

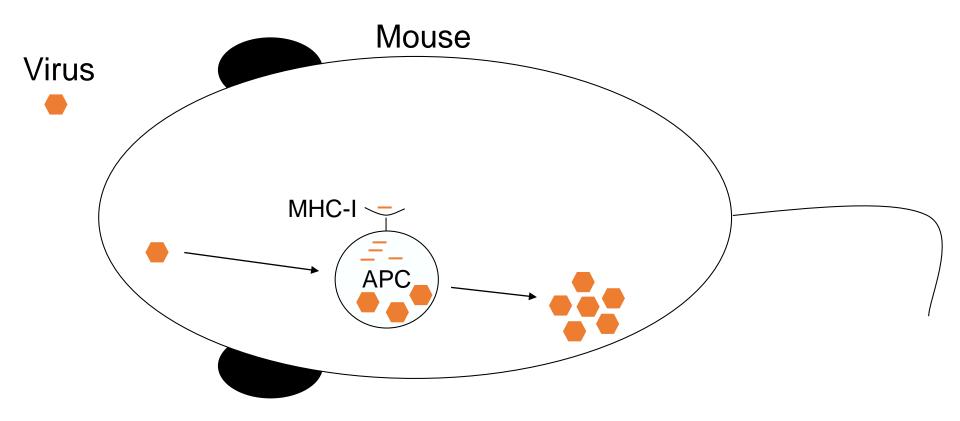
# T Cell processing & immunogenicity predictions

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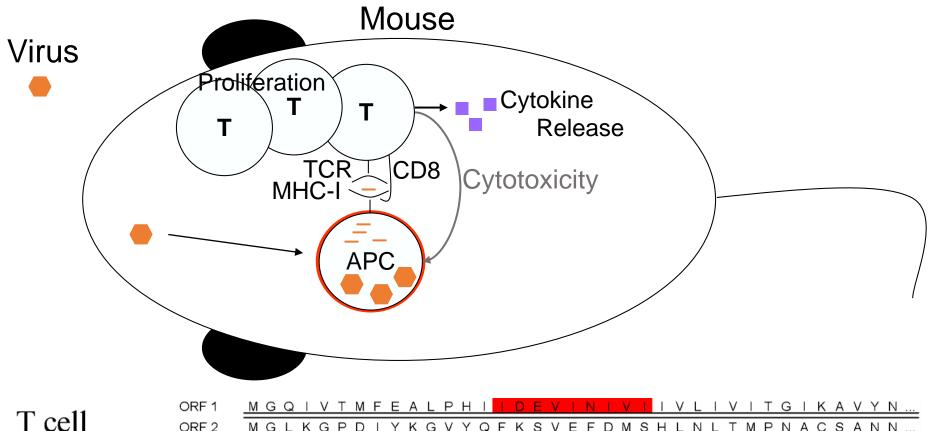
Presented by: Bjoern Peters, PI

2019 IEDB User Workshop

### CD8+ T cell epitopes in viral infection

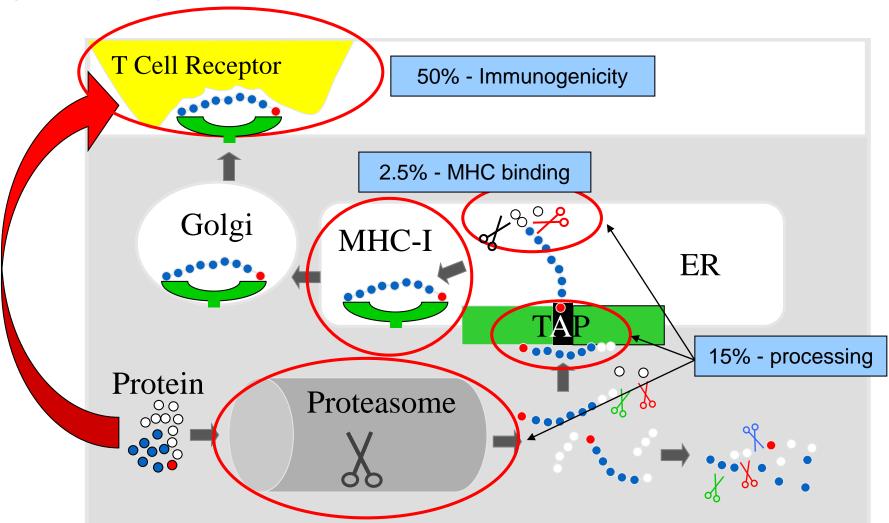


### CD8+ T cell epitopes in viral infection



TT 11	ORF 1	MGQIVIMFEALPHITDEVINIVIIVLIVIIGIKAVYN
T cell	ORF 2	MGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANN
onitono	ORF 3	MHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIDGNSNY
epitope	ORF 4	<u>MSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSD</u>
mapping	ORF 5	MHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLS
mapping	ORF 6	MKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKF
C	ORF 7	MLMRNHLLDLMGVPYCNYSKFWYLEHAKTGETSVPKC

# 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

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### 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	3	
Г Cell Ep	itopes -	Processing	g Prediction								
	_		ites based upon		<u> </u>		ne cell.				_
			ort/MHC class I					inding to produ		ll score for each	
peptid	e's intrinsi	c potential of	f being a T cell	epitope.	iy, iAP i	transport, a		inding to produ		Il score for each	
			f proteasomal cl							<u>*</u>	
			teasomal proce Jence. It also e					CTL and NetCT	Lpan are pro	edictors of T cell	
/HC-NP: F	Prediction o	of peptides na	aturally process	ed by the M	1HC						
MHC-N	IP employs	data obtain	ed from MHC e	lution expe	eriments	in order to	assess th	e probability th	at a given p	peptide is naturally ion in Immunolog	
MHCII-											
	INP.										
		MHC II ligan	d elution data	to predict r	naturally	v processed	MHC II lig	ands by scann	ing the giver	n peptide sequenc	es.
This to	ool utilizes	5			naturally	v processed	MHC II lig	ands by scann	ing the give	n peptide sequenc	es.
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# Class I 'combined predictor'

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	мнс-	-l Pro	ocessi	ng Prec	dictions	•	
	Prediction				013-02-22 [ <mark>Old</mark>		
						Specify	Sequence(s)
	Enter prot (Browse f	iein sequ or seque	ence(s) in FA nces in NCB	ASTA format			
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						TAP transp	port predictions
	Maximum	precurso	or extension	1			
	Alpha fact	tor		0	.2		
						Spec	ify Output
	Output for	mat		2	KHTML table 🔽	·	
							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 form (Browse for sequences in NCBI)	>LCMV Armstrong, Protein GP MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFLLLAGRSCGM YGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHYISMGTSGLELTFTNDSII SHNFCNLTSAFNKKTFDHILMSIVSSLHLSIKGNSNYKAVSCDFNNGITIQYNLTFSDA QSAQSQCRFFRGRVULDMFRTAFGGYVMRSGWGWGTGSDGFTWCSQTSIQVLIIQNRTWE NHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVENFGGYCLTKWMILAAE LKCFGNTAVAKCNVNHDAEFCDMLRLIDVNKAALSKFKEDVESALHLFKTTVNSLISDQ LMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEA DNMITEMLRKDYRQGSTPIALMDLLMFSTSAYLVSIFLHLVKIFTHRHIKGGSCPKP 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 O 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	MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFLLLAGRS CGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHYISMGTSGLELTF TNDSIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITI QYNLTFSDAQSAQSQCRTFRGRVLDMFRTAFGGKYMRSGWGWTGSDGKTTWCSQTS YQYLIIQNRTWENHCTYAGPFGMSRILLSQEKTKFFTRRLAGTFTWTLSDSSGVEN PGGYCLTKWMILAAELKCFGNTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDV ESALHLFKTTVNSLISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCW LVTNGSYLNETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYL VSIFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR
2	LCMV Armstrong, Protein NP	MSLSKEVKSFQWTQALRRELQSFTSDVKAAVIKDATNLLNGLDFSEVSNVQRIMRK EKRDDKDLQRLRSLNQTVHSLVDLKSTSKKNVLKVGRLSAEELMSLAADLEKLKAK IMRSERPQASGVYMGNLTTQQLDQRSQILQIVGMRKPQQGASGVVRVWDVKDSSLL NNQFGTMPSLTMACMAKQSQTPLNDVVQALTDLGLLYTVKYPNLNDLERLKDKHPV LGVITEQQSSINISGYNFSLGAAVKAGAALLDGGNMLESILIKPSNSEDLLKAVLG AKRKLNMFVSDQVGDRNPYENILYKVCLSGEGWPYIACRTSIVGRAWENTTIDLTS EKPAVNSPRAPGAAGPPQVGLSYSQTMLLKDLMGGIDPNAPTWIDIEGRFNDPVE IAIFQPQNGQFIHFYREPVDQKQFKQDSKYSHGMDLADLFNAQPGLTSSVIGALPQ GMVLSCQGSDDIRKLLDSQNRKDIKLIDVEMTREASREYEDKVWDKYGWLCKMHTG IVRDKKKKEITPHCALMDCIIFESASKARLPDLKTVHNILPHDLIFRGPNVVTL

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

#### Citations

Allele 🔶	# <sub>\$</sub>	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	VSTELHLVKT	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

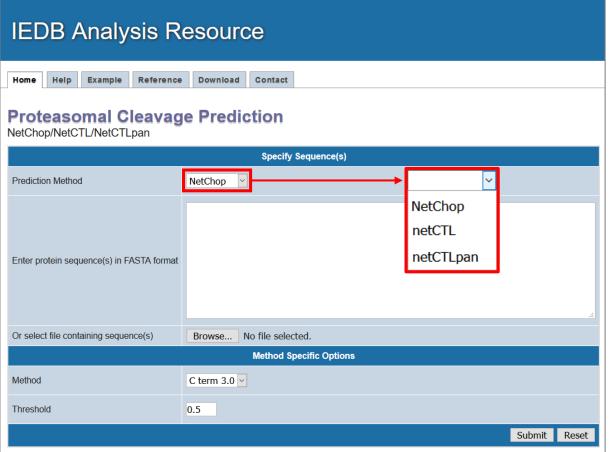
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#### **IEDB** Analysis Resource

Overview	T Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute Tools	References		
T Cell F	nitones -	Processin	g Prediction								
			tes based upon	the proces	sing of p	eptides in th	ne cell.				
			ort/MHC class I			-				<i>c</i> , ,	
l his pepti	tool combin ide's intrinsi	es predictors c potential of	f being a T cell	al processir epitope.	ng, IAP I	transport, a	and MHC b	inding to produc	e an overall	score for each	
								es (NetCTL and			
NetC	hop is a pre	dictor of prot	teasomal proce ience. It also e	essing base	ed upon a	a neural ne	twork. Net	tCTL and NetCTL	pan are pre	dictors of T cell	
		· ·	turally process				incoccure.				
MHC	-NP employs	data obtain	ed from MHC e	lution expe	eriments	in order to	assess th	e probability the	at a given pe	ptide is naturally	
	II-NP:										
		MHC II ligan	d elution data	to predict i	naturally	v processed	MHC II lig	jands by scannii	ng the given	peptide sequence	es.
This	tool utilizes				naturally	v processed	MHC II lig	jands by scannii	ng the given	peptide sequence	es.
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# **Neural Network based predictors**

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



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

### Key difference is the use of NetChop

### NetChop -example

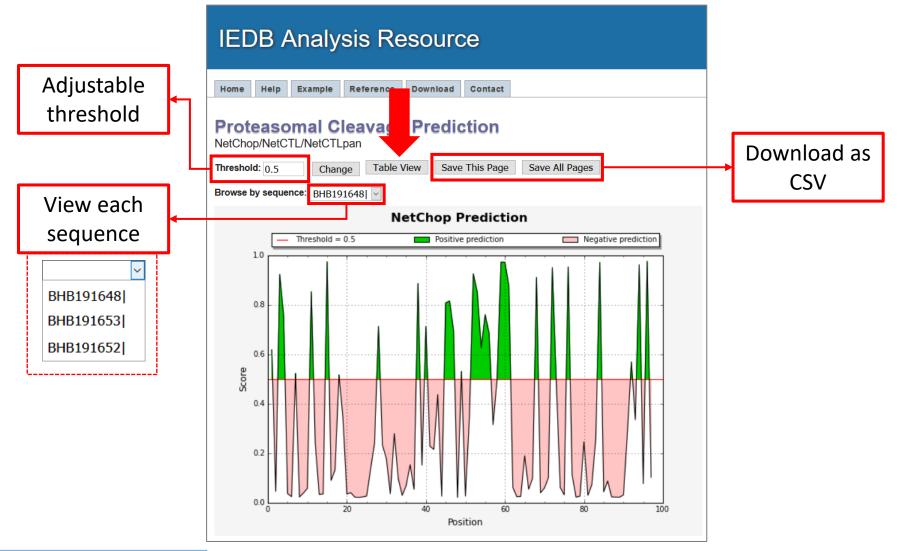
### http://tools.iedb.org/netchop/

#### **IEDB** Analysis Resource Help Example Reference Download Contact Home **Proteasomal Cleavage Prediction** NetChop/NetCTL/NetCTLpan Specify Sequence(s) Prediction Method NetChop >BHB191648|gi:90572034|gb:CY010133|UniProtKB:Q1WPY8|Gene Symbol:M2|Protein Name:Matrix protein 2|Organism:Influenza A Virus A/Canterbury/100/2000|Segment:7|Subtype:H1N1|Host:Human MSLLTEVETPIRNEWGCRCNDSSDPLVVAASIIGIVHLILWIIDRLFSKSIYRIFKHGLKRGPSTEGVPE SMREEYREEQONAVDADDGHFVSIELE Enter protein sequence(s) in FASTA format >BHB191653|gi:90572040|gb:CY010136|UniProtKB:Q1WPY3|Gene Symbol:NS1|Protein Name:Nonstructural protein 1|Organism:Influenza A Virus A/Canterbury/100/2000|Segment:8|Subtype:H1N1|Host:Human MDSHTVSSFOVDCFLWHVRKOVADODLGDAPFLDRLRRDOKSLKGRGSTLGLNIETATCVGKOIVERILK 🗸 EESDEAFKMTMASALASRYLTDMTIEEMSRDWFMLMPKQKVAGPLCVRMDQAIMDKNIILKANFSVIFDR Or select file containing sequence(s) Browse... No file selected. Method Specific Options C term 3.0 v Method C term 3.0 C term 3.0 Threshold 0.5 20S 3.0 Submit Reset

- Predicts C-terminal cleavage based on two approaches
  - **C-term 3.0**: Cterminal residues found for MHC ligands
  - 20S 3.0: Cleavage sites based on in vitro protein digests
- C-term 3.0 is not truly a proteasome predictor but performs better
- NetCTL and NetCTLPan use Cterm 3.0 by default

### NetChop -example

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



### NetChop -example

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

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#	# 🔶 🛛 An	nino acid 🔶	Prediction score \$	
	1	М	0.618935	
	2	S	0.046833	
	3	L	0.923545	
	4	L	0.756254	
	5	Т	0.037353	
	6	E	0.025171	
	7	V	0.523328	
	8	E	0.023849	
	9	т	0.039626	
	10	Р	0.058487	
	11	I.	0.853657	
	12	R	0.239428	
	13	Ν	0.034104	
	14	E	0.036200	
	15	w	0.974923	
	16	G	0.090532	
	17	С	0.132901	
	18	R	0.517519	
	19	С	0.345002	

### References

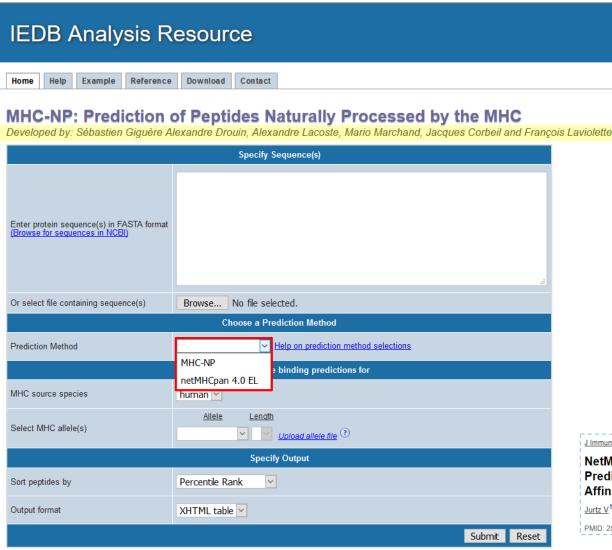
- Peters et al, JMB 2002 (proteasome)
- Peters et al, J Immunol 2003 (TAP)
- Tenzer et al, CMLS, 2005 (combined)
- Nielsen, Immunogenetics, 2005 (NetChop)
- Larsen, BMC Bioinformatics, 2007 (NetCTL)
- Stranzl, Immunogenetics, 2010 (NetCTLPan)

### 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 Tool	s Reference	S	
Cell E	Epitopes -	Processing	g Prediction								
			tes based upon				ne cell.				
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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!

i 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 V<sup>1</sup>, Paul S<sup>2</sup>, Andreatta M<sup>3</sup>, Marcatili P<sup>1</sup>, Peters B<sup>2</sup>, Nielsen M<sup>4,3</sup>

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

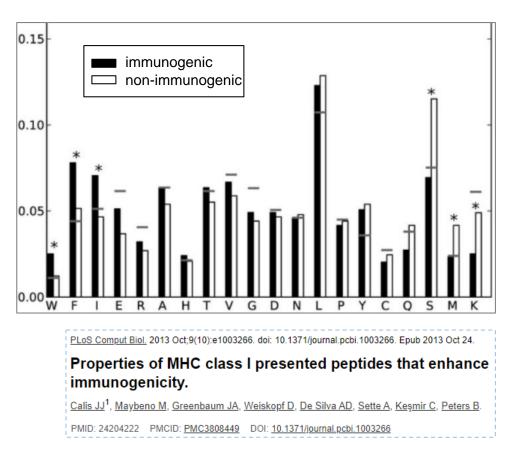
### Class I Processing + immunogenicity tools available in the IEDB <u>http://tools.iedb.org/main/tcell/</u>

#### **IEDB** Analysis Resource

Overview	T Cell Tools	B Cell Tools	Analysis Tools	Tools-API	Usage	Download	Datasets	Contribute To	ols	References			
	Initopos	Processin	n Prodiction										
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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/

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Class I Immun	ogenicity	
	Specify sequence(s) *	
Enter peptide sequence(s) (Browse for sequences in NCBI)	FIAGLIAIV LITGRLQSL RLMEVAKNL 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

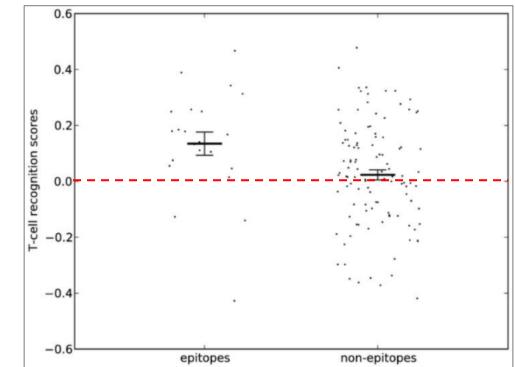
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IEDB Analysis Resource									
Home Help Example Reference Download Contact									
Class Masking: def Masked varia Peptide \$	ault		genicit	У					
FIAGLIAIV	9	0.27206							
KAVYNFATC	9	0.16928							
RLNEVAKNL	9	-0.0101							
LITGRLQSL 9 -0.10776									
FQPQNGQFI	9	-0.12392							
Download I	result 🗷								

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

# Class I immunogenicity prediction caveats / performance

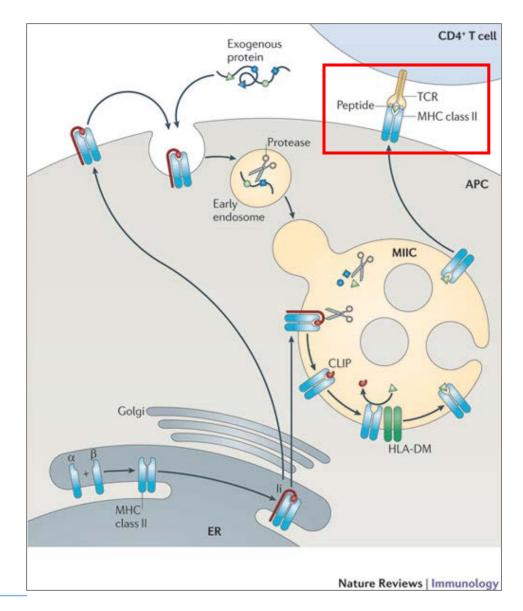
- Experimentally, many MHC binding peptides can be immunogenic (~50%)
- Cross validation gave AUC values ~ 0.65. Test on independent blind set gave AUC = 0.69
- Recommendation: Use as filter (cutoff 0) if high specificity is desired. Suggested cutoff is 0



### **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>

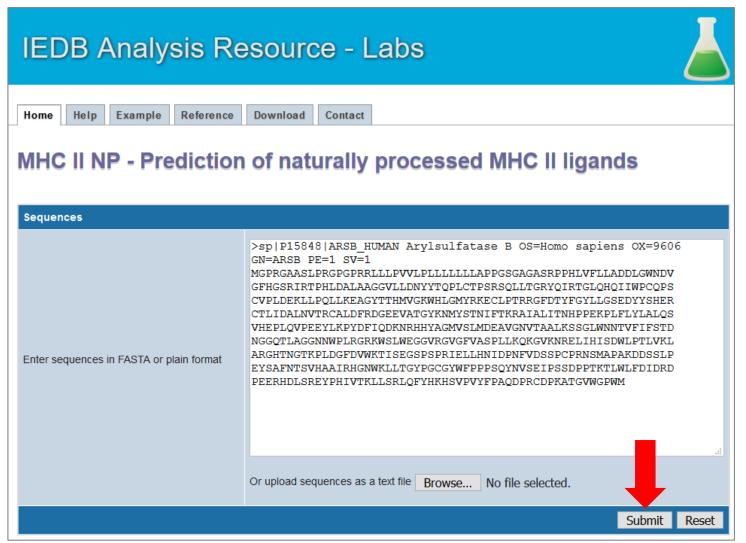
#### **IEDB** Analysis Resource

### 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/

### **IEDB Analysis Resource - Labs**

[	

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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	SP P15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	510	524	15	VPVYFPAQDPRCDPK	SVP	РКА	1.75814	0.00			
2	SP P15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	2	16	15	GPRGAASLPRGPGPR	MGP	PRR	1.73735	0.02			
3	SP P15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	247	261	15	VPEEYLKPYDFIQDK	QVP	DKN	1.48840	0.04			
4	SP P15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	384	398	15	SPSPRIELLHNIDPN	GSP	PNF	1.40420	0.05			
5	SP P15848 ARSB_HUMAN ARYLSULFATASE B OS=HOMO S	12	26	15	GPGPRRLLLPVVLPL	RGP	PLL	1.33714	0.07			
<u>Cor</u>	Complete 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

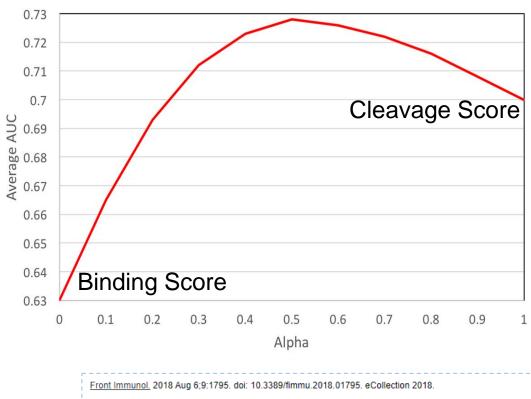
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#### RESEARCH

Footprints of antigen processing boost MHC class II natural ligand predictions

Carolina Barra<sup>1+</sup>©, Bruno Alvarez<sup>1+</sup>, Sinu Paul<sup>2</sup>, Alessandro Sette<sup>2</sup>, Bjoern Peters<sup>2</sup>, Massimo Andreatta<sup>1</sup>, Søren Buus<sup>3</sup> and Morten Nielsen<sup>1,4\*</sup> Combined score =  $\alpha \times$  cleavage probability score +  $(1 - \alpha) \times$  binding score



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

 $\underline{\mathsf{Paul S}^1}, \underline{\mathsf{Karosiene E}^1}, \underline{\mathsf{Dhanda SK}^1}, \underline{\mathsf{Jurtz V}^2}, \underline{\mathsf{Edwards L}^1}, \underline{\mathsf{Nielsen M}^{2,3}}, \underline{\mathsf{Sette A}^{1,4}}, \underline{\mathsf{Peters B}^{1,4}}.$ 

PMID: 30127785 PMCID: PMC6087742 DOI: 10.3389/fimmu.2018.01795

### 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 C	Analysis Tools	Tools-API Usage	Download Da	atasets	Contribute Tools	References		
T Cell Epitopes - Pro	cessing Prediction	า						
These tools predict epitope			peptides in the c	cell.				
Proteasomal cleavage/TAF								
This tool combines pr peptide's intrinsic pot	edictors of proteasom ential of being a T cel	nal processing, TA Il epitope.	o transport, and	MHC bi	nding to produc	e an overall	score for each	
Neural network based pred	liction of proteasomal	cleavage sites (Ne	Chop) and T cel	l epitope	es (NetCTL and I	NetCTLpan)		
NetChop is a predicto epitopes along a prot					CTL and NetCTL	pan are pre	lictors of T cell	
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### 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										
	Specify Sequence(s)									
Specify Sequence(s)         >sp P01588 EP0_HUMAN Erythropoietin OS=Homo sapiens GN=EP0 PE=1 SV=1         MGVHECPAWLWLLS_LLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHC         SLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL         HVDKAVSGLRS.LTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKL         KLYTGEACRTGDR										
Or upload epitope sequence(s) from a file	Browse No file selected.									
	Choose a prediction method									
Prediction method:	IEDB recommended (combined)									
Sort Peptides by:	Position in Protein Score/Percentile Rank									
Select maximum percentile rank threshold:	50 V Position in Protein									
Enter the Job Name (Optional)										
Email address (optional)										
	Submit Reset									

### Class II immunogenicity prediction -example

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

#### IEDB Analysis Resource - Labs

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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 🔳

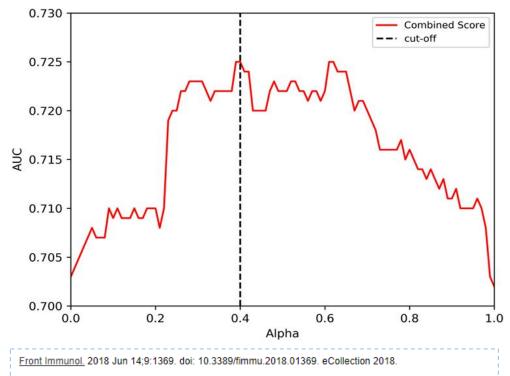
#### Citations

Protein Number	Protein Description	Peptide ¢	Start \$	End ¢	Combined Score <sub>\$</sub>	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- DRB4:01:01	HLA- DRB5:01:0 <u>1</u>
1	splP01588 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	splP01588 EPO_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	splP01588 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	splP01588 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.

Dhanda SK<sup>1</sup>, Karosiene E<sup>1</sup>, Edwards L<sup>1</sup>, Grifoni A<sup>1</sup>, Paul S<sup>1</sup>, Andreatta M<sup>2</sup>, Weiskopf D<sup>1</sup>, Sidney J<sup>1</sup>, Nielsen M<sup>2,3</sup>, Peters B<sup>1,4</sup>, Sette A<sup>1,4</sup>.

PMID: 29963059 PMCID: PMC6010533 DOI: 10.3389/fimmu.2018.01369

### **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