQR	9	17	DLDDKGCPA	82	9
HER3	LYISAWPDSLPDLSVFQNLQ	12	20	DLSVFQNLQ	41	4
HER1	AVLDNGDPLNNTTPVTGASPGG	3	11	LDNGDPLNN	32	3
HER2	FLPESFDGDPASNTAPLQPE	12	20	SNTAPLQPE	27	3

Viewing and saving the Results

Report: Relative Immunogenicity of Linear B-epitopes (B-Scanner)

Date & Time: 05.11.2020 13:46:35

Project name:

Program: EpiQuest B-Scanner v1.0.0.1

Species: Human/Mouse

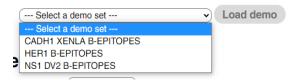
Matrix: B7.1 Peptide size: 9 Frame size: 6 Threshold: 0

Sorted by: AGI BEST

Code	Sequence	Start	End	Peptide	AGI	APB
HER4	INCTHSCVDLDDKGCPAEQR	9	17	DLDDKGCPA	82	9
HER3	LYISAWPDSLPDLSVFQNLQ	12	20	DLSVFQNLQ	41	4
HER1	AVLDNGDPLNNTTPVTGASPGG	3	11	LDNGDPLNN	32	3
HER2	FLPESFDGDPASNTAPLQPE	12	20	SNTAPLQPE	27	3

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

Demo Sequences



We supply several files for Demos that either have tested selections of epitopes or selected sets of sequences for training.

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