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How to…

Choosing an immunogenic epitope using B-Scanner

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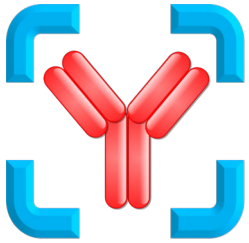
Selecting the most antigenic epitope from several peptides (using B-scanner)

Question: We have selected a number of B-epitopes (based on our own selection method) for a protein or several proteins. How can we identify the highly antigenic ones among them?



We will illustrate how to use B-scanner to analyse the epitopes it by using data from a published article of *Dakappagari et al, Cancer Res 2000;60:3782-3789.*

The ability of epitopes, described in this article, to elicit weak or strong immune response has been tested experimentally and shall allow us to control the quality of the *in silico* analysis.

Dakappagari et al wanted to find strong linear epitopes within the sequence of human ErbB2 to further incorporate them into their erbB2-vaccine. As they stated, the selection of "*candidate B-cell epitopes expressed within the human HER-2 ECD was accomplished by computer-aided analysis using various correlates of protein antigenicity as reviewed by Kaumaya et al**",* and the exact criteria for selection were not disclosed.

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**Kaumaya, P. T. P., Kobs-Conrad, S., DiGeorge, A. M., and Stevens, V. De novo engineering of protein immunogenic and antigenic determinants. In: G. M. Anantharamaiah and C. Basava (Eds.), Peptides, Design, Synthesis & Biological Activity. pp. 133–164. Boston: Birkhauser, 1994*

Candidate epitopes to compare and select the best

Thus, by certain methods, four epitopes were selected out of the human erbB2* sequence. Can we predict which one will be immunogenic and suitable for the vaccine or epitope to raise an erbB2-reactive hyperimmune serum?

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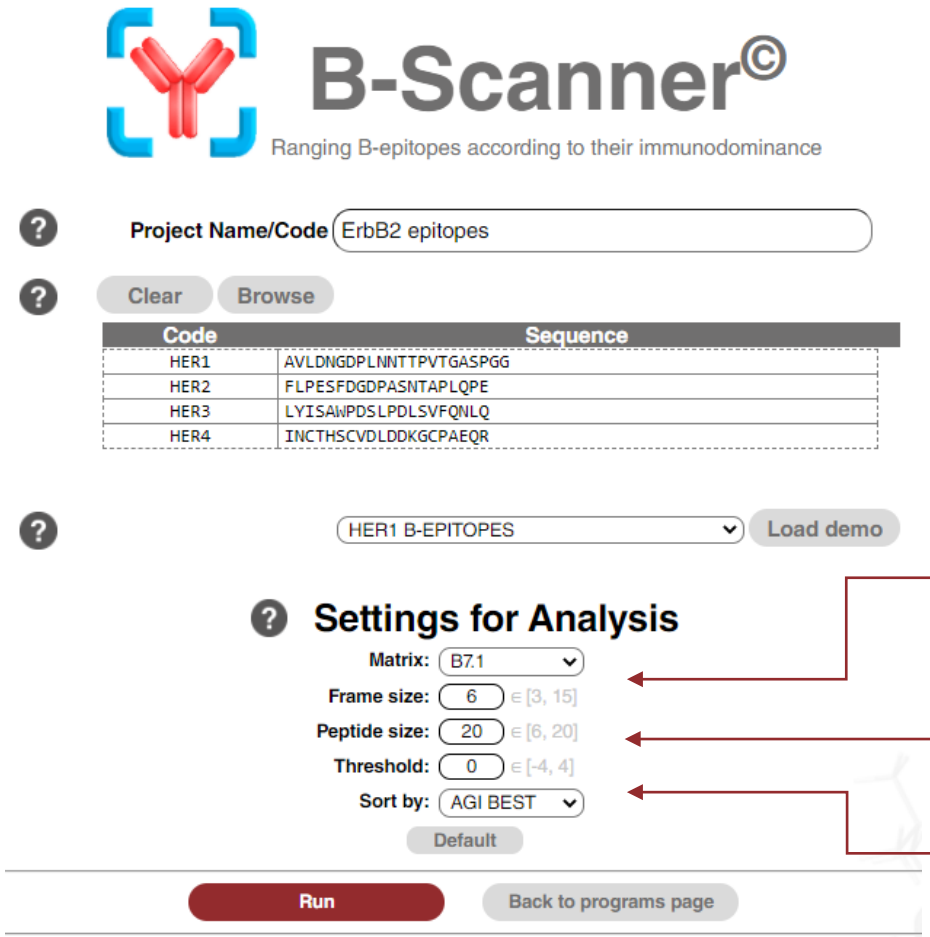
* *Dakappagari et al, Cancer Res 2000;60:3782-3789*

Epitope	Start	End	Length	Sequence
HER1	115	136	22	AVLDNGDPLNNTTPVTGASPGG
HER2	376	395	20	FLPESFDGDPASNTAPLQPE
HER3	410	429	20	LYISAWPDSLPDLSVFQNLQ
HER4	628	647	20	INCTHSCVDLDDKGCPAEQR

Comparing the peptide epitopes in B-scanner



Here we show how to choose the best epitope out of a group of peptide antigens using B-Scanner.
Since linear epitopes are generally 8-10aa long and analysed sequences are at least twice as long, they may contain at least two independent epitopes. Hence, one approach will be to compare the best epitopes that can be found in the analysed sequences, another - to compare the overall antigenicity of the entire sequences.
Frist, let's compare the overall antigenicity of the sequences (the comments on the settings are below):

The sequences are present among demo sequences, and can be opened by "Load demo"

We used the Default frame size =6, but for such large sequences (20aa), fame 9 can be used as well.

As 3 epitopes are 20 aa in length and one - 22aa, we have chosen the maximal size of 20aa as the size of the compared sequences

"AGI Best" was chosen as the type of report. Only one best 20aa sequence with the highest AGI (antigenicity) will be shown for every analysed sequence.

Results in B-scanner

a

Code	Sequence	Start	End	Peptide	AGI	APB
HER4	INCTHSCVDLDDKGCPAEQR	1	20	INCTHSCVDLDDKGCPAEQR	*130*	*6*
HER1	AVLDNGDPLNNTTPVTGASPGG	1	20	AVLDNGDPLNNTTPVTGASP	*25*	*1*
HER2	FLPESFDGDPASNTAPLQPE	1	20	FLPESFDGDPASNTAPLQPE	*-11*	*0*
HER3	LYISAWPDSLPDLSVFQNLQ	1	20	LYISAWPDSLPDLSVFQNLQ	*-33*	*-1*

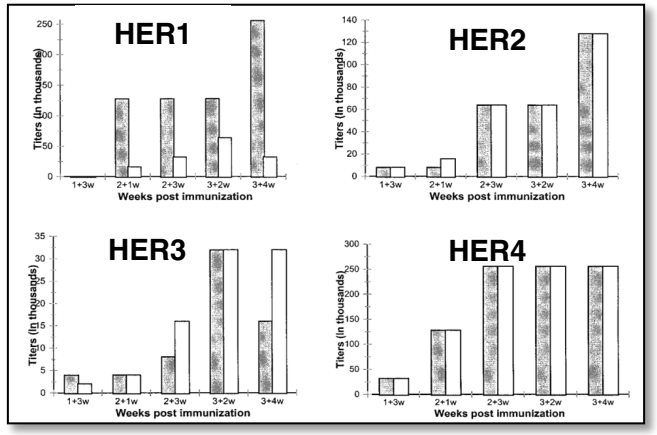
b

Code	Sequence	Start	End	Peptide	AGI	APB
HER4	INCTHSCVDLDDKGCPAEQR	9	20	DLDDKGCPAEQR	*91*	*7*
HER1	AVLDNGDPLNNTTPVTGASPGG	1	12	AVLDNGDPLNNT	*36*	*3*
HER2	FLPESFDGDPASNTAPLQPE	7	18	DGDPASNTAPLQ	*29*	*2*
HER3	LYISAWPDSLPDLSVFQNLQ	8	19	DSLPDLSVFQNL	*25*	*2*

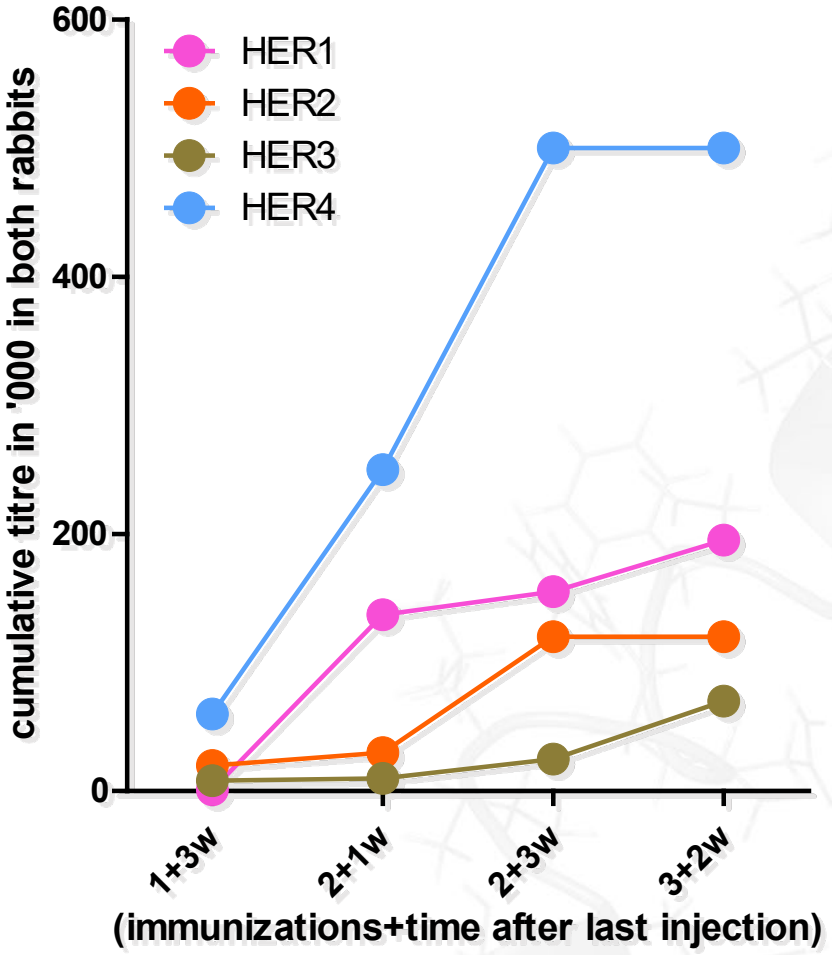
Here we show the results as they were produced in *Table* format by B-scanner. The first analysis (**a**) was performed with *Peptide Size* 20 (the length of the shortest of the peptides), the results in (**b**) obtained with *Peptide Size* 12 which is the maximal size or a standard linear B-epitope including the flanking sequences. In both runs the results were requested as *AGI BEST*, which means that for every analysed sequence will be shown the best peptides of the defined *Peptide Size*, and the results will be sorted according to their AGI (*antigenicity index*)

Actual antigenicity of epitopes

To analyse the correctness of our prediction, lets compare the *in silico* rating of the peptide with the actual experimental data. Each of 4 peptides was used, in a carrier-coupled form, to immunize 2 rabbits (data from *Dakappagari et al, Cancer Res 2000;60:3782-3789*). Below we show the original data provided by authors, on the right – averaged response in 2 rabbits.



Please, note that the scale is different in each graph



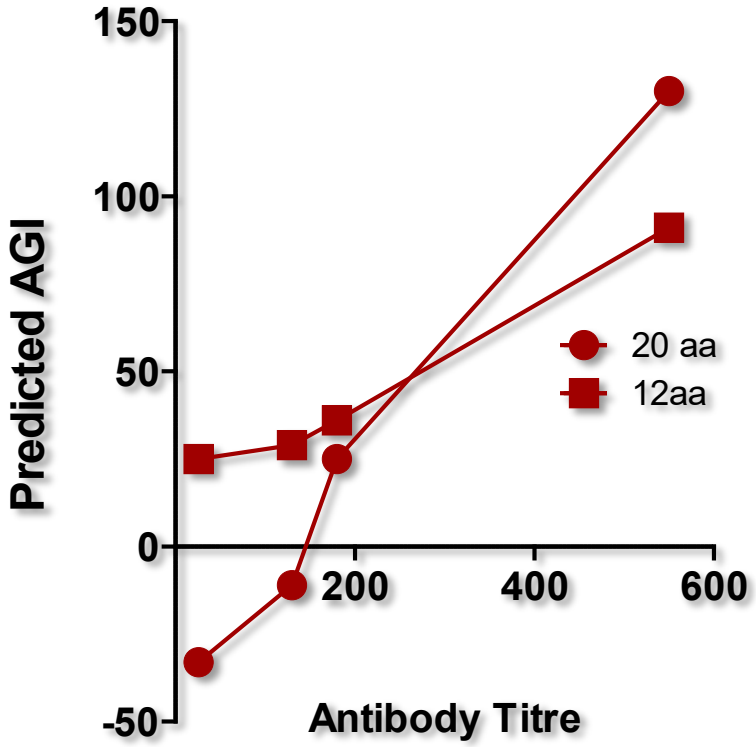
Correlation of the predicted and observed antigenicity

Relative Epitope Strength:

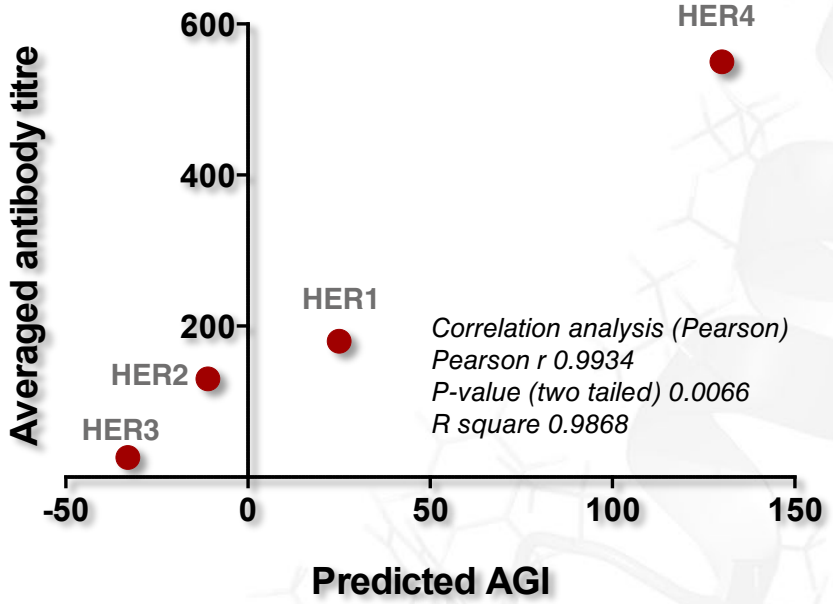
Prediction: HER4 > HER1 >HER2 > HER 3

Experiment: HER4 > HER1 >HER2 > HER 3

a



b



As shown here, the predicted antigenicity for both 20 mers and 12 mers fully correlates with ability of the peptide to elicit specific antibodies (**a**). For 20-mers the prediction is more correct and correlates with P-value of <0.01 for all 4 peptides (**b**).

In Conclusion

Concluding remarks:

- B-scanner correctly predicts the antigenicity of the analysed peptide epitopes, allowing the best choice;
- We compare the antigenicity of the epitopes they show when an animal is immunized by a synthetic peptide (in conjugate form). In other words, the antigenicity is defined only for a given peptide sequence outside the context of the original protein. This is the case when the antigenicity of the peptide equates its immunogenicity', whereas in the native protein this sequence may be poorly exposed to the surface, glycosylated, or otherwise altered the way it affects its ability to elicit antibodies.
- The method shall be used to rank the epitopes by antigenicity, which have already been preselected by other means.
- When comparing multiple epitopes of different lengths, we recommend using the size of the shortest peptide in the selected group (thus comparing the overall antigenicity)

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