

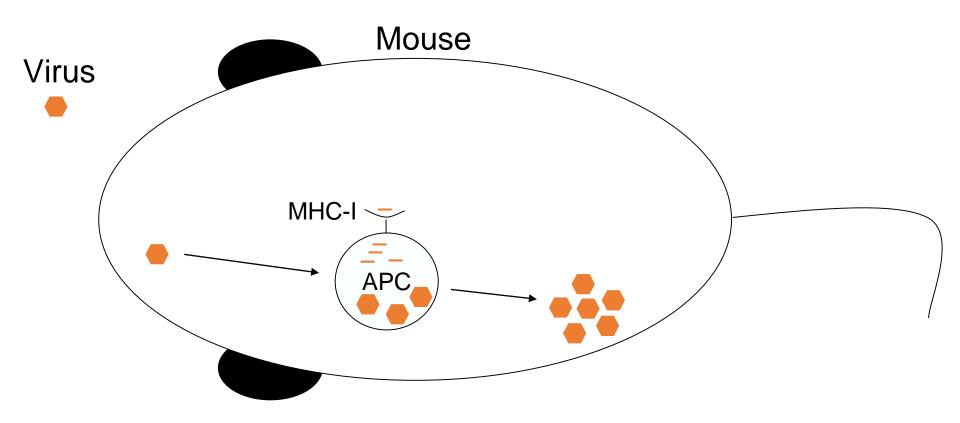
T Cell processing & immunogenicity predictions

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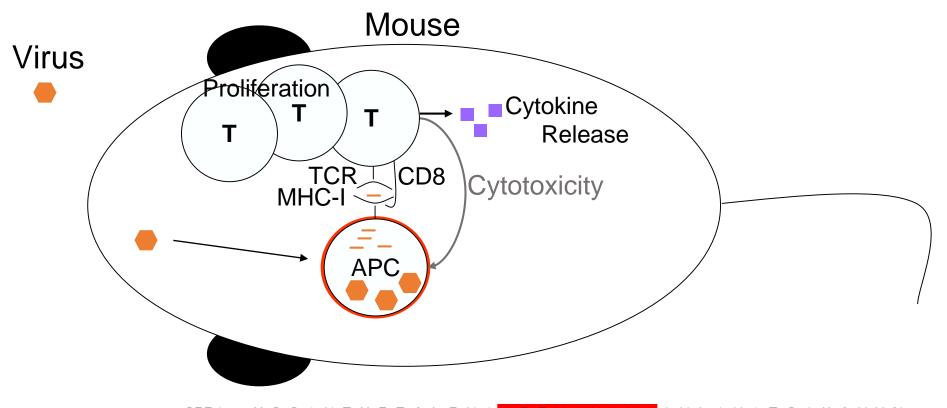
Presented by: Bjoern Peters, PI Austin Crinklaw, Research Technician

2021 IEDB User Workshop

CD8+ T cell epitopes in viral infection

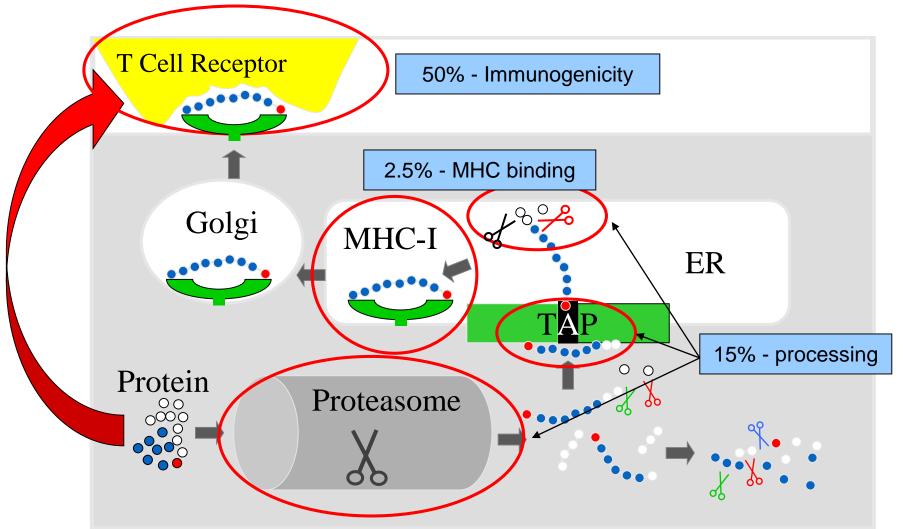


CD8+ T cell epitopes in viral infection



TT 11	ORF 1	<u>MGQIVTMFEALPHIIDEVINIVI</u> IVLIVITGIKAVYN
T cell	ORF 2	MGLKGPD I YKGVYQFKSVEFDMSHLNLTMPNACSANN
onitono	ORF 3	MHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIDGNSNY
epitope	ORF 4	<u>MSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSD</u>
manning	ORF 5	MHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLS
mapping	ORF 6	MKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKF
-	ORF 7	MLMRNHLLDLMGVPYCNYSKFWYLEHAKTGETSVPKC 3

MHC I - Antigen processing and presentation pathway



Peters et al, J Mol Biol 2002, Bioinformatics 2003, J Immunol. 2003; CMLS 2005; Assarson, J Immunol 2007

4

Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

Overview T Cell To	ols B Cell Tools	Analysis Tools	Tools-API	Usage [Download [Datasets	Contribute Tools	References	i
	s - Processin								
	ct epitope candida				otides in the	e cell.			
	vage/TAP transpo nbines predictors				ansport, and	d MHC bi	indina to produ	ce an overa	ll score for each
peptide's intr	rinsic potential of	f being a T cell	epitope.		• •		5 .		
	ased prediction of								÷
	predictor of pro						CIL and NetC	Lpan are pr	edictors of T cell
	ion of peptides na								
MHC-NP emp	oloys data obtain	ed from MHC e	lution exper	iments in	n order to a	assess th	e probability t	nat a given p	eptide is naturally ion in Immunology.
MHCII-NP:		d all design dates							
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Class I 'combined predictor'

Home Help Example Reference	e Download Contact
MHC-I Processing Pro	edictions
Prediction Method Version	2013-02-22 [Older versions]
	Specify Sequence(s)
Enter protein sequence(s) in FASTA format	
Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)	
Or select file containing sequence(s)	Browse No file selected.
Choose sequence format	auto detect format
	Choose a Prediction Method
Prediction Method	IEDB recommended V Help on prediction method selections
	Specify what to make binding predictions for
MHC source species	human 🗸
Show only frequently occuring alleles: 🗹 🕐 Select MHC allele(s)	Allele Length Upload allele file ?
	Proteasomal cleavage prediction
Specify proteasome type	immuno
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2
	Specify Output
Output format	XHTML table V
	Submit Reset

- Combines predictions for:
 - proteasomal cleavage
 - TAP transport
- Trained on specific in vitro datasets

Proteasomal cleavage

	Proteasomal cleavage prediction		
Specify proteasome type	immuno	~	
	TAP transport predictions	immuno	
Maximum precursor extension	1	constitutive	
Alpha factor	0.2		

- Proteasomes create the C-terminal end of peptides
- Prediction looks for sequence motive up and downstream of potential cleavage site
- Cleavage sequence motif determined based on in vitro protein digests by proteasomes

TAP Transport

	Proteasomal cleavage prediction
Specify proteasome type	immuno
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2

- TAP transport efficiency of peptides is sequence dependent; motif derived based on in vitro assays
- Overall TAP transport efficiency of a presented MHC ligand can be result of a collection of precursors
- Unless paper specifically read and details about the precursor length distribution are known, keep parameters unchanged

Class I 'combined predictor' - example

MHC-I Processing Predictions

Prediction Method Version	2013-02-22 [Older versions]
	Specify Sequence(s)
Enter protein sequence(s) in FASTA format (Browse for sequences in NCBI)	>LCMV Armstrong, Protein GP MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFLLLAGRSCGM YGLKGPDIYKSVYQFKSVEFDMSHLMLTMENACSANNSHHYISMGTSGLELTFTNDSII SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSDA QSAQSQCRTFRGRVLDMFRTAFGGKYMRSGMGWTGSDGKTTWCSQTSYQYLIIQNRTWE NHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVENPGGYCLTKWMILAAE LKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDVESALHLFKTTVNSLISDQ LLMRNHLRDDIMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEA DNMITEMLRKDYIKQGSTFLALMDLLMFSTSAYLVSIFLHLVKIPTHRHIKGGSCPKP HRLTNKGICSCGAFKVPGVKTVWKRR
Or select file containing sequence(s)	Browse No file selected.
Choose sequence format	auto detect format
	Choose a Prediction Method
Prediction Method	IEDB recommended Help on prediction method selections
	Specify what to make binding predictions for
MHC source species	mouse
Select MHC allele(s)	Allele Length H-2-Kb 10 S V Upload allele file ?
	Proteasomal cleavage prediction
Specify proteasome type	immuno
	TAP transport predictions
Maximum precursor extension	1
Alpha factor	0.2
	Specify Output
Output format	XHTML table
	Submit Reset

Class I 'combined predictor' - example

MHC-I Processing Prediction Results

http://tools.iedb.org/processing/

Input Sequences

#	Name	Sequence
1	LCMV Armstrong, Protein GP	eq:mgqivtmfealphiideviniviivlivlivlivdikavynfatcgifalisfllagrs CGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHYISMGTSGLELTF TNDSIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITI QYNLTFSDAQSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSDGKTTWCSQTS YQYLIIQNRTWENHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVEN PGGYCLTKWMILAAELKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDV ESALHLFKTTVNSLISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCW LVTNGSYLNETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYL VSIFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR
2	LCMV Armstrong, Protein NP	MSLSKEVKSFQWTQALRRELQSFTSDVKAAVIKDATNLLNGLDFSEVSNVQRIMRK EKRDDKDLQRLRSLNQTVHSLVDLKSTSKKNVLKVGRLSAEELMSLAADLEKLKAK IMRSERPQASGVYMGNLTTQQLDQRSQILQIVGMRKPQQGASGVVRVWDVKDSSLL NNQFGTMPSLTMACMAKQSQTPLNDVVQALTDLGLLYTVKYPNLNDLERLKDKHPV LGVITEQQSSINISGYNFSLGAAVKAGAALLDGGNMLESILIKPSNSEDLLKAVLG AKRKLNMFVSDQVGDRNPYENILYKVCLSGEGWPYIACRTSIVGRAWENTTIDLTS EKPAVNSPRPAPGAAGPPQVGLSYSQTMLLKDLMGGIDPNAPTWIDIEGRFNDPVE IAIFQPQNGQFIHFYREPVDQKQFKQDSKYSHGMDLADLFNAQPGLTSSVIGALPQ GMVLSCQGSDDIRKLLDSQNRKDIKLIDVEMTREASREYEDKVWDKYGWLCKMHTG IVRDKKKKEITPHCALMDCIIFESASKARLPDLKTVHNILPHDLIFRGPNVVTL

Prediction method: recommended | High Score = high efficiency Download result

Citations

Allele 🔶	# _{\$}	Start 🔶	End 🔶	Peptide Length	Peptide 🔶	Proteasome 🔶 Score	TAP Score 🗢	MHC Score 🗘	Processing 🔶 Score	Total Score	MHC IC50[nM]
H-2-Kb	2	203	212	10	LLYTVKYPNL	1.79	0.50	-2.01	2.28	0.27	103.5
H-2-Kb	1	116	125	10	SIISHNFCNL	1.51	0.46	-1.90	1.97	0.08	78.6
H-2-Kb	2	7	16	10	VKSFQWTQAL	1.42	0.39	-2.01	1.81	-0.21	102.9
H-2-Kb	2	235	244	10	INISGYNFSL	1.53	0.41	-2.18	1.95	-0.23	149.7
H-2-Kb	1	35	44	10	VYNFATCGIF	1.42	1.27	-3.12	2.68	-0.44	1327
H-2-Kb	1	75	84	10	KSVEFDMSHL	1.50	0.47	-2.45	1.96	-0.49	283.6
H-2-Kb	1	369	378	10	YCNYSKFWYL	1.45	0.31	-2.32	1.76	-0.56	206.8
H-2-Kb	1	449	458	10	VSTFLHLVKT	1.33	0.33	-2.25	1.66	-0.59	178.2

Class I 'combined predictor' - example

Allele 🔶	# ⇔	Start 🔶	End 🔶	Peptide Length	🔶 Peptide 🔶	Proteasome	TAP Score ♦	MHC Score 🔶	Processing Score \$	Total Score	MHC IC50[nM] ≑
H-2-Kb	2	203	212	10	LLYTVKYPNL	1.79	0.50	-2.01	2.28	0.27	103.5
H-2-Kb	1	116	125	10	SIISHNFCNL	1.51	0.46	-1.90	1.97	0.08	78.6
H-2-Kb	2	7	16	10	VKSFQWTQAL	1.42	0.39	-2.01	1.81	-0.21	102.9
H-2-Kb	2	235	244	10	INISGYNFSL	1.53	0.41	-2.18	1.95	-0.23	149.7
H-2-Kb	1	35	44	10	VYNFATCGIF	1.42	1.27	-3.12	2.68	-0.44	1327
H-2-Kb	1	75	84	10	KSVEFDMSHL	1.50	0.47	-2.45	1.96	-0.49	283.6
H-2-Kb	1	369	378	10	YCNYSKFWYL	1.45	0.31	-2.32	1.76	-0.56	206.8
H-2-Kb	1	449	458	10	VSIFLHLVKI	1.33	0.33	-2.25	1.66	-0.59	178.2

- Higher scores = higher efficiency for MHC-I presentation
- MHC binding score = -log10(IC50) (sign change)
- Combined scores are additive
 - Processing = proteasome + TAP
 - Total = proteasome + TAP + MHC
- Different variance in scores reflects different selectivity

Caveats / performance of processing predictions

- Processing predictions beat MHC binding predictions when predicting eluted peptides
- No clear evidence that processing predictions are better at predicting epitopes
- Eluted peptides may over represent 'best possible' ligands, and the difference in processing may not be relevant in practice
- Co-evolution of MHC molecules to bind peptides with motifs that are generated by proteasome and TAP means that most high affinity MHC binders are also efficiently processed
- <u>Recommendation</u>: Use MHC binding predictions alone by default
 - If resources require limiting the number of peptides considered, use total score of processing predictions as additional filter

Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

IEDB Analysis						
Overview T Cell Tools B Cell	Tools Analysis Tools	Tools-API Usage	Download Datase	ets Contribute Tools	References	
T Cell Epitopes - Proce	ssing Prediction					
These tools predict epitope c		the processing of p	eptides in the cell.			
Proteasomal cleavage/TAP t						
This tool combines pree peptide's intrinsic poter			ransport, and MH	C binding to produc	e an overall sc	ore for each
Neural network based predic	tion of proteasomal cl	eavage sites (NetC	hop) and T cell ep	topes (NetCTL and	NetCTLpan)	
NetChop is a predictor epitopes along a protei					pan are predic	tors of T cell
MHC-NP: Prediction of pepti	les naturally processe	ed by the MHC				
MHC-NP employs data processed and binds to	btained from MHC el	ution experiments	in order to asses	s the probability the	at a given pepti	de is naturally
<u> </u>						
MHCII-NP:						
This tool utilizes MHC I	ligand elution data t	o predict naturally	processed MHC I	I ligands by scannii	ng the given pe	ptide sequences.
<u> </u>	ligand elution data t	o predict naturally	processed MHC I	I ligands by scannii	ng the given pe	ptide sequences.
This tool utilizes MHC I	2		processed MHC I	I ligands by scanniı	ng the given pe	ptide sequences.
This tool utilizes MHC II	nogenicity Predic	tion		5 .	ng the given pe	ptide sequences.
This tool utilizes MHC II	nogenicity Predic ability of a peptide/MH	tion		5 .	ng the given pe	ptide sequences.
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Neural Network based predictors

http://tools.iedb.org/netchop/

IEDB Analysis R	esource		
Home Help Example Reference	Download Contact		
Proteasomal Cleavag NetChop/NetCTL/NetCTLpan	e Prediction		
	Specify Sequence(s)		
Prediction Method	NetChop	~	
Enter protein sequence(s) in FASTA format		NetChop netCTL netCTLpan	н
Or select file containing sequence(s)	Browse No file selected.		
	Method Specific Options		
Method	C term 3.0 V		
Threshold	0.5		
			Submit Reset

- NetChop: proteasomal cleavage
- NetCTL: combines NetChop, TAP transport, NetMHC
- NetCTLpan: combines NetChop, TAP transport, NetMHCpan

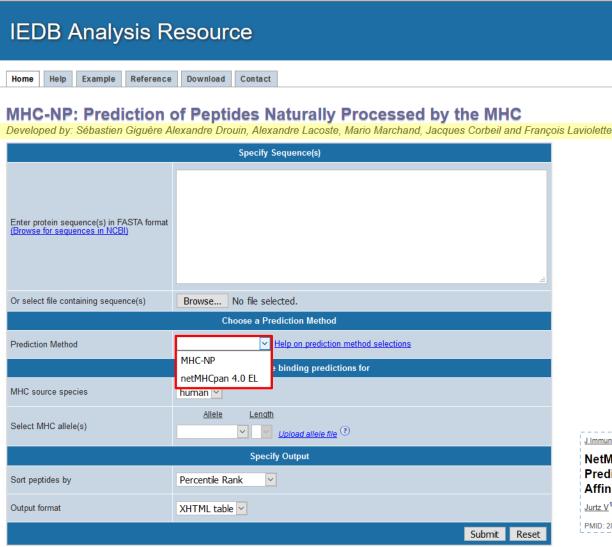
Key difference is the use of NetChop

Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

T Cell Epitopes - Processing Prediction These tools predict epitope candidates based upon the processing of peptides in the cell. Proteasomal cleavage/TAP transport/MHC class I combined predictor This tool combines predictors of proteasomal processing, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope. Neural network based prediction of proteasomal cleavage sites (NetChop) and T cell epitopes (NetCTL and NetCTLpan) NetChop is a predictor of proteasomal processing based upon a neural network. NetCTL and NetCTLpan are predictors of T cell epitopes along a protein sequence. It also employs a neural network architecture. MHC-NP: Prediction of peptides naturally processed by the MHC MHC-NP employs data obtained from MHC elution experiments in order to assess the probability that a given peptide is naturally processed and binds to a given MHC molecule. This tool was the winner of the 2nd Machine Learning Competition in Immunology. MHCI-NP: This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences.
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MHC-NP: Prediction of peptides naturally processed by the MHC <u>http://tools.iedb.org/mhcnp/</u>



Pan-predictions trained on both binding + eluted ligand data now available!

J. Immunol. 2017 Nov 1;199(9):3360-3368. doi: 10.4049/jimmunol.1700893. Epub 2017 Oct 4.

NetMHCpan-4.0: Improved Peptide-MHC Class I Interaction Predictions Integrating Eluted Ligand and Peptide Binding Affinity Data.

Jurtz V¹, Paul S², Andreatta M³, Marcatili P¹, Peters B², Nielsen M^{4,3}.

PMID: 28978689 PMCID: PMC5679736 DOI: 10.4049/jimmunol.1700893

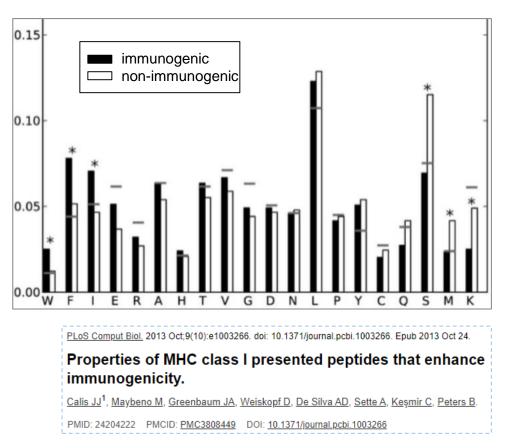
Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

Overview T Cell Tools B Cell Tools Analysis Tools Tools-API Usage Download Datasets Contribute Tools References	
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Class I immunogenicity prediction

- Approach: Assemble two datasets of peptides with similar MHC binding affinity, that are (i) recognized or (ii) not recognized by T cells
- Enrichment of W,F,I and depletion of S,M,K in immunogenic peptides
- Use enrichments to calculate propensity scores



Class I immunogenicity prediction -example

http://tools.iedb.org/immunogenicity/

IEDB Anal	ysis Resource	
Home Help Example	e Reference Download Contact	
Class I Immur	nogenicity	
	Specify sequence(s) *	
Enter peptide sequence(s) (Browse for sequences in NCBI)	FIAGLIAIV LITGRLQSL RLNEVAKNL KAVYNFATC FQPQNGQFI	
Or select file containing sequence(s)	Browse No file selected.	
	Choose which positions to mask	
Specify which positions to mask	Default (1st, 2nd, and C-terminus amino acids) Custom User Defined (Comma separated numbers) Peptide lengths must be equal when using custom masking.	Mask positions that are MHC anchors
	Submit Reset	
*The tool was only valida	ted for 9-mer peptides. However, predictions can be made for peptides of any length.	

Class I immunogenicity prediction -example

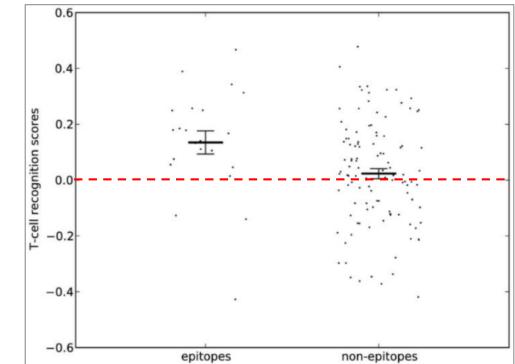
http://tools.iedb.org/immunogenicity/

IEDB Analysis Resource										
Home H	elp Exa	mple	Reference	Download	Contact					
/asking: def /asked varia	ault bles: [1, 2,	'cterm']	genicit	·y						
Peptide 🔶	Length \$	Score •								
FIAGLIAIV	9	0.27206	_							
KAVYNFATC	-									
RLNEVAKNL	9	-0.0101								
LITGRLQSL	9	-0.10776	5							
FQPQNGQFI	9	-0.12392	2							

- Scores are sums of propensity scores at all unmasked positions
- High scores = peptide is more likely to be immunogenic

Class I immunogenicity prediction caveats / performance

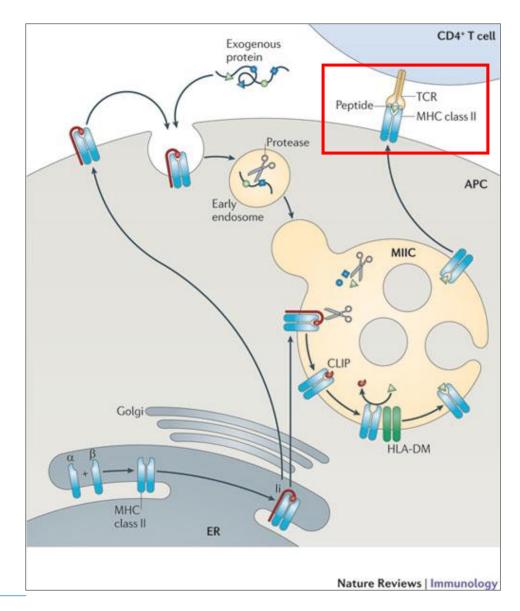
- Experimentally, many MHC binding peptides can be immunogenic (~50%)
- Cross validation gave AUC values ~ 0.65. Test on independent blind set gave AUC = 0.69
- Recommendation: Use as filter (cutoff 0) if high specificity is desired. Suggested cutoff is 0



Class I Summary

- Processing predictions are better at identifying naturally processed ligands, but have not been shown to be superior in identifying epitopes compared to MHC binding predictions
- Specific processing and immunogenicity predictions are good additional filters if the only goal is to select high likelihood T cell epitopes
- NetMHCPan 4.0 EL scores, which are trained on both MHC binding and ligand elution data are a straightforward replacement of MHC binding predictions, and show some enhanced performance
 Use these, and consider pairing with immunogenicity scores, when predicting epitope candidates

CD4 T cell epitopes (MHC class II)



Class II Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

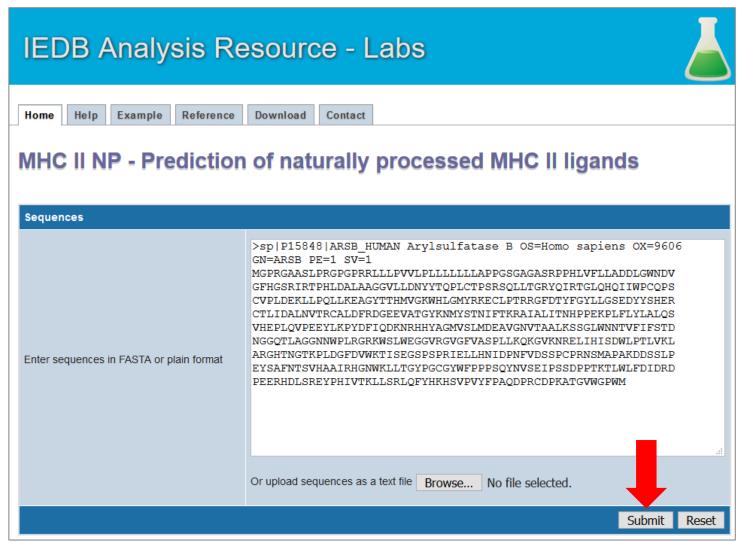
Overview	T Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Tools	References		
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			ites based upo		sing of p	eptides in th	ne cell.				
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This t peptio	ool combin de's intrinsi	es predictors potential of	of proteasom f being a T cel	al processin l epitope.	ng, TAP t	transport, a	nd MHC bi	inding to produc	e an overall	score for each	
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MHCII-NP

- Predicting the naturally processed peptides for MHC class II
- Based on
 - Cleavage motif analysis at C and N terminal of peptides
 - Ligand elution data derived from IEDB
- Ligand predictions is improved markedly when combining the binding and cleavage motifs
- T cell epitope prediction is not significantly improved

MHCII-NP - example

http://tools.iedb.org/mhciinp/



MHCII-NP -example

http://tools.iedb.org/mhciinp/

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MHC II NP results

Top 5 peptides per protein:

	Seq name	Peptide start	Peptide end	Peptide length	Peptide	N motif	C motif	Cleavage probability score	Cleavage probability percentile rank
1	SPIP15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	510	524	15	VPVYFPAQDPRCDPK	SVP	PKA	1.75814	0.00
2	SPIP15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	2	16	15	GPRGAASLPRGPGPR	MGP	PRR	1.73735	0.02
3	SPIP15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	247	261	15	VPEEYLKPYDFIQDK	QVP	DKN	1.48840	0.04
4	SPIP15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	384	398	15	SPSPRIELLHNIDPN	GSP	PNF	1.40420	0.05
ę	SPIP15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	12	26	15	GPGPRRLLLPVVLPL	RGP	PLL	1.33714	0.07
<u>Co</u>	mplete results								

MHCII-NP scores

- Cleavage Score: Derived from the cleavage motif analysis in ligand elution data
- Binding Score: Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

Barra et al. Genome Medicine (2018) 10:84 https://doi.org/10.1186/s13073-018-0594-6

Genome Medicine

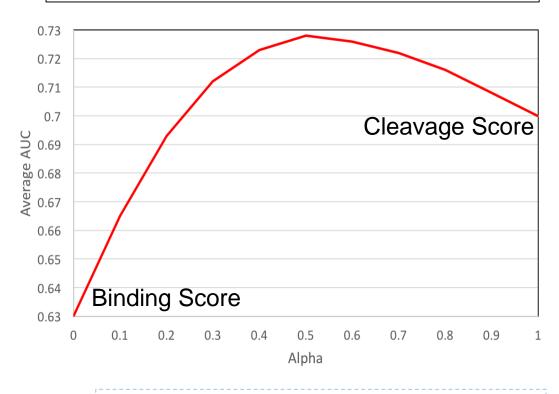
RESEARCH

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Footprints of antigen processing boost MHC class II natural ligand predictions

Carolina Barra^{1*}©, Bruno Alvarez¹⁺, Sinu Paul², Alessandro Sette², Bjoern Peters², Massimo Andreatta¹, Søren Buus³ and Morten Nielsen^{1,4*}

Combined score = $\alpha \times$ cleavage probability score + $(1 - \alpha) \times$ binding score



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Determination of a Predictive Cleavage Motif for Eluted Major Histocompatibility Complex Class II Ligands.

Paul S¹, Karosiene E¹, Dhanda SK¹, Jurtz V², Edwards L¹, Nielsen M^{2,3}, Sette A^{1,4}, Peters B^{1,4}.

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Class II Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

IEDB Analysis Resource

Dverview T Cell Tools B Cell Too	ls Analysis Tools To	ols-API Usage D	ownload Datasets	Contribute Tools	References	
Cell Epitopes - Process						
hese tools predict epitope cano			tides in the cell.			
roteasomal cleavage/TAP tran						
This tool combines predict peptide's intrinsic potentia	ors of proteasomal p I of being a T cell epi	rocessing, TAP trai tope.	nsport, and MHC bi	inding to produce	e an overall sco	ore for each
eural network based prediction	n of proteasomal cleav	age sites (NetCho	p) and T cell epitope	es (NetCTL and N	letCTLpan)	
NetChop is a predictor of p epitopes along a protein s				CTL and NetCTL	pan are predict	ors of T cell
IHC-NP: Prediction of peptides	naturally processed t	by the MHC				
MHC-NP employs data obt processed and binds to a d	ained from MHC eluti	on experiments in	order to assess th	e probability that	t a given pepti	de is naturally
MHCII-NP: This tool utilizes MHC II light MHC II light			rocessed MHC II lig	ands by scannin	g the given pe	otide sequences.
This tool utilizes MHC II lig	genicity Predictio	on		ands by scannin	g the given pe	otide sequences.
This tool utilizes MHC II lig Cell Epitopes - Immuno This tool predicts the relative abo	genicity Predictic	on		ands by scannin	g the given pe	otide sequences.
This tool utilizes MHC II lig Cell Epitopes - Immuno his tool predicts the relative ab	genicity Predictic	on complex to elicit an	immune response.		<u> </u>	
This tool utilizes MHC II lig Cell Epitopes - Immuno This tool predicts the relative ablication of the relative of the rel	genicity Predictic	on complex to elicit an	immune response.		<u> </u>	
This tool utilizes MHC II lig Cell Epitopes - Immuno his tool predicts the relative abi cell class I pMHC immunogen This tool uses amino acid MHC (pMHC) complex.	genicity Predictic lity of a peptide/MHC of icity predictor properties as well as a attempt to identify that create non-immut tion tool will list all th protein with 15mer results and final res 5 (which is difference e tools will also take nunogenic site in the	on complex to elicit an their position with immunodominant nogenic versions of the immunogenic re window size and 1 ult window will dis the median of care of the fact th	immune response. in the peptide to p of the proteins. So egions or peptides Omer overlap. 2) I splay the non-immu percentile rank fro at non-immunoger	redict the immur therapeutically ir we have opted a based on selecte n the second ste unogenic substitu m 26 reference a ic substitution in	nogenicity of a mportant prote two steps pro d threshold. Tl p, the user car lition of each se alleles set for N the immunog	class I peptide in, and suggest cess; 1) In the ese peptides select one or elected peptides. IHC class II). In enic peptides.
This tool utilizes MHC II light Cell Epitopes - Immuno This tool predicts the relative able <u>Cell class I pMHC immunoger</u> This tool uses amino acid MHC (pMHC) complex. <u>Deimmunization</u> : The deimmunization tool i amino-acid substitutions t first step, the deimmunization tob will be generated from the more peptides listed in the The default threshold is 8. the final result window, the should not create new imm	genicity Predictic lity of a peptide/MHC of <u>icity predictor</u> properties as well as attempt to identify that create non-immu tion tool will list all the protein with 15mer a results and final res 5 (which is difference tools will also take nunogenic site in the poring peptides.	on complex to elicit an their position with immunodominant nogenic versions of the immunogenic re window size and 1 ult window will dis the median of care of the fact th	immune response. in the peptide to p of the proteins. So egions or peptides Omer overlap. 2) I splay the non-immu percentile rank fro at non-immunoger	redict the immur therapeutically ir we have opted a based on selecte n the second ste unogenic substitu m 26 reference a ic substitution in	nogenicity of a mportant prote two steps pro d threshold. Tl p, the user car lition of each se alleles set for N the immunog	class I peptide in, and suggest cess; 1) In the ese peptides select one or elected peptides. IHC class II). In enic peptides.

MHC-II restricted immunogenicity prediction

- Extracted datasets of proteins from the IEDB for which overlapping peptides were tested for immunogenicity
- Utilized these datasets to train a Neural Network to learn 'motifs' associated with immunogenicity independent of specific MHC alleles expressed
- Resulting score can be combined with '7 allele method' quantifying MHC binding across alleles to predict overall immunogenicity

Class II immunogenicity prediction

- Based on Neural network model trained on
 - In house dataset for different antigens tested on different population cohorts
 - Tetramer dataset- derived from IEDB
- Validated on 57 independent studies from different groups across the world
- Implemented three approaches
 - 7-allele method (*Paul et. al. 2015*)
 - Immunogenicity predictions
 - Hybrid approach

Class II immunogenicity prediction -example

http://tools.iedb.org/CD4episcore/

IEDB Analysis Resource - Labs									
Home Help Example	Reference Contact								
CD4 T cell imm	unogenicity prediction								
Specify Sequence(s)									
Specify Sequence(s) >sp P01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1 MGVHECPAWLWLLISLISLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHC SLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL HVDKAVSGLRSITTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKL KLYTGEACRTGDR									
Or upload epitope sequence(s) from a file	Browse No file selected.								
	Choose a prediction method								
Prediction method:	IEDB recommended (combined)								
	7-allele								
Sort Peptides by:	Position in Protein Score/Percentile Rank								
Select maximum percentile rank threshold:	50 Position in Protein								
Enter the Job Name (Optional)									
Email address (optional)									
	Submit Reset								

Class II immunogenicity prediction - example

http://tools.iedb.org/CD4episcore/

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CD4 Immunogenicity prediction results

Number of proteins: 1

Number of 15mer (overlapping 10mer): 37

Threshold: 50.0%

Method : combined

Download result 🔳

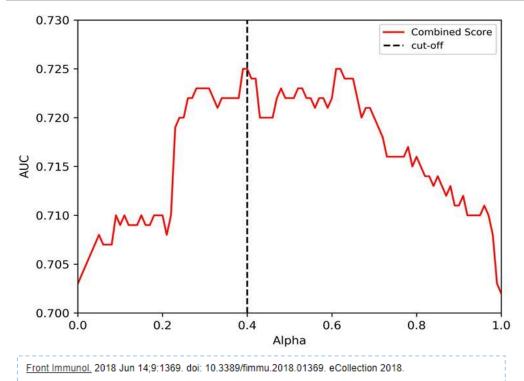
Citations

Protein Number	Protein Description	Peptide ¢	Start ¢	End ¢	Combined Score _¢	Immunogenicity Score	Peptide core	Median Percentile Rank (7- allele)	HLA- DRB1:03:01	HLA- DRB1:07:01	HLA- DRB1:15:01	HLA- DRB3:01:01	HLA- DRB3:02:02	HLA- DRB4:01:010	HLA- DRB5:01:010
1	spIP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	WLLLSLLSLPLGLPV	11	25	42.16452	95.0613	LLSLLSLPL	6.9	25.0	3.2	3.6	73.0	33.0	6.9	6.5
1	spIP01588IEPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	TKVNFYAWKRMEVGQ	71	85	47.39488	67.4872	TKVNFYAWK	34.0	52.0	22.0	15.0	71.0	30.0	65.0	34.0
1	splP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	EPLQLHVDKAVSGLR	116	130	32.55636	43.8909	LHVDKAVSG	25.0	5.4	59.0	40.0	22.0	7.0	38.0	25.0
1	splP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	VSGLRSLTTLLRALG	126	140	44.95964	86.8991	LTTLLRALG	17.0	12.0	17.0	9.3	70.0	20.0	20.0	1.3
1	splP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	SLTTLLRALGAQKEA	131	145	42.78744	69.4686	LLRALGAQK	25.0	47.0	46.0	21.0	89.0	25.0	14.0	1.6
1	spIP01588/EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	PLRTITADTFRKLFR	156	170	46.18064	85.4516	LRTITADTF	20.0	6.1	47.0	40.0	8.0	20.0	20.0	20.0
1	spIP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	TADTFRKLFRVYSNF	161	175	46.66984	44.6746	FRKLFRVYS	48.0	63.0	58.0	23.0	48.0	33.0	53.0	24.0
1	splP01588 EPO_HUMAN Erythropoietin OS=Homo sapiens GN=EPO PE=1 SV=1	RKLFRVYSNFLRGKL	166	180	13.8966	22.4415	FRVYSNFLR	8.2	53.0	8.2	0.12	27.0	4.8	26.0	1.5

Class II immunogenicity prediction scores

- Immunogenicity Score: Derived from the neural network model trained on Immunogenicity data
- HLA Score: Derived from HLA binding affinity using 7-allele method (Paul et. al. 2015).

Combined score = $\alpha \times$ Imm score + $(1 - \alpha) \times$ HLA score.



Predicting HLA CD4 Immunogenicity in Human Populations.

<u>Dhanda SK¹, Karosiene E¹, Edwards L¹, Grifoni A¹, Paul S¹, Andreatta M², Weiskopf D¹, Sidney J¹, Nielsen M^{2,3}, Peters B^{1,4}, Sette A^{1,4}.</u>

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Class II Summary

- Similar to MHC class I, enhancement of epitope prediction efficacy is minor compared to using MHC binding predictions alone
- Prediction of naturally eluted ligands is greatly improved with processing predictions
- As of now, recommendation is to stick to allele specific MHC binding predictions (NetMHCPanII), or the 7-allele method for broad populations

→ Both class I and II epitope predictions are constantly being re-evaluated, and these recommendations are subject to change