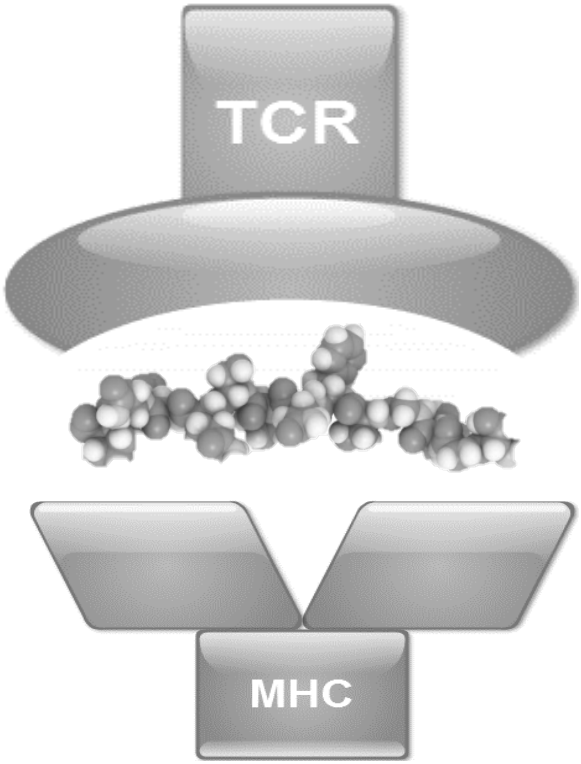
EpiQuest Support Library

EpiQuest-T

Finding *functional* CTL epitopes

DOC.REF. EPQ2021-001

How are T-epitopes usually selected?



All informatics' efforts are currently focused on defining in a protein sequence of peptide T-epitopes capable of binding to the groove of the respected MHC class I or II molecules. However, there is a T - cell receptor, and it also defines the strength (or affinity) to the peptide in the context of the MHC. Based on our experience of sequences capable of eliciting high-affinity antibodies, we focused on the structural characteristics of the peptide epitope: **whether it is capable of eliciting a strongly binding TCR.**

The T (CTL) epitope sequences differ in their capability to induce strongly binding TCR. Irrespective of the fact that they may bind well to the respective MHC.
This strongly affects the FUNCTIONAL strength of an epitope. Please remember that:

Less than 5% of MHC-binding epitopes show *any* functional activity
(95 % shown no functional activity at all)

Therefore, in search of sequences for T-cell epitopes, the program should pick-up the unique features of the sequence that allow it to elicit TCR that would strongly bind to it in the context of the respective MHC.

This is what EpiQuest-T does.

A danger of weak T-epitopes

As was clearly demonstrated by *Chung et al (2014)* in their article, the presence of many low avidity epitopes inhibit the development of high avidity response:

Cell Reports
Article

Cell Reports 8, 871–882, August 7, 2014

Antigen-Specific Inhibition of High-Avidity T Cell Target Lysis by Low-Avidity T Cells via Trogocytosis

Brile Chung,[1,6] Tor B. Stuge,[2,6,8] John P. Murad,[1] Georg Beilhack,[3,7] Emily Andersen,[1] Brian D. Armstrong,[1,5] Jeffrey S. Weber,[4] and Peter P. Lee[1,*]
[1]Department of Cancer Immunotherapeutics and Tumor Immunology (CITI), City of Hope National Medical Center, 1500 East Duarte Road Duarte, CA 91010, USA

Highlights

- Low-avidity CTLs inhibit high-avidity CTL target lysis in an antigen-specific manner
- Low-avidity CTLs strip specific pMHCs from target cells without lysis
- Peptide repertoire on the cell surface is dynamic and shaped by interaction with T cells
- Design of T cell vaccines needs to account for avidity

We need to aim to find and use **strong** T-epitopes for immunization, as the weaker one's lead to the development of low-affinity T-cells which result in ineffective CTL defense against the target.

So, it is critical to predict the CTL epitopes capable to elicit strongly binding N-cell receptors through *in silico* analysis.

Thus, the question is whether we can identify the features of peptide sequences that can have strong interactions with TCR.

Do we really need to know the precise T-epitopes?

There is one key question we are often asked about our approach used in EpiQuest-T: the program does not give the exact peptide, only the area of its potential location. However(see the findings of Bijker et al. 2007), for the purpose of vaccination, we do not need to know the precise epitope, but rather the area containing it.

For those who wish to establish the absence of the epitopes in the sequence, our approach is also more relevant, as it demonstrates (at different levels of sensitivity) all sequences where strong epitopes may be located.

J Immunol October 15, 2007, 179 (8) 5033-5040
DOI: https://doi.org/10.4049/jimmunol.179.8.5033

$CD8^+$ CTL Priming by Exact Peptide Epitopes in Incomplete Freund's Adjuvant Induces a Vanishing CTL Response, whereas Long Peptides Induce Sustained CTL Reactivity

Martijn S. Bijker, Susan J. F. van den Eeden, Kees L. Franken, Cornelis J. M. Melief, Rienk Offringa and Sjoerd H. van der Burg

"Our data clearly show why ***priming of CTL with minimal peptide epitopes*** in IFA ***is suboptimal***, and demonstrate that the use of longer versions of these CTL peptide epitopes ensures the induction of sustained effector $CD8^+$ T cell reactivity in vivo"

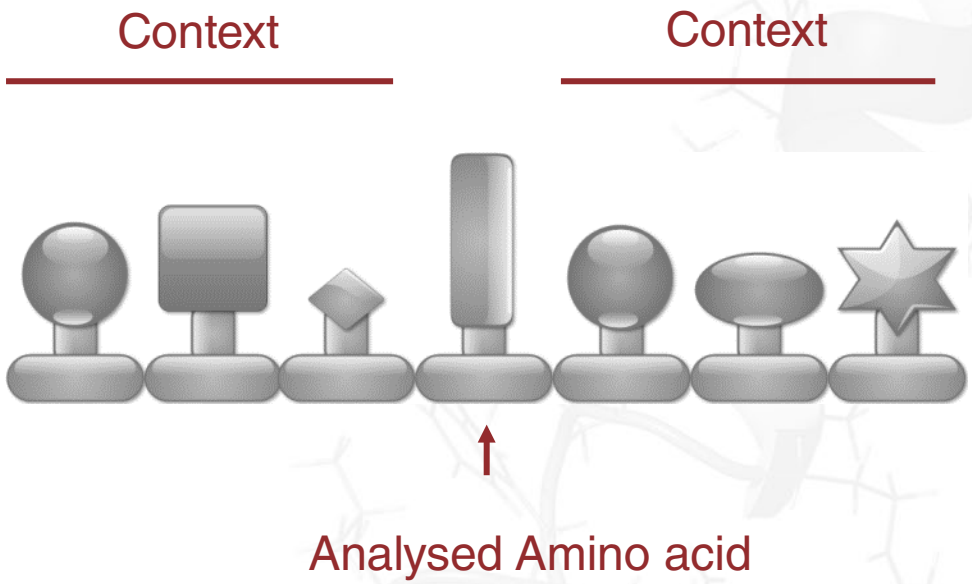
What does EpiQuest-T search for?

EpiQuest-T is based on our unique algorithm that has identified the typical structural features of a strong T-epitope that should be recognized by TCR in the context of a particular type of MHC molecule.

The software analyzes the linear protein sequence and calculates the probability of an amino acid at a given position in the sequence, depending on the context, to be involved into high affinity (immuno-dominant) CTL epitope depending on its context.

The algorithm calculates the value of an individual amino acid defined by its context using a Matrix of values. It finds the amino acids that may participate in HLA and TCR binding.

In other words, the Matrix defines the relative value of amino acid in sequence on the basis of close and distant neighbors. It was developed experimentally by analysis of the context for over 10.000 amino acids in known epitopes (strong, low and non-immunogenic).



Score for epitopes by EpiQuest-T and their actual activity

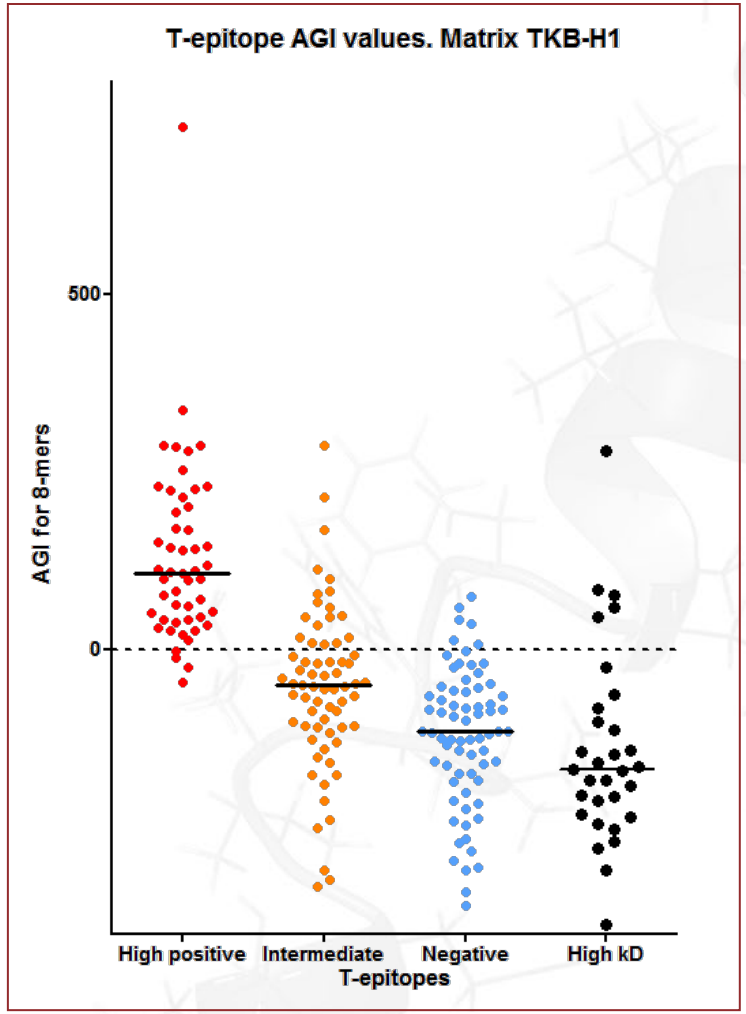
All reported high and intermediate activity epitopes*, as well as a random selection of the negative ones, and high activity epitopes for kD haplotype were analyzed for their AGI. The data presented is for the highest AGI 8-mer present within the epitope sequence.

Matrix H1 (H2-kB haplotype) allows to clearly discriminate between highly positive and negative epitopes (92% correct prediction), and that the prediction is MHCI allele-specific.

Statistical analysis of the groups

Dunn's Multiple Comparison Test	Significant? P < 0.01?	Summary
High positive vs Intermediate	Yes	***
High positive vs Negative	Yes	***
High positive vs High kD	Yes	***
Intermediate vs Negative	No	*
Intermediate vs High kD	Yes	**
Negative vs High kD	No	ns

The spread of the AGI values for the epitope within each group should be expected, as the values were determined for only the best 8-mer within each epitope, as their classification in the IEDB according to the category of the functional strength is very relative (they were not compared in the same experiment)

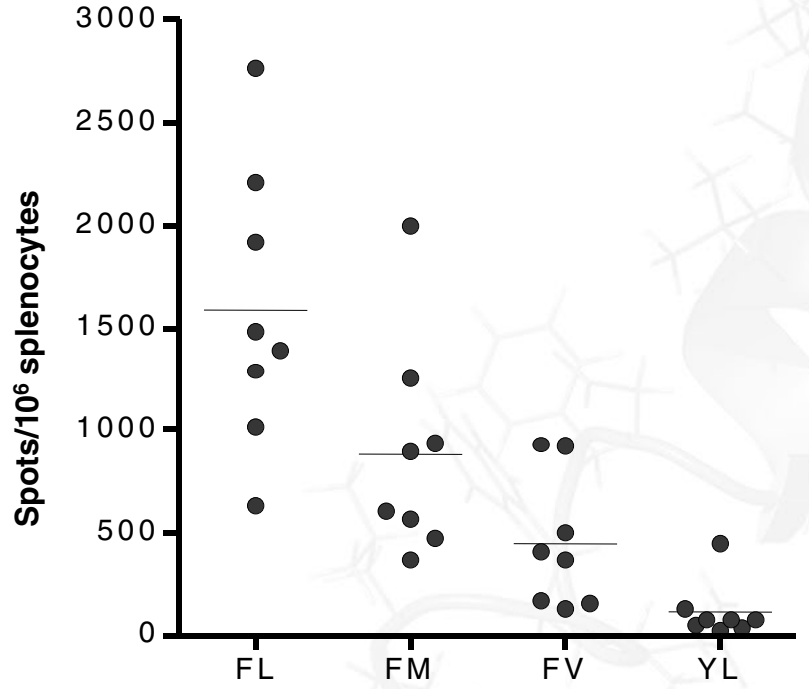


Predicted CTL epitope strength agrees with experimental data

To test whether the program correctly detects the immunodominance of epitopes, we have designed 4 mutants of the same CTL epitopes (H2-kB). The functional strength of the epitopes is presented as the proliferation of splenocytes from primed mice (each individual mouse corresponds to one dot). The high and low functional activity of CTL epitopes directly corresponds to the Antigenicity Index defined for each epitope.

The analysis performed using two matrixes for H2kB haplotype: H1, focused on features of high activity epitopes and HI2 that is also sensitive for the epitopes with intermediate activity.

Immunodominance hierarchy in C57Bl/6 primed with pDUO (peptide)



AGI	FL	FM	FV	YL
Matrix H1	112	40	40	-20
Matrix HI2	312	278	204	107

pDUO-peptide **SIINFEKL** > **SIINFEKV** > **SIINFEKM** > **SIINYEKL**

EpiQuest-T prediction vs MHCI-binding prediction (IEDB)

Further we will show you some examples illustrating the difference in results you obtain with EpiQuest-T and MHC-binding prediction programs (IEDB and similar), and how these results relate to finding the functional CTL epitopes.

Example 1: Glycoprotein [Lymphocytic choriomeningitis virus]

GenBank: ABC96001.2

Analysis using IEDB tool (allele H2-kB)

Allele	seq_num	start	end	length	peptide	method	Percentile Rank	ann_ic50	ann_rank	smm_ic50	smm_rank
H-2-Kb	**1**	**264**	**271**	**8**	**ISHYFRNI**	**Consensus (ann/smm)**	**0.11**	**6.56**	**0.02**	**35.79**	**0.2**
H-2-Kb	1	198	205	8	ISYLLVGV	Consensus (ann/smm)	0.12	9.57	0.03	28.56	0.2
H-2-Kb	1	211	218	8	ICYTFSTV	Consensus (ann/smm)	0.12	12.48	0.04	35.71	0.2
H-2-Kb	1	45	52	8	VTFLCGFL	Consensus (ann/smm)	0.13	22.51	0.06	19.62	0.2
H-2-Kb	1	12	19	8	IFLMFLSL	Consensus (ann/smm)	0.14	33.05	0.09	17.25	0.2
H-2-Kb	1	274	282	9	KAYFYFLFF	Consensus (ann/smm)	0.21	95.94	0.23	48.43	0.2
H-2-Kb	1	277	285	9	FYFLFFTSF	Consensus (ann/smm)	0.24	126.58	0.29	47.88	0.2
H-2-Kb	1	250	259	10	TSFKAHYIAL	Consensus (ann/smm)	0.26	39.38	0.11	1893.78	0.4
H-2-Kb	1	444	451	8	IGSNFKNL	Consensus (ann/smm)	0.26	43.89	0.12	83.51	0.4
H-2-Kb	1	14	22	9	LMFLSLTML	Consensus (ann/smm)	0.27	99.12	0.23	93.78	0.3
H-2-Kb	1	17	25	9	LSLTMLSPL	Consensus (ann/smm)	0.27	47.32	0.13	144.91	0.4
H-2-Kb	1	187	195	9	MVYLLLTFL	Consensus (ann/smm)	0.28	67.42	0.17	122.21	0.4
H-2-Kb	1	248	259	12	GATSFKAHYIAL	ann	0.3	128.37	0.3-		-
H-2-Kb	1	336	343	8	IMLMFIAI	Consensus (ann/smm)	0.3	42.34	0.11	90.52	0.5

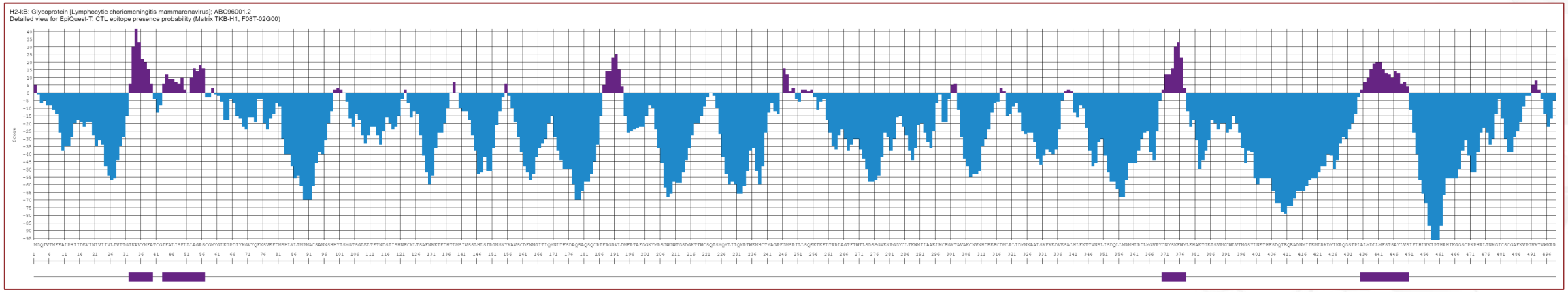
RANK

The analysis was performed using the recommended IEDB settings, the data presented in accordance to the "consensus" rank of the peptide. Please note that the rank, as well as ic50 values for the top peptide is critically higher for the 2 top peptides.

Example 1: Glycoprotein [Lymphocytic choriomeningitis virus]

GenBank: ABC96001.2

Analysis using EpiQuest-T (allele H2-kB)



Matrix = TKB-H1 / F = 8 / G = 0 / T = -2 / Peptide size = 8+					
Start	End	Length	AGI	AGR	Sequence
32	**39**	**8**	**174.00**	**21.75**	IKAVYNFA
370	377	8	131.00	16.38	CNYSKFWY
435	450	16	187.00	11.69	ALMDLLMFSTSAYLVS
43	56	14	135.00	9.64	IFALISFLLLAGRS

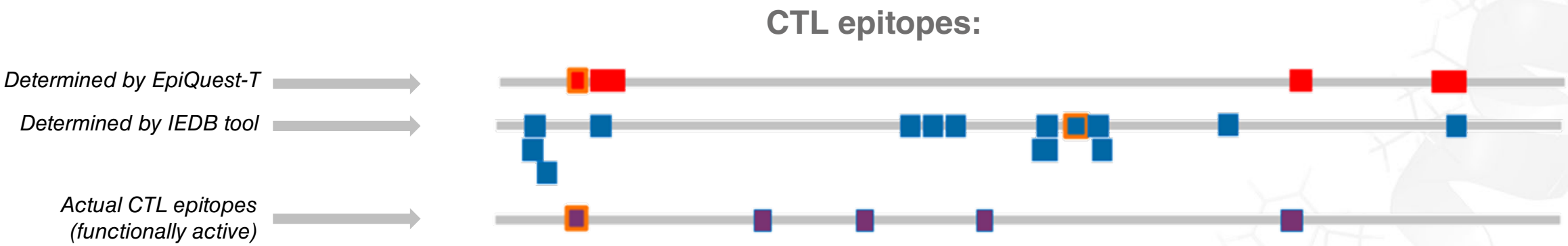
RANK

The analysis was performed using the default setting of EpiQuest-T with increased sensitivity (Threshold was -2). The results suggest the area 32-39 (+43-56) as the likely to contain the active CTL epitopes

Example 1: Glycoprotein [Lymphocytic choriomeningitis virus]

GenBank: ABC96001.2

Comparing the prediction to the actual presence of CTL epitopes with biological activity



An Orange line marks the best epitope for every group

There were 5 experimentally established functional CTL epitopes for H2-Kb within this protein. The best one corresponds to the best detected by EpiQuest-T. The second best was partially detected by EpiQuest-T.

None of the functional epitopes were present among those found by IEDB approach.

Example 2:Potassium uptake protein [Francisella tularensis]

WP_003017163.1

Analysis using IEDB tool (allele H2-kB)

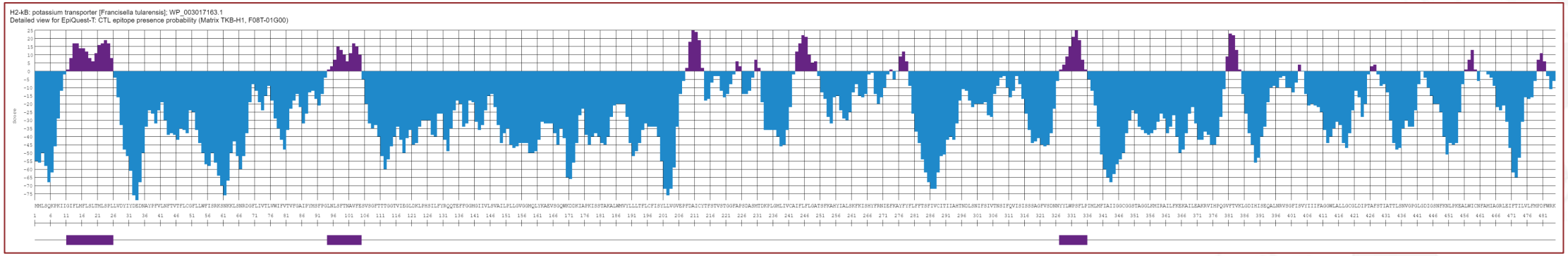
Allele	#	Start	End	Length	Peptide	Core	Icore	Score	Percentile Rank
H-2-Kb	1	35	43	9	NAYPFVLNF	NAYPFVLNF	NAYPFVLNF	0.839769	0.02
H-2-Kb	3	58	65	8	ISYLLVGV	ISYL-LVGV	ISYLLVGV	0.787577	0.02
H-2-Kb	4	54	61	8	ISHYFRNI	ISH-YFRNI	ISHYFRNI	0.78054	0.03
H-2-Kb	4	1	8	8	ICYTFSTV	ICYT-FSTV	ICYTFSTV	0.752617	0.03
H-2-Kb	4	59	67	9	RNIEFKAYF	RNIEFKAYF	RNIEFKAYF	0.69186	0.05
H-2-Kb	2	19	26	8	MSFPGLNL	MSFP-GLNL	MSFPGLNL	0.652491	0.06
H-2-Kb	7	24	31	8	IGSNFKNL	IGSN-FKNL	IGSNFKNL	0.632271	0.06
H-2-Kb	1	41	48	8	LNFTVTFL	LNFT-VTFL	LNFTVTFL	0.616936	0.06
H-2-Kb	5	28	35	8	SIFQVISI	SIFQ-VISI	SIFQVISI	0.564377	0.09
H-2-Kb	1	12	19	8	IFLMFLSL	IFLM-FLSL	IFLMFLSL	0.494301	0.13

*The analysis was performed using **the latest** recommended IEDB settings: Prediction method: NetMHCpan EL 4.1 | High Score = good binder*

Example 2:Potassium uptake protein [Francisella Tularensis]

WP_003017163.1

Analysis using EpiQuest-T (allele H2-kB)



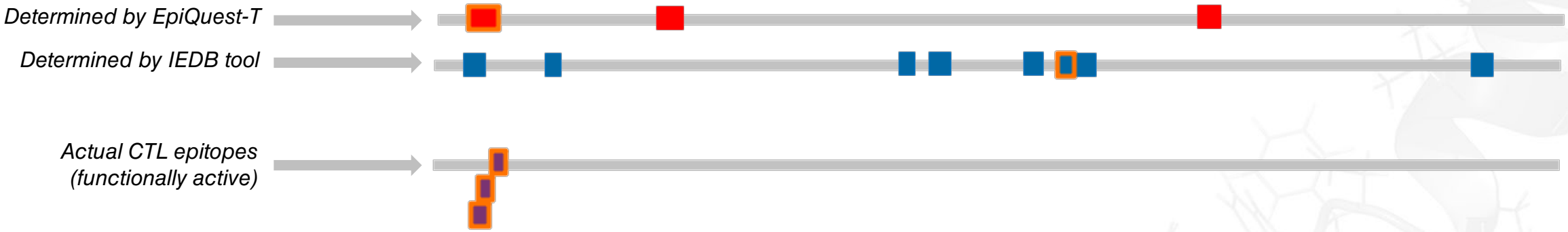
Matrix = TKB-H1 / F = 8 / G = 0 / T = -1 / Peptide size = 8+					
Start	End	Length	AGI	AGR	Sequence
11	25	15	185.00	12.33	GIFLMFLSLTMLSPL
327	335	9	102.00	11.33	YYLWPSFLP
94	104	11	108.00	9.82	LNLSFTNAVFE

RANK

The analysis was performed using the default setting of EpiQuest-T with increased sensitivity (Threshold was -2). The results suggest the area 11-25 as the likely to contain the active CTL epitopes

Example 2:Potassium uptake protein [Francisella Tularensis]

Comparing the prediction to the actual presence of CTL epitopes with biological activity



An Orange line marks the best epitope for every group

The actual functional epitopes for H2-kB mice are located 22-30, 15-22 and 11-21. They all (one partially) located in the best area detected by EpiQuest-T.
IEDB software predicted epitope 12-19, which falls within the borders of 2 actual epitopes, but does not correspond exactly to any of them. Moreover, it has the *lowest* score from the best 10.

Conclusion

As demonstrated by the sequence analysis examples, EpiQuest-T can successfully detect strong and functional CTL epitope areas.

Unlike other programs to predict CTL epitopes, EpiQuest-T aims to predict functional epitopes, able to induce an immune response. The search for MHC-binding epitopes often leads to the discovery of multiple high-score sequences with no actual functional activity.

As a minimum both approaches should be used when looking for functional CTL epitopes.

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