

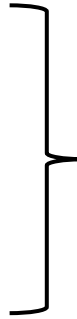


# MHC Binding Predictions

[tools.iedb.org](https://tools.iedb.org)

Presented by: Sinu Paul, Bioinformatics Scientist

# Outline

- MHC class I binding prediction
  - MHC class II binding prediction
  - TepiTool
  - Datasets availability
  - Benchmarking of class I tools
  - Contributing tools
- 
- How the tool works
  - Recommendations
  - Interpreting results
  - Exercises

# MHC binding predictions

- MHC molecules are **highly polymorphic** – thousands of different variants exist
- MHC-peptide binding is **promiscuous** in nature
- Experimental characterization of peptide–MHC interactions is highly **cost-intensive**
- Prediction methods facilitate selection of potential epitopes from a pool of peptides

## Peptide binding data HLA-A\*01:01

Peptide	IC <sub>50</sub> (nM)
ASFCGSPY	51.4
LTDFGLSK	739.3
FTSFFYRY	1285.0
KSVFNSLY	1466.0
RDWAHNSL	1804.6
FSSCPVAY	1939.4
RNWAHSSL	2201.7
LSCAASGF	2830.1
LASIDLKY	3464.0

+

## Machine learning algorithms



# MHC I Binding Prediction

[tools.iedb.org/main/tcell/](https://tools.iedb.org/main/tcell/)

## IEDB Analysis Resource

Overview T Cell Tools B Cell Tools Analysis Tools Tools-API Usage Download Datasets Contribute Tools References

### T Cell Epitope Prediction Tools

#### T Cell Epitopes - MHC Binding Prediction

These tools predict IC50 values for peptides binding to specific MHC molecules. Note that binding to MHC is necessary but not sufficient for recognition by T cells.

##### [Peptide binding to MHC class I molecules](#)

This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

##### [Peptide binding to MHC class II molecules](#)

This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.

##### [TepiTool:](#)

The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per your specific needs. You can go back to previous steps to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.

#### 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](#).



##### [MHCII-NP:](#)

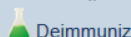
This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences.

#### T Cell Epitopes - Immunogenicity Prediction

This tool predicts the relative ability of a peptide/MHC complex to elicit an immune response.

##### [T cell class I pMHC immunogenicity predictor](#)

This tool uses amino acid properties as well as their position within the peptide to predict the immunogenicity of a class I peptide MHC (pMHC) complex.

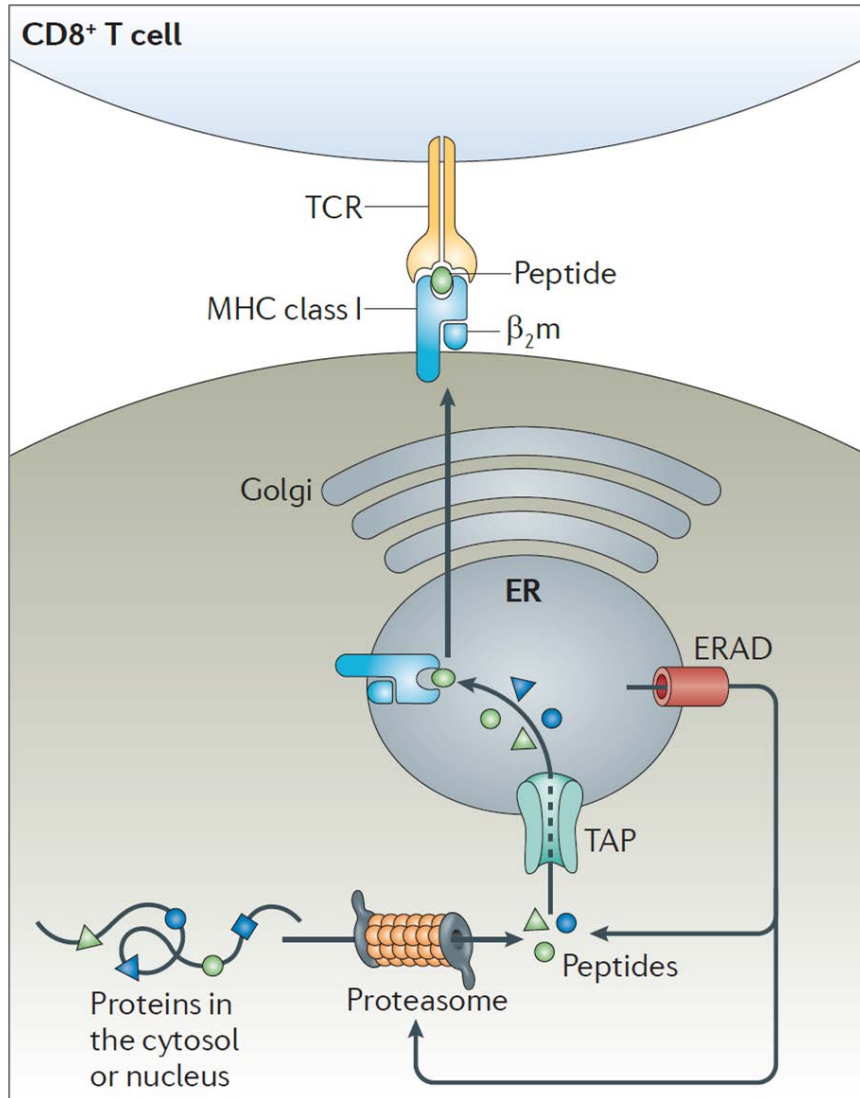


##### [Deimmunization:](#)

The deimmunization tool is attempt to identify immunodominant regions in a given therapeutically important protein, and suggest amino-acid substitutions that create non-immunogenic versions of the proteins. So we have opted a two steps process; 1) In the first step, the deimmunization tool will list all the immunogenic regions or peptides based on selected threshold. These peptides will be generated from the protein with 15mer window size and 10mer overlap. 2) In the second step, the user can select one or more



# Endogenous antigen processing pathway (class I)



- Antigens generated within the cell
  - Viral particles
  - Self proteins
  - DRiPs (Defective Ribosomal Particles)
- Different factors influence peptide being “epitope”

[Nat Rev Immunol. 2011 Nov 11;11\(12\):823-36. doi: 10.1038/nri3084.](#)

**Towards a systems understanding of MHC class I and MHC class II antigen presentation.**

[Neefjes J<sup>1</sup>, Jongsma ML, Paul P, Bakke O.](#)

PMID: 22076556 DOI: [10.1038/nri3084](#)

# Class I MHC molecule

- Expressed by almost all nucleated cells
- Presents antigen to **CD8+ T cells** (Cytotoxic T cells)
- One MHC encoded polymorphic chain ( $\alpha$ ) (2nd chain –  $\beta$ 2-microglobulin)
- The binding groove is **closed** at both ends and can accommodate peptides of **8-15 AA**
- Only  **$\alpha$  chain** impacts binding

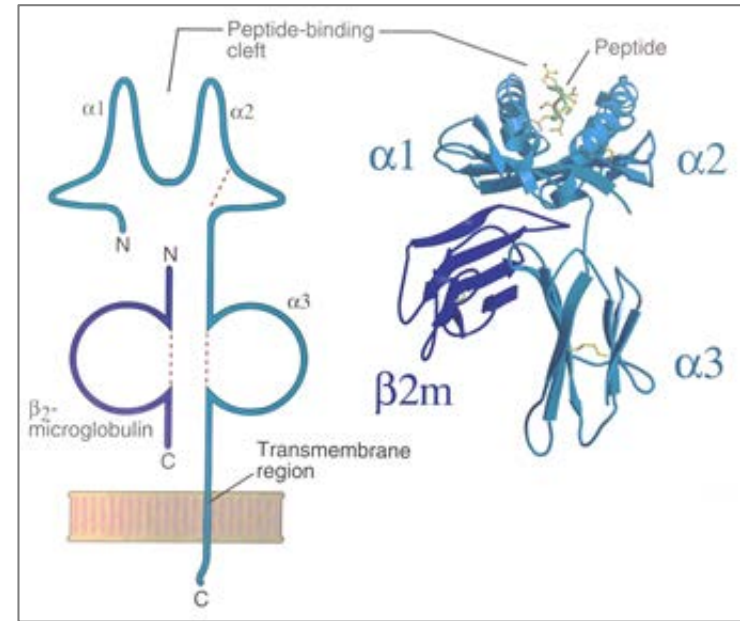


Figure Source  
*Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman*

# MHC-I binding prediction - example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Predictions

Prediction Method Version 2013-02-22 [\[Older versions\]](#)

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences.  
[\(Browse for sequences in NCBI\)](#)

```
>LCMV Armstrong, Protein GP
MGQIVTMFEALPHIIDVINIVIIIVLIVITGKAVYNFATCGIFALISFLLLAGRSCGM
YGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSAANSHHYISMGTSGLELFTNDSII
SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSDA
QSAQSQRCRTFRGRVLDMFRTAFGGKYMRSWGWTGSDGKTTWCSQTSYQYLIIQNRTWE
NHCTYAGPFGMSRILLSQEKTKFFRRLAGFTWTLSDSSGVENPGGYCLTKWMILAAE
LKCFGNTAVAKCNVNHDAEFCMDMLRLIDYNKAALSKFKEDVESALHLFKTTVNSLISDQ
LLMRNHLRDLMGVPPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSQDIEQEA
DNMITEMLRDYIKRQGSTPLALMDLLMFSTAYSIVSIFLHLVKIPTRHRHKGGSCKPK
HRLTNKGICSCGAFKVPGVKTVWKR
```

Or select file containing sequence(s) Choose File No file chosen

Epitope  
sequence  
(copy or upload)

### How to obtain FASTA sequences for a given organism

1. On the [taxonomy browser](#) page (by default, the taxonomy browser here is set to start at the virus level), click on the red number following an organism name to view the protein sequences available in NCBI. To go to a specific taxonomic level, click on the organism name. To go to the highest taxonomic level, click on the [root](#) link.
2. On the protein sequence page, select "FASTA" in the "Display" selection list. By default, only 20 sequences are displayed on one page. To view more sequences, select the appropriate number on the "Show" selection list. Next, change the "Send to" to "Text". Finally, copy and paste the sequences into the MHC-I or MHC-II-binding tool.



NCBI Taxonomy Browser

Search for Viruses as complete name lock Go Clear

Display 3 levels using filter: none

- Nucleotide
- Protein
- Structure
- Genome
- Popsit
- SNP
- Conserved Domains
- GEO Datasets
- PubMed Central
- Gene
- HomoloGene
- SRA Experiments
- LinkOut
- BLAST
- GEO Profiles
- Protein Clusters
- Identical Protein Groups
- SPARCLE
- Bio Project
- Bio Sample
- Bio Systems
- Assembly
- dbVar
- Genetic Testing Registry
- Host
- Viral Host
- Probe
- PubChem
- BioAssay

- o [Viruses](#) [LinkOut 5,769,963](#) *Click on organism name to get more information.*
  - o [Adenoviridae](#) [LinkOut 50,516](#)
    - [Atadenovirus](#) [LinkOut 1,205](#)
      - o [Bovine atadenovirus D](#) [LinkOut 115](#)
      - o [Deer atadenovirus A](#) [275](#)
      - o [Duck atadenovirus A](#) [LinkOut 307](#)
      - o [Lizard atadenovirus A](#) [75](#)
      - o [Ovine atadenovirus D](#) [LinkOut 69](#)
      - o [Possum atadenovirus A](#) [LinkOut 8](#)

# MHC-I binding prediction - example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Predictions

Prediction Method Version: 2013-02-22 [\[Older versions\]](#)

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [\(Browse for sequences in NCB\)](#)

```
>LCMV Armstrong, Protein GP
MGQIVTMFEALPHIIDEVINIIVLIVITGKAVYNFATCGIFALISFLLLAGRSCGM
YGLKGPDIYQKGVYQFQKSVFEDMSHLNLTMPNACSAANSHHYISMGTSGLELFTNDSII
SHNFCNLTSAFNKKTDFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIYNLTFSDA
QSAQSQCRTRFRGRVLDMFRTAFFGGKYMRSQWGWWTGSDGKTTWCSQTSYQYLIQNRTWE
NHCTYAGPFGMSRILLSQEKTFFTRRLAGTFTWTLSDSSGVENPGGYCLTKWWMILAAE
LKCFGNTAVAKCNVNHDAEFCMDMLRLIDYNKAALSKFKEDVESALHLFKTTVNSLISDQ
LLMRNHLRDLMGVPCNYSKFWYLEHAKTGETSVPKCWLVNNGSYLNETHFSDQIEQEA
DNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAVLVSIFLHLVKIPIHRHIKGGSCP KP
HRLTNKGICSCGAFKVPVGVKTVWKR
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

**Choose a Prediction Method**

Prediction Method <sup>?</sup>  
Show all the method versions:

MHC source species

Show only frequently occurring alleles:  <sup>?</sup>  
Select MHC allele(s)

Select HLA allele reference set:  <sup>?</sup>

Sort peptides by

Show

Output format

Email address (optional)  <sup>?</sup>

**IEDB recommended 2.22** <sup>?</sup> [Help on prediction method selections](#)

- IEDB recommended 2.22
- IEDB recommended 2.19
- IEDB recommended 2.18
- Consensus
- NetMHCpan EL 4.0
- NetMHCpan BA 4.0
- NetMHCpan 2.8
- NetMHCpan 3.0
- ANN 4.0
- ANN 3.4
- SMPMBEC
- SMM
- CombLib\_Sidney2008
- PickPocket
- netMHCcons
- netMHCstabpan

Prediction method



# MHC class I binding prediction methods available

Method	Reference	Performance Reported
Consensus	Moutaftsi et al., 2006	
NetMHCpan-4.0	Jurtz et al., 2017	0.960 AUC (average)
NetMHCpan-3.0	Nielsen & Andreatta, 2016	0.890 AUC (average)
ANN (NetMHC-4.0)	Andreatta & Nielsen, 2016	0.887 AUC (average)
SMM with Peptide:MHC Binding Energy Covariance matrix (SMMPMBEC)	Kim et al., 2009	0.894 AUC (average)
Stabilized matrix method (SMM)	Peters & Sette, 2005	0.887 AUC (average) (Kim et. al., 2009)
Combinatorial library (CombLib)	Sidney et al., 2008	0.909 AUC (HLA-A*0201)
PickPocket-1.1	Zhang et al., 2009	0.895 AUC (average)
NetMHCcons-1.1	Karosiene et al., 2012	0.729 PCC (average)
NetMHCstabpan-1.0	Rasmussen et al., 2016	0.980 AUC (average)

# Guidelines: Choosing the prediction method

- Suggested method = “IEDB recommended”
  - Employs Consensus (Combination of ANN, SMM & CombLib) or NetMHCpan depending on the allele
  - Provides binding affinity & percentile rank for each method separately as well
- Recommendation will change with the new benchmark studies

# MHC-I binding prediction – example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Predictions

Prediction Method Version: 2013-02-22 [\[Older versions\]](#)

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [\(Browse for sequences in NCBI\)](#)

```
>LCMV Armstrong, Protein GP
MGQIVTMFEALPHIIDEVINIVIVLITVIGIKAVYNFATCGIFALISFLLLAGRSCGM
YGLKGPDIYKGVYQFKSVFEDMSHLNLTMPNACSAANSHHYISMGTSGLELTFNDSII
SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSDA
QSAQSQCRTRFRGRVLDMFRTAFGGKYMRSWGWTGSDGKTTWCSQTSYQYLIQNRWE
NHCTYAGPFGMSRILLSQEKTFFTRRLAGTFTWTLSDSGVENPGGYCLTKWMILAAE
LKCFGNTAVAKCNVNHDAEFCMDMLRLIDYNKAALSFKFEDVESALHLFKTTVNSLISDQ
LLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVNNGSYLNETHFSDQIEQEA
DNMITEMLRKDYIKRQGSTPLALMDLLMFSTASLVLSIFLHLVKIPTRHIKGGSCPKP
HRLTNKGICSCGAFKVPGVKTVWKR
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

**Choose a Prediction Method**

Prediction Method <sup>?</sup> IEDB recommended 2.22 [Help on prediction method selections](#)  
Show all the method versions:

**Specify what to make binding predictions for**

MHC source species  ← Choose species

Show only frequently occurring alleles:  <sup>?</sup>  
Select MHC allele(s)  [Upload allele file](#) <sup>?</sup>  
[Select HLA allele reference set](#):  <sup>?</sup>

**Specify Output**

Sort peptides by

Show

Output format

Email address (optional)  <sup>?</sup>

Choose species

# MHC-I binding prediction – example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Predictions

Prediction Method Version 2013-02-22 [Older versions](#)

### Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [\(Browse for sequences in NCBI\)](#)

```
>LCMV Armstrong, Protein GP
MGQIVTMFEALPHIIDVINIIVLIVITGIKAVYNFATCGIFALISFLLAGRSCGM
YGLKGPDIYKGVYQFKSVEFDMShLNLTMPNACSANNHHYISMGTSGLELFTNDSII
SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITIQYNLTFSDA
QSAQSQCRTRFRGRVLDMFRATFGGKYMRSGWGWGTGSDGKTTWCSTSYQYLIQNRTWE
NHCTYAGPFGMSRILLSQEKTFFTRRLAGFTFTWTLSDSSGVENPGGYCLTKWMILAAE
LKCFGNTAVAKCNVNHDAEFCMDMLRLIDYNKAALSFKFEDVESALHLFKTTVNSLISDQ
LLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEA
DNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYLVSIFLHLVKIPTRHIKGGSCP
HRLTNKGICSCGAFKVPGVKTVVWRRR
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

### Choose a Prediction Method

Prediction Method  IEDB recommended 2.22 [Help on prediction method selections](#)

Show all the method versions:

### Specify what to make binding predictions for

MHC source species

Allele	Length	
HLA-A*01:01	9	<input type="checkbox"/>
HLA-B*07:02	10	<input type="checkbox"/>

Show only frequently occurring alleles

Select MHC allele(s)

Select HLA allele reference set

### Specify Output

Sort peptides by

Show

Output format

Email address (optional)

Complete set

Reference alleles

Specify allele(s) & peptide length (select or upload)

Upload format:  
HLA-A\*01:01,9  
HLA-B\*07:02,10

# Natural length distribution in epitope prediction

- Alleles differ in their preference for lengths on binding and presentation of peptides

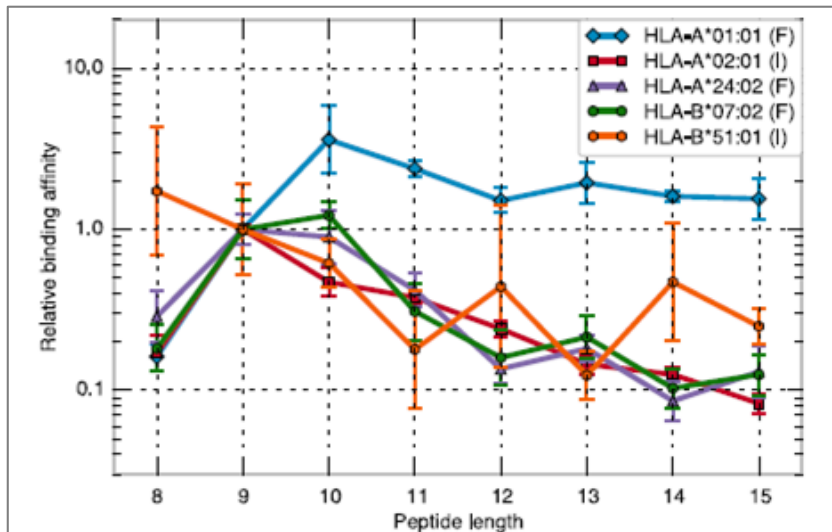


FIGURE 1. Peptide binding-length preference for five common HLA alleles. The length preference for each HLA was determined by measuring

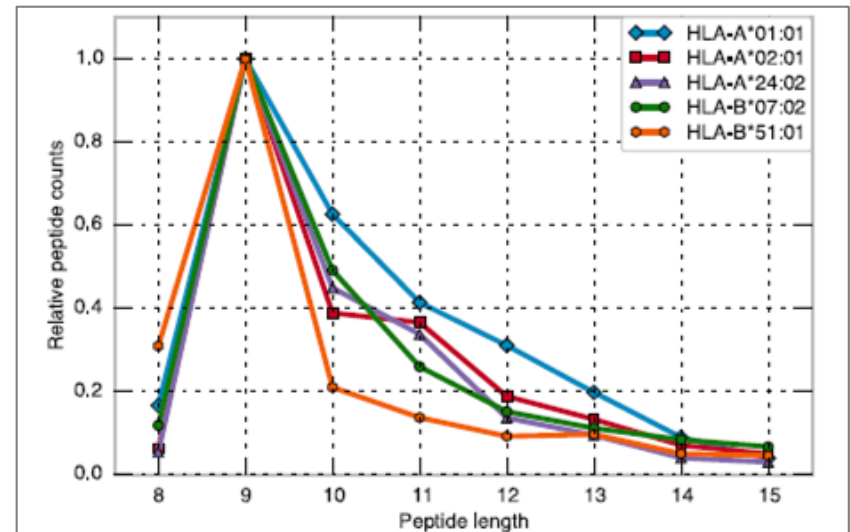


FIGURE 2. Length profiles of naturally presented peptides for five HLA molecules. Large datasets of HLA-I ligands were determined by the elu-

*J. Immunol.* 2016 Feb 15;196(4):1480-7. doi: 10.4049/jimmunol.1501721. Epub 2016 Jan 18.

**The Length Distribution of Class I-Restricted T Cell Epitopes Is Determined by Both Peptide Supply and MHC Allele-Specific Binding Preference.**

Trolle T<sup>1</sup>, McMurtrey CP<sup>2</sup>, Sidney J<sup>3</sup>, Bardet W<sup>2</sup>, Osborn SC<sup>2</sup>, Kaever T<sup>3</sup>, Sette A<sup>3</sup>, Hildebrand WH<sup>2</sup>, Nielsen M<sup>4</sup>, Peters B<sup>5</sup>.

PMID: 26783342 PMCID: PMC4744552 DOI: 10.4049/jimmunol.1501721

# Allele selection – Reference set for global coverage

- Reference set of 27 alleles
- Covers > 97% of population

HLA-A	Frequency	HLA-B	Frequency
A*01:01	16.2	B*07:02	13.3
A*02:01	25.2	B*08:01	11.5
A*02:03	3.3	B*15:01	5.2
A*02:06	4.9	B*35:01	6.5
A*03:01	15.4	B*40:01	10.3
A*11:01	12.9	B*44:02	9.2
A*23:01	6.4	B*44:03	7.6
A*24:02	16.8	B*51:01	5.5
A*26:01	4.7	B*53:01	5.4
A*30:01	5.1	B*57:01	3.2
A*30:02	5.0	B*58:01	3.6
A*31:01	4.7		
A*32:01	5.7		
A*33:01	3.2		
A*68:01	4.6		
A*68:02	3.3		

<http://iedb.zendesk.com/entries/25054538-HLA-allele-frequencies>

# Prediction method dependent allele selection

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

NetMHCpan prediction methods allow FASTA sequence input

Choose a Prediction Method

Prediction Method <sup>?</sup> NetMHCpan EL 4.0 [Help on prediction method selections](#)

Show all the method versions:

Specify what to make binding predictions for

MHC source species human

Input FASTA sequence [\(Select MHC allele\(s\)\)](#)

Paste a single full length MHC protein sequence in [FASTA](#) format:

Peptide length: --choose--

# MHC-I binding prediction – example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Predictions

Prediction Method Version 2013-02-22 [Older versions](#)

### Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [Browse for sequences in NCBI](#)

```
>LCMV Armstrong, Protein GP
MGQIVTMFEALPHIIDEVINIIVLIVITGIKAVYNFATCGIFALISFLLLAGRSCGM
YGLKGPDIYKGVYQFQSVEFDMSHLNLTMPNACSANNHHYISMGTSGLELFTNDSII
SHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIRGNSNYKAVSCDFNNGITQYNLTFSDA
QSAQSQCRTRFRGRVLDMFRTAFGGKYMRSWGWTGSDGKTTWCQSQTSYQLIQRNRTWE
NHCTYAGPFGMSRILLSQEKTFFTRRLAGFTFTWTLSDSSGVENPGGYCLTKWMILAAE
LKCFGNTAVAKCNVNHDAEFCMDLRLIDYNKAALSFKFEDVESALHLFKTTVNSLISDQ
LLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYLNETHFSDQIEQEA
DNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYLVSIFLHLVKIPTRHIKGGSCPKE
HRLTNKGICSCGAFKVPGVKTVVWRR
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

### Choose a Prediction Method

Prediction Method [?](#) IEDB recommended 2.22 [Help on prediction method selections](#)  
Show all the method versions:

### Specify what to make binding predictions for

MHC source species human

Allele	Length	
HLA-A*01:01	9	<input type="checkbox"/>
HLA-B*07:02	10	<input type="checkbox"/>

Show only frequently occurring alleles:  [?](#)  
Select MHC allele(s)  
[Select HLA allele reference set](#):  [?](#)  
  [Upload allele file](#) [?](#)

### Specify Output

Sort peptides by

Show

Output format

Email address (optional)

Input

Output



# How the tool works

- Breaks the sequence into all possible peptides (of chosen length).
- Predicts the binding affinity for each peptide based on the method.
- Compares the predicted affinity to that of a large set of randomly selected peptides.
- Assigns a percentile rank depending on individual predicted affinity.
- Consensus picks the median rank of the methods used.

# MHC-I binding prediction – example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

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## MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	LCMV Armstrong, Protein GP	MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFL LLAGRSCGMYGLKGPDIYKGVYQFKSVFEFMSHLNLTMPNACSAANSHHY ISMGTSGLLELTFNDSIIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIR GNSNYKAVSCDFNNGITIQYNLTFSDAQAQSQCRTRFRGRVDMFRTAFG GKYMRSQGWGTGSDGKTTWCSQTSYQYLIIQNRTWENHCTYAGPFGMSRI LLSQEKTFFFRRLAGTFTWTLSDSSGVENPGGYCLTKWMILAAELKCFG NTAVAKCNVNHDAEFCDFMLRLIDYNKAALSKFKEDVESALHLFKTTVNSL ISDQLMRNHLRDLMGVPYPCNYSKFWYLEHAKTGETSVPKCWLVTNGSYL NETHFSQDIEQEAADNMIETMLRKDYIKRQGSTPLALMDLLMFSTAYSLS IFLHLVKIPTHRIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKR

Prediction method: **IEDB recommended 2.22** | **Low Percentile Rank = good binders**  
[Download result](#)

Citations  
 Check to expand the result:

Allele	#	Start	End	Length	Peptide	Method used	Percentile Rank
HLA-A*01:01	1	92	100	9	CSANNSHHY	Consensus (ann/smm)	0.2
HLA-A*01:01	1	405	413	9	FSDQIEQEA	Consensus (ann/smm)	0.34
HLA-B*07:02	1	471	480	10	KPHRLTNKGI	Consensus (ann/smm)	0.35
HLA-A*01:01	1	417	425	9	ITEMLRKDY	Consensus (ann/smm)	0.6
HLA-A*01:01	1	361	369	9	LRDLMGVPY	Consensus (ann/smm)	0.68
HLA-A*01:01	1	233	241	9	RTWENHCTY	Consensus (ann/smm)	0.69
HLA-A*01:01	1	217	225	9	TTWCSQTSY	Consensus (ann/smm)	0.71
HLA-A*01:01	1	439	447	9	LLMFSTSAY	Consensus (ann/smm)	0.75
HLA-A*01:01	1	147	155	9	LSIRGNSNY	Consensus (ann/smm)	1.25
HLA-B*07:02	1	425	434	10	YIKRQGSTPL	Consensus (ann/smm)	1.27
HLA-B*07:02	1	243	252	10	GPFMGSRILL	Consensus (ann/smm)	1.35
HLA-A*01:01	1	191	199	9	VLDMFRTAF	Consensus (ann/smm)	1.6

Input sequence

Output  
 (sorted low-to-high by percentile rank)

A **percentile rank** for a peptide is the percentage of randomly sampled peptides scoring better than the peptide.

# MHC-I binding prediction – example

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

Individual scores for different methods

Prediction method: IEDB recommended 2.22 | Low Percentile Rank = good binders

[Download result](#) 

## Citations

Check to expand the result:

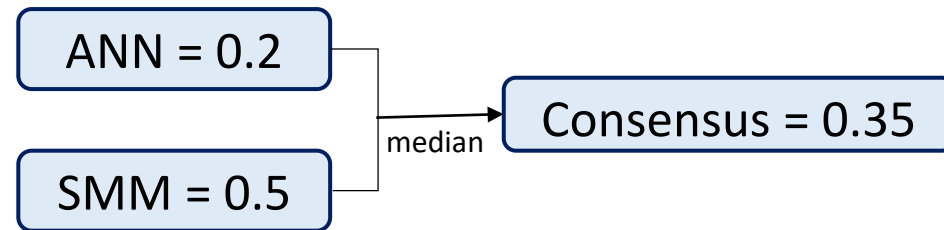
Allele	#	Start	End	Length	Peptide	Method used	Percentile Rank	ANN IC50(nM)	ANN rank	SMM IC50(nM)	SMM rank
HLA-A*01:01	1	92	100	9	CSANNSHHY	Consensus (ann/smm)	0.2	25.62	0.09	173.60	0.3
HLA-A*01:01	1	405	413	9	FSDQIEQEA	Consensus (ann/smm)	0.34	121.15	0.27	360.21	0.4
HLA-B*07:02	1	471	480	10	KPHRLTNKGI	Consensus (ann/smm)	0.35	46.84	0.2	112.67	0.5
HLA-A*01:01	1	417	425	9	ITEMLRKDY	Consensus (ann/smm)	0.6	591.06	0.71	426.14	0.5
HLA-A*01:01	1	361	369	9	LRDLMGVPY	Consensus (ann/smm)	0.68	799.14	0.85	421.26	0.5
HLA-A*01:01	1	233	241	9	RTWENHCTY	Consensus (ann/smm)	0.69	552.60	0.68	694.30	0.7
HLA-A*01:01	1	217	225	9	TTWCSQTSY	Consensus (ann/smm)	0.71	604.36	0.72	653.96	0.7
HLA-A*01:01	1	439	447	9	LLMFSTSAY	Consensus (ann/smm)	0.75	724.33	0.8	728.70	0.7
HLA-A*01:01	1	147	155	9	LSIRGNSNY	Consensus (ann/smm)	1.25	3116.42	2.0	448.28	0.5
HLA-B*07:02	1	425	434	10	YIKRQGSTPL	Consensus (ann/smm)	1.27	59.83	0.24	575.20	2.3
HLA-B*07:02	1	243	252	10	GPFGMSRILL	Consensus (ann/smm)	1.35	418.14	1.2	351.41	1.5
HLA-A*01:01	1	191	199	9	VLDMFRTAF	Consensus (ann/smm)	1.6	2586.86	1.8	1457.30	1.4
HLA-A*01:01	1	174	182	9	FSDAQAQS	Consensus (ann/smm)	1.75	2437.12	1.7	1934.42	1.8



# Consensus (ann/smm)

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

- Requires that both methods give predictions on the same scale – percentile ranks

Nat Biotechnol. 2006 Jul;24(7):817-9. Epub 2006 Jun 11.  
**A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus.**  
Moutafsi M<sup>1</sup>, Peters B, Pasquetto V, Tschärke DC, Sidney J, Bui HH, Grey H, Settle A.  
PMID: 16767078 DOI: 10.1038/nbt1215



Prediction method: IEDB recommended 2.22 | Low Percentile Rank = good binders  
[Download result](#)  



Citations  
Check to expand the result:





Allele	#	Start	End	Length	Peptide	Method used	Percentile Rank	ANN IC50(nM)	ANN rank	SMM IC50(nM)	SMM rank
HLA-A*01:01	1	92	100	9	CSANNSHHY	Consensus (ann/smm)	0.2	25.62	0.09	173.60	0.3
HLA-A*01:01	1	405	413	9	FSDQIEQEA	Consensus (ann/smm)	0.34	121.15	0.27	360.21	0.4
HLA-B*07:02	1	471	480	10	KPHRLTNKGI	Consensus (ann/smm)	0.35	46.84	0.2	112.67	0.5
HLA-A*01:01	1	417	425	9	ITEMLRKDY	Consensus (ann/smm)	0.6	591.06	0.71	426.14	0.5
HLA-A*01:01	1	361	369	9	LRDLMGVPY	Consensus (ann/smm)	0.68	799.14	0.85	421.26	0.5
HLA-A*01:01	1	233	241	9	RTWENHCTY	Consensus (ann/smm)	0.69	552.60	0.68	694.30	0.7

# Downloaded prediction results

	A	B	C	D	E	F	G	H	I	J	K	L
1	allele	seq_num	start	end	length	peptide	method	Percentile Rank	ann_ic50	ann_rank	smm_ic50	smm_rank
2	HLA-A*01:01	1	92	100	9	CSANNSHHY	Consensus (ann/smm)	0.2	25.62	0.09	173.6	0.3
3	HLA-A*01:01	1	405	413	9	FSDQIEQEA	Consensus (ann/smm)	0.34	121.15	0.27	360.21	0.4
4	HLA-B*07:02	1	471	480	10	KPHRLTNKGI	Consensus (ann/smm)	0.35	46.84	0.2	112.67	0.5
5	HLA-A*01:01	1	417	425	9	ITEMLRKDY	Consensus (ann/smm)	0.6	591.06	0.71	426.14	0.5
6	HLA-A*01:01	1	361	369	9	LRDLMGVPI	Consensus (ann/smm)	0.68	799.14	0.85	421.26	0.5
7	HLA-A*01:01	1	233	241	9	RTWENHCTY	Consensus (ann/smm)	0.69	552.6	0.68	694.3	0.7
8	HLA-A*01:01	1	217	225	9	TTWCSQTSY	Consensus (ann/smm)	0.71	604.36	0.72	653.96	0.7
9	HLA-A*01:01	1	439	447	9	LLMFSTSAY	Consensus (ann/smm)	0.75	724.33	0.8	728.7	0.7
10	HLA-A*01:01	1	147	155	9	LSIRGNSNY	Consensus (ann/smm)	1.25	3116.42	2	448.28	0.5
11	HLA-B*07:02	1	425	434	10	YIKRQGSTPL	Consensus (ann/smm)	1.27	59.83	0.24	575.2	2.3
12	HLA-B*07:02	1	243	252	10	GPFGMSTRILL	Consensus (ann/smm)	1.35	418.14	1.2	351.41	1.5
13	HLA-A*01:01	1	191	199	9	VLDMFRTAF	Consensus (ann/smm)	1.6	2586.86	1.8	1457.3	1.4
14	HLA-A*01:01	1	174	182	9	FSDAQSAQS	Consensus (ann/smm)	1.75	2437.12	1.7	1934.42	1.8
15	HLA-A*01:01	1	52	60	9	LAGRSCGMY	Consensus (ann/smm)	2.05	4721.07	2.5	1692.58	1.6
16	HLA-A*01:01	1	220	228	9	CSQTSYQYL	Consensus (ann/smm)	2.15	5007.72	2.6	1826.21	1.7
17	HLA-A*01:01	1	219	227	9	WCSQTSYQY	Consensus (ann/smm)	2.2	2051.4	1.6	3009.89	2.8
18	HLA-A*01:01	1	86	94	9	LTMPNACSA	Consensus (ann/smm)	2.25	4423.31	2.4	2215.9	2.1
19	HLA-B*07:02	1	320	329	10	RLIDYNKAAL	Consensus (ann/smm)	2.25	1113.26	2.2	595.42	2.3
20	HLA-B*07:02	1	190	199	10	RVLDMFRTAF	Consensus (ann/smm)	2.4	567.7	1.5	816.24	3.3
21	HLA-A*01:01	1	272	280	9	LSDSSGVEN	Consensus (ann/smm)	2.45	8300.79	3.9	913.17	1
22	HLA-A*01:01	1	369	377	9	YCNYSKFWY	Consensus (ann/smm)	2.45	5677.63	2.9	2145.61	2
23	HLA-A*01:01	1	436	444	9	LMDLLMFST	Consensus (ann/smm)	2.5	3758.17	2.2	3037.74	2.8
24	HLA-B*07:02	1	432	441	10	TPLALMDLLM	Consensus (ann/smm)	2.6	767.22	1.8	854.71	3.4
25	HLA-A*01:01	1	166	174	9	ITIQYNLTF	Consensus (ann/smm)	2.75	8692.54	4	1583.25	1.5
26	HLA-A*01:01	1	364	372	9	LMGVPIYCN	Consensus (ann/smm)	2.75	5142.58	2.7	3009.89	2.8
27	HLA-A*01:01	1	104	112	9	GTSGLLETF	Consensus (ann/smm)	2.8	7192.3	3.4	2374.38	2.2
28	HLA-A*01:01	1	222	230	9	QTSYQYLII	Consensus (ann/smm)	2.9	8442.18	4	1873.05	1.8
29	HLA-A*01:01	1	448	456	9	LVSIFLHLV	Consensus (ann/smm)	2.95	5023.73	2.7	3424.13	3.2

# Emailed prediction results

IEDB Tools MHC class I prediction result (2019-10-07 10:05:35) Inbox x  

**IEDB Tools** <Prediction-results-noreply@iedb.org>  10:05 AM (0 minutes ago)   



to me ▾

Your MHC class I prediction completed on the IEDB servers (<http://tools.iedb.org/mhci/>) and the result is attached in csv format.



Input parameters  
Method: recommended  
Number of sequences: 1  
Input sequences: attached  
Alleles: HLA-A\*01:01, HLA-B\*07:02  
Lengths: 9, 10

Job parameters  
Submission date: 2019-10-07 10:05:35  
Completion date: 2019-10-07 10:05:51  
Total walltime since submission: 16 seconds

---

**2 Attachments**  

Allele	seq_len	start	end	length	allele	method	Percent
HLA-A*01:01	9	86	100	15	R QDRRBRW	Consensus.com	
HLA-A*01:01	9	86	100	15	R FRRGRWGA	Consensus.com	
HLA-A*01:01	9	87	100	14	R QPRLS R RAS	Consensus.com	
HLA-A*01:01	9	87	100	14	R FTR R RWF	Consensus.com	
HLA-A*01:01	9	88	100	13	R LTR R RWF	Consensus.com	
HLA-A*01:01	9	276	289	14	R R FRRGRW	Consensus.com	
HLA-A*01:01	9	277	289	13	R TRGRGRW	Consensus.com	
HLA-A*01:01	9	428	437	10	R LRRRGRW	Consensus.com	
HLA-A*01:01	9	102	111	10	R LRRRGRW	Consensus.com	
HLA-A*01:01	9	425	434	10	R RRRGRW	Consensus.com	
HLA-A*01:01	9	461	470	10	R RRRGRW	Consensus.com	
HLA-A*01:01	9	19	100	10	R VLRRRGRW	Consensus.com	
HLA-A*01:01	9	176	185	10	R TRRRGRW	Consensus.com	
HLA-A*01:01	9	52	60	9	R LRRRGRW	Consensus.com	
HLA-A*01:01	9	200	209	9	R QRRRGRW	Consensus.com	
HLA-A*01:01	9	275	284	9	R LRRRGRW	Consensus.com	

 **predict\_result.csv**  **input\_sequences.txt**

# Selection of “binders”

- Pick peptides **below percentile rank 1.0**
- Pick peptides **below predicted binding affinity of 500 nM**
  - IC50 < 50 nM - high affinity
  - IC50 < 500 nM - intermediate affinity
  - IC50 < 5000 nM - low affinity
  - Sette et al. 1994, J. Immunology (PMID: 7527444)
  - Ensures that all peptides have reasonable affinity
- Pick **top 1% of peptides** for each allele/length combination to cover most of immune responses
  - Moutaftsi et al. 2006 (PMID: 16767078)
  - Kotturi et al. 2007 (PMID: 17329346)
  - Ensures equal number of peptides per allele
- Select based on **allele specific binding affinity** threshold

# Different peptide-binding repertoires

- The size of the peptide repertoire binding at a given affinity varies between alleles

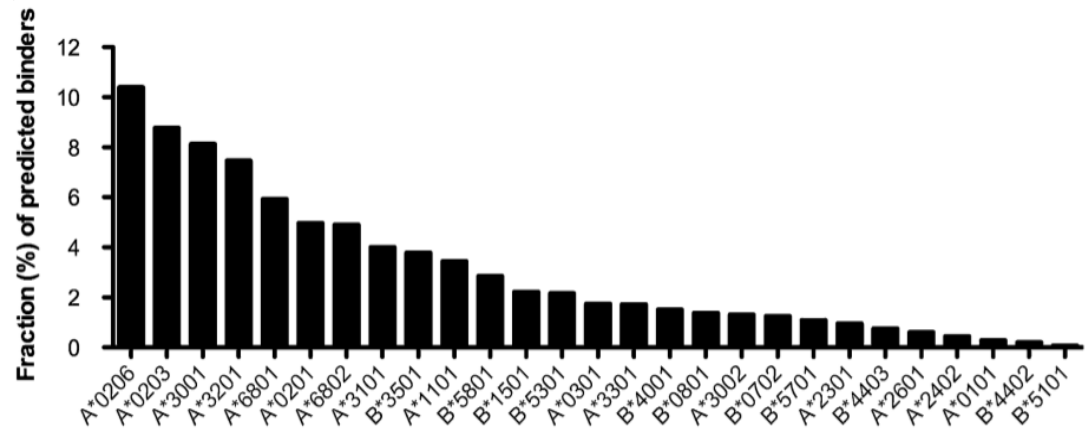
● All peptides

★ Binders

HLA-A\*02:01



HLA-A\*01:01



**Allele-specific affinity cutoffs**

J Immunol. 2013 Dec 15;191(12):5831-9. doi: 10.4049/jimmunol.1302101. Epub 2013 Nov 4.

**HLA class I alleles are associated with peptide-binding repertoires of different size, affinity, and immunogenicity.**

Paul S<sup>#1</sup>, Weiskopf D<sup>#1</sup>, Angelo MA<sup>1</sup>, Sidney J<sup>1</sup>, Peters B<sup>1</sup>, Sette A<sup>1</sup>.

PMID: 24190657 PMCID: PMC3872965 DOI: 10.4049/jimmunol.1302101



# Allele-specific thresholds

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## MHC-I binding predictions - Tutorial

Guidelines for selecting thresholds (cut-offs) for predictions can be found [here](#).

### How to obtain predictions

This website provides access to predictions

## Selecting thresholds (cut-offs) for MHC class I and II binding predictions



Ward Fleri  
posted this on May 21, 2013 04:33 PM

### MHC class I

For MHC class I T cell epitope predictions, selection of predicted binders can be done based on the percentile rank or MHC binding affinity. The IEDB currently recommends making selections based on a percentile rank of  $\leq 1\%$  for each (MHC allele, length) combination to cover most of the immune responses.<sup>1,2</sup> Alternatively, a binding affinity (IC50) threshold of 500 nM identifies peptide binders recognized by T cells and this threshold can be used to select peptides.<sup>3</sup> Recently, a paper from our group showed that absolute binding affinity threshold correlates better with immunogenicity and also that, for even better correlation, MHC-specific thresholds should be used.<sup>4</sup> The tables below show the allele-specific thresholds for the 38 most common HLA-A and HLA-B alleles, representative of the nine major supertypes. The tables can also be downloaded as an RTF file (see attached file).

Alleles sorted by population frequency			Alleles sorted by name		
Allele	Population frequency of allele	Allele specific affinity cutoff (IC50 nM)	Allele	Population frequency of allele	Allele specific affinity cutoff (IC50 nM)
A*0201	25.2	255	A*0101	16.2	884
A*2402	16.8	849	A*0201	25.2	255
A*0101	16.2	884	A*0203	3.3	92
A*0301	15.4	602	A*0206	4.9	60
B*0702	13.3	687	A*0301	15.4	602
A*1101	12.9	382	A*1101	12.9	382
B*0801	11.5	663	A*2301	6.4	740
B*4001	10.3	639	A*2402	16.8	849
B*4402	9.2	904	A*2501	2.5	795
B*4403	7.6	780	A*2601	4.7	815
B*3501	6.5	348	A*2902	2.9	641
A*2301	6.4	740	A*3001	5.1	109
A*3201	5.7	131	A*3002	5	674
B*5101	5.5	939	A*3101	4.7	329
B*5301	5.4	538	A*3201	5.7	131
B*1501	5.2	528	A*3301	3.2	606
A*3001	5.1	109	A*6801	4.6	197
A*3002	5	674	A*6802	3.3	259

# Recommendations

- Both approaches (affinity and ranking) are reasonable, and have been applied in numerous studies
- Thresholds can be combined (peptides in top 1% and  $IC_{50} < 500nM$ )
- Current studies suggest that allele specific thresholds can be derived

# Alternate approaches for selecting binders

- Change threshold values depending on your need
  - e.g. in case you have too few or too many predicted binders.
- Set a desired percentage within your peptide set (irrespective of IEDB percentile rank) in case you want to study a fixed number of best possible peptides.

# Walk through exercise

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

Find the best epitope candidate of length 9 for HLA-A\*02:01 from SARS spike glycoprotein (GenBank accession no: ABD72984.1)

Solution:

1. Copy sequence from **GenBank** (NCBI Protein) into the prediction tool
2. Select prediction method as **“IEDB recommended”**
3. Select species as **“Human”**
4. Select the allele as **HLA-A\*02:01 & length as 9**
5. Submit

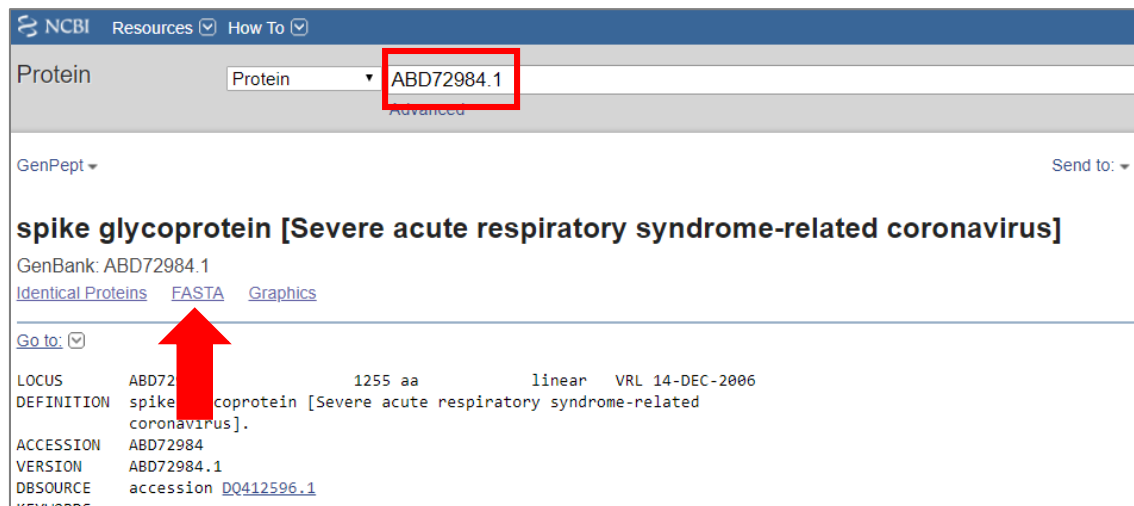
# Walk through exercise

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

Find the best epitope candidate of length 9 for HLA-A\*02:01 from SARS spike glycoprotein (GenBank accession no: **ABD72984.1**)

Solution:

1. Collect sequence from GenBank (NCBI Protein) - <https://www.ncbi.nlm.nih.gov/protein/>



NCBI Resources How To

Protein Protein **ABD72984.1**

GenPept Send to:

**spike glycoprotein [Severe acute respiratory syndrome-related coronavirus]**

GenBank: ABD72984.1

[Identical Proteins](#) [FASTA](#) [Graphics](#)

Go to:

LOCUS ABD72984.1 1255 aa linear VRL 14-DEC-2006

DEFINITION spike glycoprotein [Severe acute respiratory syndrome-related coronavirus].

ACCESSION ABD72984

VERSION ABD72984.1

DBSOURCE accession [DQ412596.1](#)

KEYWORDS

# Practice Exercise

[tools.iedb.org/mhci/](https://tools.iedb.org/mhci/)

## MHC-I Binding Predictions

Prediction Method Version: 2013-02-22 [\[Older versions\]](#)

### Specify Sequence(s)

Enter protein sequence(s) in FASTA format or as whitespace-separated sequences. [\(Browse for sequences in NCBI\)](#)

```
>ABD72984.1 spike glycoprotein [Severe acute respiratory syndrome-related coronavirus]
MFIFILFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVVYPDEIFRSDTLYLTQDLFLPFYSNVTGF
H
TINHTFGNPVVPFKDGIYFAATEKSNVVRGWVFGSTMNKSQSVIIINNSTNVVIRACNFELCDNPFPA
V
SKPMGTQHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFNKDKGFLYVYKGYQPIDVVRDL
P
SGFNLTLPKIFKPLPLGINITNFRAILTAFSPAQDIWGTSAAYFVGYLKPTTFMLKYDENGITIDAVDCS
Q
```

FASTA format detected.

Or select file containing sequence(s):  No file chosen

### Choose a Prediction Method

Prediction Method <sup>?</sup>: IEDB recommended 2.22 [Help on prediction method selections](#)

Show all the method versions:

### Specify what to make binding predictions for

MHC source species: human

Show only frequently occurring alleles:  <sup>?</sup>  
Select MHC allele(s):  
Select HLA allele reference set:  <sup>?</sup>

Allele	Length
HLA-A*02:01	9

<sup>?</sup>

### Specify Output

Sort peptides by: Percentile Rank

Show: All predictions

Output format: XHTML table

Email address (optional):  <sup>?</sup>

Insert protein sequence

Select prediction method

Select species

Select allele & length

# Practice Exercise

## MHC-I Binding Prediction Results

### Input Sequences

#	Name	Sequence
1	ABD72984.1 spike glycoprotein [Severe acute respiratory syndrome-related coronavirus]	MFIFILFLTLTSGSDLRCTTFDDVQAPNYTQHTSSMRGVVYPDEIFRSD TLYLTQDLFLPFYSNVTGFHTINHDFGNPVIPIFKDGIYFAATEKSNVVRG WFGSTMNKSQSVIIINNSTNVVIRACNFELCDNPF FAVSKPMGTQHT MIFDNFNCTFEYISDAFSLDVSEKSGNFKHLREFVFNKKGFLVYKGY QPIDVVRDLPSGFNTLKPFIKPLGINITNFRAILTAFSPAQDIWGTSA AYFVGYLKPTTFMLKYDENGITDAVDCSQNPLAELKCSVKSFEDKGIY QTSNFRVVPDGVVRFNITNLCPFGEVFNATKFPVYAWERKKISNCVA DYSVLYNSTFFSTFKCYGVSATKLNLCF SNVYADSFVVKGDVVRQIAPG QTGVIADYNYKL PDDFMGCVLAWNTRNIDATSTGNVNYKYRYLRHGKLRP FERDISNVFSPDGKPCPPALNCYWPLNDYGFYTTTGIGYQPYRVVVL FELLNAPATVCGPKLSTDLIKNQCVNFNENGLTGTGVLTPSSKRFQPFQ FGRDVSDF TDSVRDPKTSEILDISPCSFGGVSVITPGTNASSEVAVLYQD VNC TDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAHEVDTSYECDI PIGAGICASYHTVSLLRSTSQKSI VAYTMSLGADSSIAYSNNTIAIPTNF <b>FSISITTEV</b> MPVSMAKTSVDCNMYICGDSTECANLLQYGSFCQLNRALS GIAAEQDRNTRREVFAQVKQMYKTP TLKYFGGFNF SQILPDPLKPTKRSFI EDLLFNKVTLADAGFMKQYGECLGDINARDL ICAQKFNGLTVLPPLTDD MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMYRFGNIGVTQNVLYE NQKQIANQFNKAI S QIQESL TTTSTALGKLDQVVNQNAQALNTLVKQLSS NFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI RASANLAATKMSECVLQSKRVDFCGKGYHLSMFPQAAPHGVVFLHVTVV PSQERNFTTAPAI CHEGKAYFPREGVVFVNGTSWFITQRNFFSPQIITTD NTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPVDLGD ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPVYWL GFIAGLIAIVMVTILLCMTSCCCLKGACSCGSCCKFDEDDSEPVKGV KLHYT

Prediction method: IEDB recommended 2.22 | Low Percentile Rank = good binders

[Download result](#)

### Citations

Check to expand the result:

Allele	#	Start	End	Length	Peptide	Method used	Percentile Rank
HLA-A*02:01	1	700	708	9	FSISITTEV	Consensus (ann/comblib_sidney2008/smm)	0.34
HLA-A*02:01	1	1202	1210	9	FIAGLIAIV	Consensus (ann/comblib_sidney2008/smm)	0.4
HLA-A*02:01	1	982	990	9	RLQSLQTYV	Consensus (ann/comblib_sidney2008/smm)	0.7
HLA-A*02:01	1	354	362	9	VLYNSTFFS	Consensus (ann/comblib_sidney2008/smm)	0.73
HLA-A*02:01	1	2	10	9	FIFILFLTL	Consensus (ann/comblib_sidney2008/smm)	0.83
HLA-A*02:01	1	404	412	9	VIADYNYKL	Consensus (ann/comblib_sidney2008/smm)	0.9
HLA-A*02:01	1	965	973	9	RLDKVEAEV	Consensus (ann/comblib_sidney2008/smm)	0.9
HLA-A*02:01	1	256	264	9	YLKPTTFML	Consensus (ann/comblib_sidney2008/smm)	1.0

# Self-practice (or mini-break): 10 min

## Background

Apical membrane antigen-1 (AMA1) is a protein expressed in the membrane of *P. falciparum* sporozoite liver and blood stages. In clinical trials AMA1 gives both CD4+ & CD8+ responses and is considered a good malaria vaccine candidate.

## Methods

Five volunteers were immunized with a vaccine containing full length of AMA1. A peptide pool of 15-mers overlapping by 11 amino acids in the AMA1 sequence was constructed. ELISpot responses of the peptides from the peptide pool were tested among the volunteers. HLA typing was done for each volunteer.

## Problem statement

Use IEDB prediction tools to determine the minimal (length 9-10) epitope within the 15-mer **LLSAFEFTYMINFGR**. Volunteer's HLA set: **HLA-A\*02:01, HLA-A\*26:01, HLA-B\*18:01, HLA-B\*44:02**.

[Malar J. 2010 Aug 24;9:241. doi: 10.1186/1475-2875-9-241.](#)

**Identification and localization of minimal MHC-restricted CD8+ T cell epitopes within the Plasmodium falciparum AMA1 protein.**

[Sedegah M<sup>1</sup>, Kim Y, Peters B, McGrath S, Ganeshan H, Lejano J, Abot E, Banania G, Belmonte M, Sayo R, Farooq E, Doolan DL, Regis D, Tamminga C, Chuang J, Bruder JT, King CR, Ockenhouse CF, Faber B, Remarque E, Hollingdale MR, Richie TL, Sette A.](#)

PMID: 20735847 PMCID: [PMC2939619](#) DOI: [10.1186/1475-2875-9-241](#)




# Self-practice results

Home Help Example Reference Download Contact

## MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	ws-separated-0	LLSAFEETYMINFGR

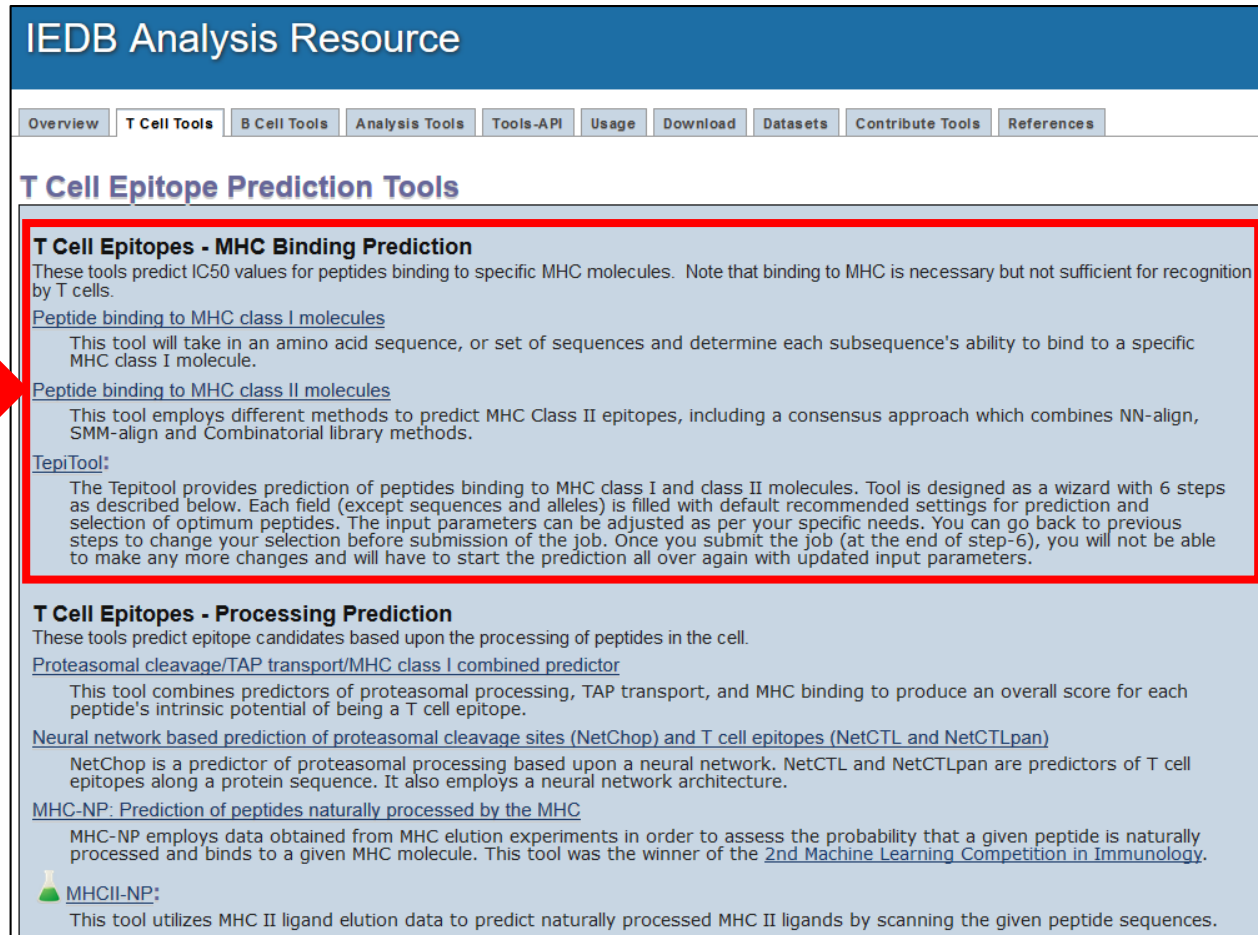
Prediction method: IEDB recommended 2.22 | Low Percentile Rank = good binders  
[Download result](#) 

Citations  
Check to expand the result:

Allele	#	Start	End	Length	Peptide	Method used	Percentile Rank
HLA-B*18:01	1	5	13	9	FEFTYMINF	Consensus (ann/smm)	0.12
HLA-B*44:02	1	5	13	9	FEFTYMINF	Consensus (ann/smm)	0.57
HLA-A*02:01	1	1	10	10	LLSAFEETYM	Consensus (ann/smm)	0.63
HLA-B*44:02	1	4	13	10	AFEFTYMINF	Consensus (ann/smm)	0.68
HLA-A*02:01	1	3	11	9	SAFEETYMI	Consensus (ann/comblib_sidney2008/smm)	2.3
HLA-B*18:01	1	5	14	10	FEFTYMINFG	Consensus (ann/smm)	2.95
HLA-A*26:01	1	2	10	9	LSAFEETYM	Consensus (ann/smm)	3.1
HLA-A*02:01	1	2	11	10	LSAFEETYMI	Consensus (ann/smm)	4.55
HLA-B*18:01	1	1	9	9	LLSAFEETY	Consensus (ann/smm)	4.8
HLA-B*44:02	1	3	11	9	SAFEETYMI	Consensus (ann/smm)	5.4
HLA-A*26:01	1	7	15	9	FTYMINFGR	Consensus (ann/smm)	6.55
HLA-A*26:01	1	5	13	9	FEFTYMINF	Consensus (ann/smm)	6.9
HLA-A*26:01	1	6	15	10	FEFTYMINFGR	Consensus (ann/smm)	6.95

# Class II MHC Binding Prediction

- Basic structure and principles same as class I binding prediction tool



**IEDB Analysis Resource**

Overview | **T Cell Tools** | B Cell Tools | Analysis Tools | Tools-API | Usage | Download | Datasets | Contribute Tools | References

## T Cell Epitope Prediction Tools

**T Cell Epitopes - MHC Binding Prediction**  
These tools predict IC50 values for peptides binding to specific MHC molecules. Note that binding to MHC is necessary but not sufficient for recognition by T cells.

[Peptide binding to MHC class I molecules](#)  
This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

[Peptide binding to MHC class II molecules](#)  
This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.


**TepiTool:**  
The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per your specific needs. You can go back to previous steps to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.

**T Cell Epitopes - Processing Prediction**  
These tools predict epitope candidates based upon the processing of peptides in the cell.

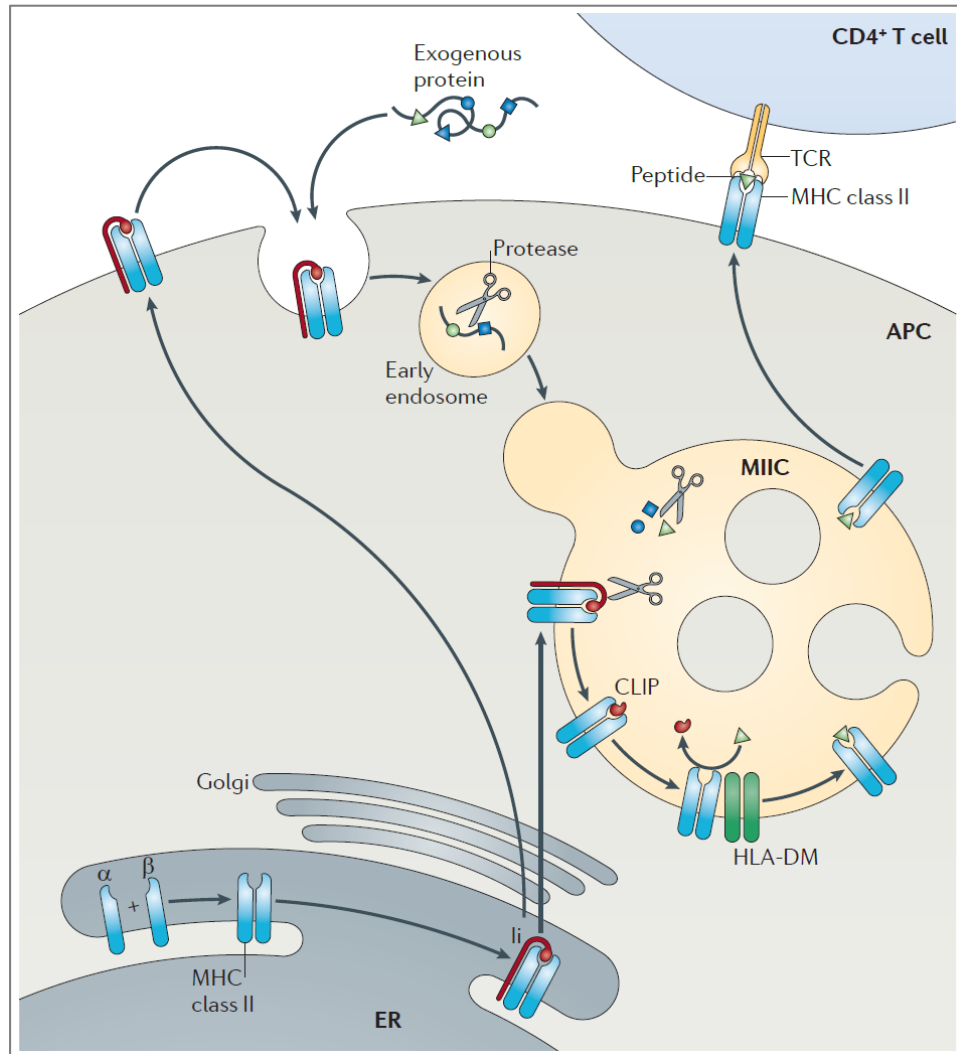
[Proteasomal cleavage/TAP transport/MHC class I combined predictor](#)  
This tool combines predictors of proteasomal processing, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope.

[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](#).

 **MHCII-NP:**  
This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences.

# Exogenous antigen processing pathway (class II)



- Antigenes generated outside the cell
  - Entered through inhalation, ingestion, injection
  - Bacteria, Allergens, Parasites etc.

Nat Rev Immunol. 2011 Nov 11;11(12):823-36. doi: 10.1038/nri3084.

**Towards a systems understanding of MHC class I and MHC class II antigen presentation.**

Neefjes J<sup>1</sup>, Jongstra ML, Paul P, Bakke O.

PMID: 22076556 DOI: 10.1038/nri3084

# Class II MHC molecule

- Only in antigen presenting cells
- Two MHC encoded polymorphic chains ( $\alpha$ ,  $\beta$ )
- Both  $\alpha$  and  $\beta$  chains impact binding
- Binding groove is open
- Can bind **longer peptides** (13-25 AA)
- Presents antigen to **CD4+ T cells**

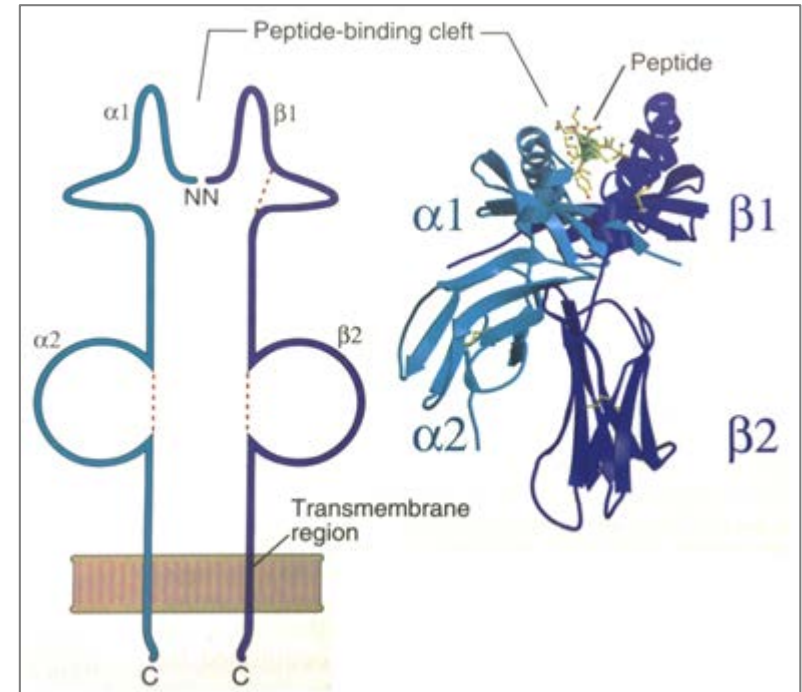


Figure Source  
*Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman*

# HLA Nomenclature

- Class I:
  - Only  $\alpha$  chain is variable
    - HLA-B\*07:02 (“B” is locus)
    - $\beta$ 2-microglobulin
- Class II:
  - Both  $\alpha$  and  $\beta$  chains are variable for DP & DQ loci
    - HLA-DPA1\*01:03/DPB1\*02:01
    - HLA-DQA1\*01:01/DQB1\*05:01
  - Only  $\beta$  chain is variable for DR locus
    - HLA-DRB1\*01:01

# Class II binding peptide “Binding core”

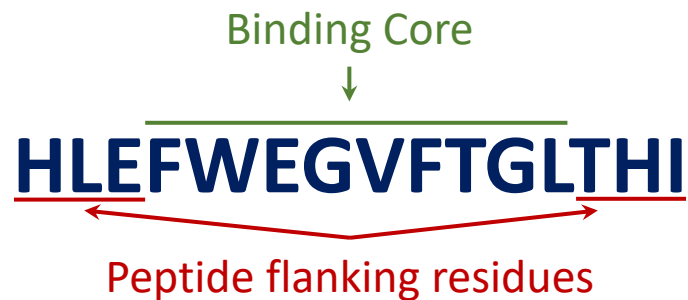
- 9 AA core within the peptide that interacts with the binding groove of MHC molecule



- Challenge: Correct identification of the binding core
- Needs proper alignment of the binding core with the binding groove

# “Peptide flanking residues” (PFR)

- Residues flanking the binding core - interacts with MHC molecule outside the groove.



- Challenge: PFR length & composition influence binding.

# Other challenges of class II binding prediction

- Availability of uniform experimentally measured binding data which can be used for training the tools - less compared to class I
- A minimum of 200 peptides with binding affinity data needed for description of binding motif in MHC class II alleles
- Fewer alleles available for class II tools compared to class I



# Other differences between class I & II tools

- Lesser accuracy compared to class-I tool

Class I		Class II	
Method	AUC*	Method	AUC*
NetMHCpan-4.0	0.960 <sup>1</sup>	NetMHCIIpan-3.2	0.781 <sup>2</sup>
SMM	0.894 <sup>3</sup>	SMM-align	0.763 <sup>4</sup>

*\* The AUCs reported here are from different studies and obtained from different data sets*

- Higher threshold for selecting binders than class-I

1. Jurtz et al., 2017, J of Immunology
2. Jensen et al. 2018, Immunology
3. Kim et al. 2009, BMC Bioinformatics
4. Wang et al. 2010, BMC Bioinformatics

# MHC-II binding prediction interface

- Tool entry point layout similar to class I

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

The screenshot shows the MHC-II Binding Predictions interface with several key features highlighted by red boxes and arrows:

- Navigation:** Home, Help, Example, Reference, Download, Contact
- Specify Sequence(s):** A large text area for entering protein sequence(s) in FASTA format. A red box and arrow point to this area with the label "Insert protein sequence(s)".
- Choose a Prediction Method:** A dropdown menu set to "IEDB recommended 2.22". A red box and arrow point to this dropdown with the label "Select prediction method".
- Specify what to make binding predictions for:**
  - Select species/locus:** A dropdown menu set to "Human, HLA-DR". A red box and arrow point to this dropdown with the label "Select species".
  - Select MHC allele(s):** A dropdown menu set to "Allele". A red box and arrow point to this dropdown with the label "Select allele(s) & length".
  - Select length(s):** A grid of buttons for selecting peptide lengths. The "15" button is highlighted. A red box and arrow point to this grid with the label "Select allele(s) & length".
- Specify Output:**
  - Sort peptides by:** A dropdown menu set to "Adjusted Rank".
  - Output format:** A dropdown menu set to "XHTML table".
  - Email address (optional):** A text input field.A red box and arrow point to these options with the label "Output options".

At the bottom of the form are "Submit" and "Reset" buttons.

# MHC-II binding prediction - example

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Home Help Example Reference Download Contact

## MHC-II Binding Predictions

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format ([Browse for sequences in NCBI](#))

```
>West Nile virus envelope glycoprotein
FNCLGMSNRDFLEGVSGATWVDLVLEGDSVITMSKDKPTIDVKMMNMEANLAEVRSYCYLATVSDLST
KAACPTMGEAHNDKRAPAFVCRQGVVDRGWGNGCGLFGKGSIDTCAKFACTKAIGRTILKENIKYEVA
IFVHGPTTVESHGNYSTQVGATQAGRFSITPAAPSYTLKLGEGEVTVDCEPRSGIDTNAVYVMTVGTKT
FLVHREWFMDLNLPPWSSAGSTVWRNRETLMEFEFPHATKQSVIALGSEQEGALHQAAGAIPVEFSNTVK
LTSGLKCRVKMEKLLQKGTTYGVCSKAFKFLGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDLT
PVGRLVTVNPFVSVATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHKSGSSIGKAFTTLTKGAQRLAA
LGDATWDFGSVGGVFTSVGKAVHQVFGGAFRSLFGGMSWITQGLLGALLLWGINARDRSIALTFLAVGG
VLLFLSVNVHA
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

**Choose a Prediction Method**

Prediction Method [?](#)  
Show all the method versions:

[help on prediction method selections](#)

Select species/locus

Select MHC allele(s)  
Select  $\alpha$  &  $\beta$  chains separately if applicable:  [?](#)

Select full HLA reference set:  [?](#)  
Select 7-allele HLA reference set:  [?](#)

Select length(s) [?](#)

default	12-16	as is							
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

**Specify Output**

Sort peptides by: Adjusted Rank

Output format: XHTML table

Email address (optional)  [?](#)

Prediction method

# Guidelines: Choosing the prediction method

- Suggested method = “IEDB recommended”
  - Employs Consensus (Combination of NN-align, SMM-align & CombLib/Sturniolo) or NetMHCIIpan depending on the allele
  - Provides binding affinity & percentile rank for each method separately as well
- Recommendation will change with the new benchmark studies

# MHC-II binding prediction – example

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Home Help Example Reference Download Contact

## MHC-II Binding Predictions

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format ([Browse for sequences in NCBI](#))

```
>West Nile virus envelope glycoprotein
FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEANLAEVRSYCYLATVSDLST
KAACPTMGEAHNDKRADPAFVCRQGVVDRGWGNGCGLFGKGSIDTCAKFACTKAIGRTILKENIKYEVA
IFVHGPTTVESHGNYSTQVGATQAGRFSITPAAPSYTLKGEYGEVTVDCEPRSGIDTNAYYVMTVGTKT
FLVHREWFMDLNLPWSSAGSTVWRNRETLMFEFEPHATKQSVIALGSQEGALHQAAGAIPEFSSNTVK
LTSGHLKCRVKMEKQLKLGTTYGVCSKAFKFLGTPADTGHGTVVLELQYGTGDPCKVPISSVASLNDLT
PVGRLVTNPPFVSATANAKVLEIEPPFGDSYIVVGRGEQQINHWHKSGSSIGKAFITTLKGAQRLAA
LGDTAWDFGSGVGVFTSVGKAVHQVFGGAFRSLFGGMSWITQGLLALLLWGMINARDRSIALTFLAVGG
VLLFLSVNVHA
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

**Choose a Prediction Method**

Prediction Method  IEDB recommended 2.22 [Help on prediction method selections](#)  
Show all the method versions:

**Specify what to make binding predictions for**

Select species/locus

Select MHC allele(s)  
Select  $\alpha$  &  $\beta$  chains separately if applicable:

[Select full HLA reference set:](#)

Select 7-allele HLA reference set:

Select length(s)

default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

**Specify Output**

Sort peptides by

Output format

Email address (optional)

Choose species & locus

Select or upload alleles

Upload format:  
H2-IAb  
HLA-DPA1\*01/DPB1\*04:01  
HLA-DRB1\*01:01

# Allele selection - $\alpha$ and $\beta$ chains separately

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Specify what to make binding predictions for

Select species/locus: Human, HLA-DQ ▾

Select MHC allele(s)  
Select  $\alpha$  &  $\beta$  chains separately if applicable  ?

Select full HLA reference set:  ?  
Select 7-allele HLA reference set:  ?

Select length(s) ?

Allele

DQA1\*01:01 ▾

Upload allele file ?

default	12-18				
11	12	13	14	19	20
21	22	23	24	29	30

Specify  $\alpha$  chain

Adjusted Rank

XHTML table ▾

Email address (optional)

Submit Reset

DQB1\*02:01  
DQB1\*02:02  
DQB1\*02:03  
DQB1\*02:04  
DQB1\*02:05  
DQB1\*02:06  
DQB1\*03:01  
DQB1\*03:02  
DQB1\*03:03  
DQB1\*03:04  
DQB1\*03:05  
DQB1\*03:06  
DQB1\*03:07  
DQB1\*03:08  
DQB1\*03:09  
DQB1\*03:10  
DQB1\*03:11  
DQB1\*03:12  
DQB1\*03:13

# Allele selection – 27 allele reference set

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Specify what to make binding predictions for

Select species/locus: Human, HLA-DQ ▾

Select MHC allele(s)

Select  $\alpha$  &  $\beta$  chains separately if applicable:  ?

Select full HLA reference set:  ?

Select 7-allele HLA reference set:  ?

Allele	
HLA-DRB1*01:01	<input type="radio"/>
HLA-DRB1*03:01	<input type="radio"/>
HLA-DRB1*04:01	<input type="radio"/>
HLA-DRB1*04:05	<input type="radio"/>
HLA-DRB1*07:01	<input type="radio"/>
HLA-DRB1*08:02	<input type="radio"/>
HLA-DRB1*09:01	<input type="radio"/>
HLA-DRB1*11:01	<input type="radio"/>
HLA-DRB1*12:01	<input type="radio"/>
HLA-DRB1*13:02	<input type="radio"/>
HLA-DRB1*15:01	<input type="radio"/>
HLA-DRB3*01:01	<input type="radio"/>
HLA-DRB3*02:02	<input type="radio"/>
HLA-DRB4*01:01	<input type="radio"/>
HLA-DRB5*01:01	<input type="radio"/>
HLA-DQA1*05:01/DQB1*02:01	<input type="radio"/>
HLA-DQA1*05:01/DQB1*03:01	<input type="radio"/>
HLA-DQA1*03:01/DQB1*03:02	<input type="radio"/>
HLA-DQA1*04:01/DQB1*04:02	<input type="radio"/>
HLA-DQA1*01:01/DQB1*05:01	<input type="radio"/>
HLA-DQA1*01:02/DQB1*06:02	<input type="radio"/>
HLA-DPA1*02:01/DPB1*01:01	<input type="radio"/>
HLA-DPA1*01:03/DPB1*02:01	<input type="radio"/>
HLA-DPA1*01/DPB1*04:01	<input type="radio"/>
HLA-DPA1*03:01/DPB1*04:02	<input type="radio"/>
HLA-DPA1*02:01/DPB1*05:01	<input type="radio"/>
HLA-DPA1*02:01/DPB1*14:01	<input type="radio"/>

DQA1\*01:01 ▾  ▾ [Upload allele file](#) ?

Select length(s)

default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

# Allele selection – 7 allele set

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Specify what to make binding predictions for

Select species/locus: Human, HLA-DR ▾

Select MHC allele(s)

Select  $\alpha$  &  $\beta$  chains separately if applicable:  ?

Select full HLA reference set:  ?

Select 7-allele HLA reference set:  ?

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\*01:01

[Upload allele file](#) ?

[J Immunol Methods](#). 2015 Jul;422:28-34. doi: 10.1016/j.jim.2015.03.022. Epub 2015 Apr 7.

## Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes.

Paul S<sup>1</sup>, Lindestam Arlehamn CS<sup>2</sup>, Scriba TJ<sup>3</sup>, Dillon MB<sup>2</sup>, Oseroff C<sup>2</sup>, Hinz D<sup>2</sup>, McKinney DM<sup>2</sup>, Carrasco Pro S<sup>4</sup>, Sidney J<sup>2</sup>, Peters B<sup>2</sup>, Sette A<sup>2</sup>.

PMID: 25862607 PMCID: [PMC4458426](#) DOI: [10.1016/j.jim.2015.03.022](#)



# MHC-II binding prediction – example

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Home Help Example Reference Download Contact

## MHC-II Binding Predictions

**Specify Sequence(s)**

Enter protein sequence(s) in FASTA format  
([Browse for sequences in NCBI](#))

```
>West Nile virus envelope glycoprotein
FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEANLA
EVRSYCYLATVSDLST
KAACPTMGEAHNDKRAPAFVCRQGVVDRGWGNGCGLFGKGSIDTCAKFACT
KAIGRTILKENIKYEVA
IFVHGPTTVESHGNYSTQVGATQAGRFSITPAAPSYTLKLGEGEVTVDCEPRSGI
DTNAYVVMTVGTKT
FLVHREWFMDLNLPWSSAGSTVWRNRETLMEFEEPHATKQSVIALGSQEGALHQ
ALAGAIPVEFSSNTVK
LTSGHLKCRVKMEKLQLKGTTYGVC SKAFKFLGTPADTGHGTVVLELQYTGTDGP
```

FASTA format detected.

Or select file containing sequence(s)  No file chosen

**Choose a Prediction Method**

Prediction Method <sup>?</sup> IEDB recommended 2.22 [Help on prediction method selections](#)  
Show all the method versions:

**Specify what to make binding predictions for**

Select species/locus Human, HLA-DP ▾

Select MHC allele(s)  
Select  $\alpha$  &  $\beta$  chains separately if applicable:  <sup>?</sup>  
Select full HLA reference set:  <sup>?</sup>  
Select 7-allele HLA reference set:  <sup>?</sup>

Allele  
DPA1\*01/DPB1\*04:01   
  
[Upload allele file](#) <sup>?</sup>

Select length(s) <sup>?</sup>  
default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

**Specify Output**

Sort peptides by Adjusted Rank ▾

Output format XHTML table ▾

Email address (optional) spaul@lji.org <sup>?</sup>

Length selection

Output format

# How the tool works

- Breaks sequence into all possible peptides of chosen lengths
- Predicts the binding affinity for each peptide based on the method
- Compares the predicted affinity to that of a large set of randomly selected peptides
- Assigns a percentile rank depending on individual predicted affinity
- Consensus picks median rank of the methods used – consensus percentile rank

# MHC-II binding prediction – example

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Home Help Example Reference Download Contact

## MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	West Nile virus envelope glycoprotein	FNCLGMSNRDFLEGVSGATWDLVLEGDSCVTIMSKDKPTIDVKMMNHEA ANLAEVRSYCYLATVSDLSTKAACP TMGEAHNDK RADPAFVCRQGVVDRG WNGNCGLFGKGSIDTCAKFACTKAIGRTILKENIKYEVAIFVHGPTTVE SHGNYSIQVGATQAGRFSITPAAPSYTLKLGVEYGEVTVDCPRSGIDTNA YYVMTVGTKTFLVHREWFMDLNLWSSAGSTVWRNRETLMEFEFPATKQ SVIALGSGEQGALHQAAGAIPEVFSNTVKLTSGLKCRVKMEKQLQKGT TYGVCSKAFKFLGTPADTGHGTVLELQYGTGDGPKVPISSVASLNDLT PVGR LVTVPFVS VATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHKS GSSIGKAF TTT LKGAQR LAALGDTAWDFG SVGGVFT SVGKAVHQVFGGAF RSLFGGMSWITQGLL GALLLWGMINARDRSIALTF LAVGGVLLFLSVNVH A

Prediction method: IEDB Recommended | Low adjusted\_rank = good binders  
[Download result](#)

Citations  
 Check to expand the result:

Allele	#	Start	End	Length	Method used	Peptide	Percentile Rank	Adjusted rank
HLA-DPA1*01/DPB1*04:01	1	476	490	15	Consensus (comb.lib./smm)	ARDRSIALTF LAVGG	2.85	2.85
HLA-DPA1*01/DPB1*04:01	1	474	488	15	Consensus (comb.lib./smm)	INARDRSIALTF LAV	2.85	2.85
HLA-DPA1*01/DPB1*04:01	1	475	489	15	Consensus (comb.lib./smm)	NARDRSIALTF LAVG	2.85	2.85
HLA-DPA1*01/DPB1*04:01	1	477	491	15	Consensus (comb.lib./smm)	RDRSIALTF LAVGGV	2.85	2.85
HLA-DPA1*01/DPB1*04:01	1	478	492	15	Consensus (comb.lib./smm)	DRSIALTF LAVGGVLL	2.95	2.95
HLA-DPA1*01/DPB1*04:01	1	207	221	15	Consensus (comb.lib./smm)	GTKTF LVHREWFMDL	3.55	3.55
HLA-DPA1*01/DPB1*04:01	1	209	223	15	Consensus (comb.lib./smm)	KTF LVHREWFMDLNL	3.60	3.60
HLA-DPA1*01/DPB1*04:01	1	208	222	15	Consensus (comb.lib./smm)	TKTF LVHREWFMDLN	3.60	3.60
HLA-DPA1*01/DPB1*04:01	1	479	493	15	Consensus (comb.lib./smm)	RSIALTF LAVGGVLL	3.95	3.95
HLA-DPA1*01/DPB1*04:01	1	200	214	15	Consensus (comb.lib./smm)	AYYVMTVGTKTF LVH	4.05	4.05
HLA-DPA1*01/DPB1*04:01	1	202	216	15	Consensus (comb.lib./smm)	YVMTVGTKTF LVHRE	4.05	4.05
HLA-DPA1*01/DPB1*04:01	1	203	217	15	Consensus (comb.lib./smm)	VMTVGTKTF LVHREW	4.10	4.10
HLA-DPA1*01/DPB1*04:01	1	201	215	15	Consensus (comb.lib./smm)	YYVMTVGTKTF LVHR	4.10	4.10
HLA-DPA1*01/DPB1*04:01	1	483	497	15	Consensus (comb.lib./smm)	LTFLAVGGVLL FLSV	4.50	4.50
HLA-DPA1*01/DPB1*04:01	1	204	218	15	Consensus (comb.lib./smm)	MTVGTKTF LVHREWF	4.71	4.71
HLA-DPA1*01/DPB1*04:01	1	440	454	15	Consensus (comb.lib./smm)	KAVHQVFGGAF RSLF	4.95	4.95
HLA-DPA1*01/DPB1*04:01	1	441	455	15	Consensus (comb.lib./smm)	AVHQVFGGAF RSLFG	5.00	5.00
HLA-DPA1*01/DPB1*04:01	1	443	457	15	Consensus (comb.lib./smm)	HQVFGGAF RSLFGGM	5.00	5.00
HLA-DPA1*01/DPB1*04:01	1	442	456	15	Consensus (comb.lib./smm)	VHQVFGGAF RSLFGG	5.10	5.10
HLA-DPA1*01/DPB1*04:01	1	439	453	15	Consensus (comb.lib./smm)	GKAVHQVFGGAF RSL	5.20	5.20

Input sequence

Output  
(sorted low-to-high by  
adjusted rank)

The **adjusted rank** is the percentile rank adjusted based on the frequency of peptide lengths.

# MHC-II binding prediction – example

[tools.iedb.org/mhcii/](https://tools.iedb.org/mhcii/)

Individual scores for different methods

Prediction method: IEDB recommended | Low adjusted\_rank = good binders  
[Download result](#)

Citations  
 Check to expand the result

Allele	#	Start	End	Length	Method used	Peptide	Percentile Rank	Adjusted rank	Comblib. core	Comblib. score	Comblib. percentile rank	Comblib. adjusted rank	SMM align core	SMM align IC50(nM)	SMM align percentile rank	SMM align adjusted rank
HLA-DPA1*01:DPB1*04:01	1	476	490	15	Consensus (comb.lib./smm)	ARDRSIALTFLAVGG	2.85	2.85	RSIALTFLA	0.01	2.80	2.80	SIALTFLAV	208.00	2.90	2.90
HLA-DPA1*01:DPB1*04:01	1	474	488	15	Consensus (comb.lib./smm)	INARDRSIALTFLAV	2.85	2.85	RSIALTFLA	0.01	2.80	2.80	RSIALTFLA	207.00	2.90	2.90
HLA-DPA1*01:DPB1*04:01	1	475	489	15	Consensus (comb.lib./smm)	NARDRSIALTFLAVG	2.85	2.85	RSIALTFLA	0.01	2.80	2.80	SIALTFLAV	203.00	2.90	2.90
HLA-DPA1*01:DPB1*04:01	1	477	491	15	Consensus (comb.lib./smm)	RDRSIALTFLAVGGV	2.85	2.85	RSIALTFLA	0.01	2.80	2.80	SIALTFLAV	205.00	2.90	2.90
HLA-DPA1*01:DPB1*04:01	1	478	492	15	Consensus (comb.lib./smm)	DRSIALTFLAVGGVL	2.95	2.95	RSIALTFLA	0.01	2.80	2.80	SIALTFLAV	221.00	3.10	3.10
HLA-DPA1*01:DPB1*04:01	1	207	221	15	Consensus (comb.lib./smm)	GKTFVLVHREWFMDL	3.55	3.55	KTFVLVHREW	0.03	3.90	3.90	FLVHREWFMDL	230.00	3.20	3.20
HLA-DPA1*01:DPB1*04:01	1	209	223	15	Consensus (comb.lib./smm)	KTFVLVHREWFMDLNL	3.60	3.60	KTFVLVHREW	0.03	3.90	3.90	VHREWFMDL	232.00	3.30	3.30
HLA-DPA1*01:DPB1*04:01	1	208	222	15	Consensus (comb.lib./smm)	TKTFVLVHREWFMDLN	3.60	3.60	KTFVLVHREW	0.03	3.90	3.90	VHREWFMDL	232.00	3.30	3.30
HLA-DPA1*01:DPB1*04:01	1	479	493	15	Consensus (comb.lib./smm)	RSIALTFLAVGGVLL	3.95	3.95	RSIALTFLA	0.01	2.80	2.80	SIALTFLAV	348.00	5.10	5.10
HLA-DPA1*01:DPB1*04:01	1	200	214	15	Consensus (comb.lib./smm)	AYVMTVGTKTFLVH	4.05	4.05	TVGKTFLV	0.01	0.01	0.01	TVGKTFLV	579.00	8.10	8.10
HLA-DPA1*01:DPB1*04:01	1	202	216	15	Consensus (comb.lib./smm)	YVMTVGTKTFLVHRE	4.05	4.05	TVGKTFLV	0.01	0.01	0.01	VGTKTFLVH	583.00	8.10	8.10
HLA-DPA1*01:DPB1*04:01	1	203	217	15	Consensus (comb.lib./smm)	VMTVGTKTFLVHREW	4.10	4.10	TVGKTFLV	0.01	0.01	0.01	VGTKTFLVH	593.00	8.20	8.20
HLA-DPA1*01:DPB1*04:01	1	201	215	15	Consensus (comb.lib./smm)	YVMTVGTKTFLVHR	4.10	4.10	TVGKTFLV	0.01	0.01	0.01	VGTKTFLVH	585.00	8.20	8.20
HLA-DPA1*01:DPB1*04:01	1	483	497	15	Consensus	LTF LAVGGVLLFLSV	4.50	4.50	AVGGVLLFL	0.03	3.90	3.90	FLAVGGVLL	346.00	5.10	5.10

# Guidelines: Selecting binders

- Based on Percentile rank or MHC binding affinity?  
Recommendation: **IEDB Percentile rank**
- Threshold guidelines:
  - Percentile rank  $\leq$  **10.0** (Percentile rank on linear scale (0-100), lower value = better binder)
  - MHC binding affinity  $IC_{50} \leq$  **1000nM**
- Select all peptides with IEDB percentile rank  $\leq$  10.0

# Alternate approaches for selecting binders

- Change threshold values depending on your need
  - e.g. in case you have too few or too many predicted binders.
- Set a desired percentage within your peptide set (irrespective of IEDB percentile rank) in case you want to study a fixed number of best possible peptides.

# Issue of overlapping peptides

- The tool breaks the sequence into all possible 15-mers - Peptides overlapping by 14 amino acid residues

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	allele	seq_n	start	end	peptide	method	percent	comblib_core	comblib	comblib	smm_align_core	smm_al	smm_al	nn_align_core	nn_a
2	HLA-DPA1*01/DPB1*0401	1	527	541	DHLEFWEGVFTGLTH	Consensus (com	2.52	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	3
3	HLA-DPA1*01/DPB1*0401	1	528	542	HLEFWEGVFTGLTHI	Consensus (com	2.57	FWEGVFTGL	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	Consensus (com	2.62	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	Consensus (com	3.13	FWEGVFTGL	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	Consensus (com	3.26	FWEGVFTGL	6.9	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	5
7	HLA-DPA1*01/DPB1*0401	1	39	53	AAQTFLATCINGVCW	Consensus (com	3.8	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	6
8	HLA-DPA1*01/DPB1*0401	1	262	276	GSPITYSTYGKFLAD	Consensus (com	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	
9	HLA-DPA1*01/DPB1*0401	1	40	54	AQTFLATCINGVCWT	Consensus (com	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	Consensus (com	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5
11	HLA-DPA1*01/DPB1*0401	1	38	52	TAAQTFLATCINGVC	Consensus (com	4.13	TAAQTFLAT	52.52	29.77	FLATCINGV	478	1.49	FLATCINGV	7
12	HLA-DPA1*01/DPB1*0401	1	37	51	STAAQTFLATCINGV	Consensus (com	4.56	TAAQTFLAT	52.52	29.77	TAAQTFLAT	464	1.41	FLATCINGV	8
13	HLA-DPA1*01/DPB1*0401	1	261	275	TGSPITYSTYGKFLA	Consensus (com	4.78	TYSTYGKFL	2.38	15.24	ITYSTYGKF	908	4.78	ITYSTYGKF	
14	HLA-DPA1*01/DPB1*0401	1	530	544	EFWEGVFTGLTHIDA	Consensus (com	5	FWEGVFTGL	6.9	19.59	FWEGVFTGL	664	2.75	FWEGVFTGL	9
15	HLA-DPA1*01/DPB1*0401	1	102	116	SDLYLVTRHADVIPV	Consensus (com	7.45	LVTRHADVI	23.49	25.43	YLVRHADV	1194	7.45	YLVRHADV	14
16	HLA-DPA1*01/DPB1*0401	1	41	55	QTFLATCINGVCWTV	Consensus (com	7.57	QTFLATCIN	728.23	45.84	FLATCINGV	829	4.09	FLATCINGV	16
17	HLA-DPA1*01/DPB1*0401	1	101	115	SSDLYLVTRHADVIP	Consensus (com	7.57	LVTRHADVI	23.49	25.43	YLVRHADV	1206	7.57	YLVRHADV	16
18	HLA-DPA1*01/DPB1*0401	1	260	274	TTGSPITYSTYGKFL	Consensus (com	7.71	TYSTYGKFL	2.38	15.24	ITYSTYGKF	1221	7.71	ITYSTYGKF	10
19	HLA-DPA1*01/DPB1*0401	1	100	114	GSSDLYLVTRHADVI	Consensus (com	7.85	GSSDLYLVT	0.74	11.33	YLVRHADV	1183	7.34	YLVRHADV	17
20	HLA-DPA1*01/DPB1*0401	1	531	545	FWEGVFTGLTHIDAH	Consensus (com	7.97	FWEGVFTGL	6.9	19.59	FWEGVFTGL	728	3.24	FWEGVFTGL	17
21	HLA-DPA1*01/DPB1*0401	1	103	117	DLYLVTRHADVIPVR	Consensus (com	8.57	LVTRHADVI	23.49	25.43	YLVRHADV	1307	8.57	YLVRHADV	16

# Issue of overlapping peptides: Solution

- Pre-processing:
  - Generate 15mers overlapping by 10 AA residues and do the prediction

```
APITAYAQQTRGLLGCIITSLTGRD
APITAYAQQTRGLLG-----
-----YAQQTRGLLGCIITS-----
-----RGLLGCIITSLTGRD
```

- 15 is mostly preferred length for class II
- 10 AA overlap captures minimal 15mers with all possible 9mer binding cores with at least 1 flanking residue
- Python/Perl script or Excel



# Issue of overlapping peptides: Solution

- Post-processing:
  - Remove largely overlapping peptides after prediction (based on same binding core or position)

	A	B	C	D	E	G	H	I	J	K	L	M	N	O
1	allele	seq_n	start	end	peptide	percent	comblib_core	comblib	comblib	smm_align_core	smm_al	smm_al	nn_align_core	nn_a
2	HLA-DPA1*01/DPB1*0401	1	527	541	DHLEFWEGVFTGLTH	2.52	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	3
3	HLA-DPA1*01/DPB1*0401	1	<del>528</del>	<del>542</del>	<del>HLEFWEGVFTGLTHI</del>	<del>2.57</del>	<del>FWEGVFTGL</del>	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	<del>526</del>	<del>540</del>	<del>QDHLEFWEGVFTGLT</del>	<del>2.62</del>	<del>FWEGVFTGL</del>	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	<del>529</del>	<del>543</del>	<del>LEFWEGVFTGLTHID</del>	<del>3.13</del>	<del>FWEGVFTGL</del>	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	<del>525</del>	<del>539</del>	<del>CQDHLEFWEGVFTGL</del>	<del>3.26</del>	<del>FWEGVFTGL</del>	6.9	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	5
7	HLA-DPA1*01/DPB1*0401	1	39	53	AAQTFLATCINGVCW	3.8	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	6
8	HLA-DPA1*01/DPB1*0401	1	262	276	GSPITYSTYGKFLAD	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	
9	HLA-DPA1*01/DPB1*0401	1	<del>40</del>	<del>54</del>	<del>AQTFLATCINGVCWT</del>	<del>4.08</del>	<del>QTFLATCIN</del>	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	<del>263</del>	<del>277</del>	<del>SPITYSTYGKFLADG</del>	<del>4.08</del>	<del>TYSTYGKFL</del>	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5

# Promiscuous binders

- Peptides that bind to more than one MHC molecule.
- Significance:
  - Associated with stronger antigenicity & larger population coverage
  - Important in reducing immunogenicity of therapeutic proteins
  - Can be predicted based on binding affinity
- Consensus percentile rank threshold  $\leq 20.0$

[J Immunol](#). 2010 Jul 15;185(2):943-55. doi: 10.4049/jimmunol.1000405. Epub 2010 Jun 16.

## **Molecular determinants of T cell epitope recognition to the common Timothy grass allergen.**

[Oseroff C](#)<sup>1</sup>, [Sidney J](#), [Kotturi MF](#), [Kolla R](#), [Alam R](#), [Broide DH](#), [Wasserman SI](#), [Weiskopf D](#), [McKinney DM](#), [Chung JL](#), [Petersen A](#), [Grey H](#), [Peters B](#), [Sette A](#).

PMID: 20554959 PMCID: PMC3310373 DOI: 10.4049/jimmunol.1000405

# Promiscuous binders - Multiple alleles

## MHC-II Binding Predictions

Specify Sequence(s)

Enter protein sequence(s) in FASTA format  
[\(Browse for sequences in NCBI\)](#)

```
>HCV_NS3
APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTATQTFLATCINGVCWTVYHGAGTRTIASPKGP
VIQMYTINVDDQLVGWPAAPQGSRLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGS
GPLLCPAGHAVGLFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPAVPQSFQVAHLHAPTGGSGK
STKVPAAYAAQGYKVLVLPNSVAATLGFAYMSKAHGVDPNIRTVRTITTTGSPITYSTYGKFLADGGCS
GGAYDIIICDECHSTDATSLIGIGTVLDQAETAGARLVVLAATATPPGSVTVSHPNIEEVALSTTGEIPFY
GKAIPLEVIKGRHLIFCHSKKKKCELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVSTDALMTGFTG
DFDSVIDCNTCVTQTVDFSLDPTFTIETTLPQDAVSRTQRRGRTRGRGKPGIYRFVAPGERPSGMFDSSV
LCECYDAGCAWYELTPAETTIVRLRAYMNTPLPVCQDHLFEWEGVFTGLTHIDAHFLSQTQSGENFPYL
VAYQATVCARAQAPPPSWDQMWKCLIRLKP TLHGPTPLLYRLGAVQNEVTLTHPITKYIMTCMSADLEVV
T
```

FASTA format detected.

Or select file containing sequence(s)

Browse...

No file selected.

Choose a Prediction Method

Prediction Method ?

Show all the method versions:

IEDB recommended 2.22
▼
[Help on prediction method selections](#)

Specify what to make binding predictions for

Select species/locus

Select MHC allele(s)

Select  $\alpha$  &  $\beta$  chains separately if applicable:  ?

Select full HLA reference set:  ?

Select 7-allele HLA reference set:  ?

Human, HLA-DR ▼

Allele

DPA1\*01/DPB1\*04:01
⊗

DPA1\*03:01/DPB1\*04:02
⊗

DPA1\*02:01/DPB1\*05:01
⊗

DRB1\*01:01
⊗

▼
[Upload allele file](#) ?

Select length(s)

?

default

12-18

as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

# Multiple alleles - result

Allele	#	Start	End	Length	Method used	Peptide	Percentile Rank	Adjusted rank
HLA-DRB1*01:01	1	222	236	15	Consensus (comb.lib./simm/nn)	GYKVLVLNPSVAATL	0.14	0.14
HLA-DRB1*01:01	1	221	235	15	Consensus (comb.lib./simm/nn)	QGYKVLVLNPSVAAT	0.14	0.14
HLA-DRB1*01:01	1	220	234	15	Consensus (comb.lib./simm/nn)	AQGYKVLVLNPSVAA	0.39	0.39
HLA-DRB1*01:01	1	223	237	15	Consensus (comb.lib./simm/nn)	YKVLVLNPSVAATLG	0.39	0.39
HLA-DRB1*01:01	1	224	238	15	Consensus (comb.lib./simm/nn)	KVLVLNPSVAATLGF	1.30	1.30
HLA-DRB1*01:01	1	219	233	15	Consensus (comb.lib./simm/nn)	AAQGYKVLVLNPSVA	1.80	1.80
HLA-DRB1*01:01	1	378	392	15	Consensus (comb.lib./simm/nn)	AAKLVALGINAVAYY	3.60	3.60
HLA-DRB1*01:01	1	379	393	15	Consensus (comb.lib./simm/nn)	AKLVALGINAVAYYR	3.60	3.60
HLA-DRB1*01:01	1	375	389	15	Consensus (comb.lib./simm/nn)	DELAAKLVALGINAV	3.60	3.60
HLA-DRB1*01:01	1	376	390	15	Consensus (comb.lib./simm/nn)	ELAAKLVALGINAVA	3.60	3.60
HLA-DRB1*01:01	1	377	391	15	Consensus (comb.lib./simm/nn)	LAAKLVALGINAVAY	3.60	3.60
HLA-DRB1*01:01	1	225	239	15	Consensus (comb.lib./simm/nn)	VLVLNPSVAATLGFG	3.90	3.90
HLA-DRB1*01:01	1	386	400	15	Consensus (comb.lib./simm/nn)	INAVAYYRGLDVSVI	6.00	6.00
HLA-DRB1*01:01	1	512	526	15	Consensus (comb.lib./simm/nn)	RLRAYMNTPLPVCQ	6.00	6.00
HLA-DRB1*01:01	1	388	402	15	Consensus (comb.lib./simm/nn)	AVAYYRGLDVSVIPT	6.30	6.30
HLA-DRB1*01:01	1	389	403	15	Consensus (comb.lib./simm/nn)	VAYYRGLDVSVIPTS	6.30	6.30
HLA-DRB1*01:01	1	511	525	15	Consensus (comb.lib./simm/nn)	VRLRAYMNTPLPVC	6.40	6.40
HLA-DRB1*01:01	1	555	569	15	Consensus (comb.lib./simm/nn)	ENFPYLVAIQATVCA	6.50	6.50
HLA-DRB1*01:01	1	557	571	15	Consensus (comb.lib./simm/nn)	FPYLVAYQATVCARA	6.50	6.50
HLA-DRB1*01:01	1	554	568	15	Consensus (comb.lib./simm/nn)	GENFPYLVAIQATVC	6.50	6.50
HLA-DRB1*01:01	1	387	401	15	Consensus (comb.lib./simm/nn)	NAVAYYRGLDVSVIP	6.50	6.50
HLA-DRB1*01:01	1	556	570	15	Consensus (comb.lib./simm/nn)	NFPYLVAIQATVCAR	6.50	6.50
HLA-DRB1*01:01	1	553	567	15	Consensus (comb.lib./simm/nn)	SGENFPYLVAIQATV	6.50	6.50
HLA-DRB1*01:01	1	380	394	15	Consensus (comb.lib./simm/nn)	KLVALGINAVAYYRG	7.30	7.30
HLA-DRB1*01:01	1	513	527	15	Consensus (comb.lib./simm/nn)	LRAYMNTPLPVCQD	7.50	7.50
HLA-DRB1*01:01	1	558	572	15	Consensus (comb.lib./simm/nn)	PYLVAIQATVCARAQ	7.60	7.60
HLA-DRB1*01:01	1	559	573	15	Consensus (comb.lib./simm/nn)	YLVAYQATVCARAQA	7.60	7.60
HLA-DRB1*01:01	1	372	386	15	Consensus (comb.lib./simm/nn)	KKCDELAAKLVALGI	8.00	8.00
HLA-DRB1*01:01	1	514	528	15	Consensus (comb.lib./simm/nn)	RAYMNTPLPVCQDH	8.00	8.00
HLA-DRB1*01:01	1	373	387	15	Consensus (comb.lib./simm/nn)	KCDELAAKLVALGIN	8.20	8.20
HLA-DPA1*03:01/DPB1*04:02	1	164	178	15	Consensus (comb.lib./simm)	AKAVDFIPVENLETT	8.30	8.30
HLA-DRB1*01:01	1	226	240	15	Consensus (comb.lib./simm/nn)	LVLNPSVAATLGFGA	8.60	8.60
HLA-DPA1*03:01/DPB1*04:02	1	37	51	15	Consensus (comb.lib./simm)	STATQTFLATCINGV	8.80	8.80
HLA-DPA1*03:01/DPB1*04:02	1	163	177	15	Consensus (comb.lib./simm)	VAKAVDFIPVENLETT	8.85	8.85

# Panel of 27 class II alleles to allow for global coverage

Locus	Molecule	Phenotype frequency
DRB1	DRB1*01:01	5.4
	DRB1*03:01	13.7
	DRB1*04:01	4.6
	DRB1*04:05	6.2
	DRB1*07:01	13.5
	DRB1*08:02	4.9
	DRB1*09:01	6.2
	DRB1*11:01	11.8
	DRB1*12:01	3.9
	DRB1*13:02	7.7
	DRB1*15:01	12.2
	<b>Combined</b>	<b>71.1</b>
DRB3/4/5	DRB3*01:01	26.1
	DRB3*02:02	34.3
	DRB4*01:01	41.8
	DRB5*01:01	16.0
		<b>Combined</b>

Locus	Molecule	Phenotype frequency
DQA1/DQB1	DQA1*05:01/DQB1*02:01	11.3
	DQA1*05:01/DQB1*03:01	35.1
	DQA1*03:01/DQB1*03:02	19.0
	DQA1*04:01/DQB1*04:02	12.8
	DQA1*01:01/DQB1*05:01	14.6
	DQA1*01:02/DQB1*06:02	14.6
	<b>Combined</b>	<b>81.6</b>
DPA1/DPB1	DPA1*02:01/DPB1*01:01	16.0
	DPA1*01:03/DPB1*02:01	17.5
	DPA1*01/DPB1*04:01	36.2
	DPA1*03:01/DPB1*04:02	41.6
	DPA1*02:01/DPB1*05:01	21.7
	DPA1*02:01/DPB1*14:01	7.4
	<b>Combined</b>	<b>94.5</b>

[Immunogenetics](#). 2011 Jun;63(6):325-35. doi: 10.1007/s00251-011-0513-0. Epub 2011 Feb 9.

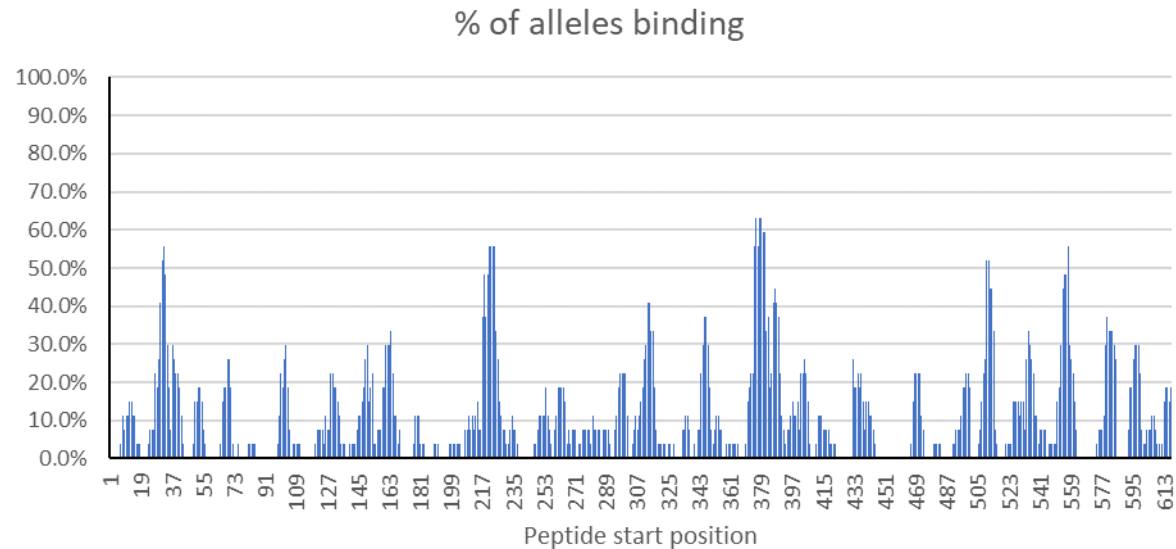
**Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes.**

[Greenbaum J<sup>1</sup>](#), [Sidney J](#), [Chung J](#), [Brander C](#), [Peters B](#), [Sette A](#).

PMID: 21305276 PMCID: [PMC3626422](#) DOI: [10.1007/s00251-011-0513-0](#)

# Promiscuous binders

- Binders with  $\geq 50\%$  alleles binding (consensus percentile  $\leq 20.0$ ) considered promiscuous binders



# “7-allele” method

- Aim was to capture maximum immune response with minimum no. of peptides
- 6 peptide datasets with measured immune responses (SFCs/106 PBMCs)
- 15 or 16mer peptide sets with 10 AA residues overlapping

Dataset	Purpose	No. of Antigens	Total peptides	No. of donors	Reference
Der p/f (House dust mite)	Training data	4	156	20	Hinz et al., 2015, CEA
Phl p (Timothy grass)	Training data	10	425	25	Oseroff et al., 2010, JI
TB-1	Training data	4	71	18	Arlehamn et al., 2012, JI
TB-2	Training data	11	499	32	Arlehamn et al., 2016, PLoS Path
Cockroach	Validation data	6	463	19	Dillon et al., 2015, CEA
Pertussis	Validation data	9	785	23	Bancroft et al., 2016, CEA
TOTAL		44	2399	137	

# “7-allele” method

- Optimal results obtained with a set of 7 alleles:
  - 3 DRB1 alleles with frequency  $\geq 12\%$  (DRB1\*03:01, DRB1\*07:01, DRB1\*15:01) and 4 DRB3/4/5 alleles (DRB3\*01:01, DRB3\*02:02, DRB4\*01:01, DRB5\*01:01)
- Top 21.41% peptides  $\approx 50\%$  response
- **The median consensus percentile rank of the 7 alleles  $\approx 20.0$  - Universal prediction threshold**

[J Immunol Methods](#). 2015 Jul;422:28-34. doi: [10.1016/j.jim.2015.03.022](#). Epub 2015 Apr 7.

## **Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes.**

[Paul S](#)<sup>1</sup>, [Lindestam Arlehamn CS](#)<sup>2</sup>, [Scriba TJ](#)<sup>3</sup>, [Dillon MB](#)<sup>2</sup>, [Oseroff C](#)<sup>2</sup>, [Hinz D](#)<sup>2</sup>, [McKinney DM](#)<sup>2</sup>, [Carrasco Pro S](#)<sup>4</sup>, [Sidney J](#)<sup>2</sup>, [Peters B](#)<sup>2</sup>, [Sette A](#)<sup>2</sup>.

PMID: 25862607 PMCID: [PMC4458426](#) DOI: [10.1016/j.jim.2015.03.022](#)



# “7-allele” method

- Generate 15mers overlapping by 10 AA residues
- Do binding prediction for the **7 selected alleles**
- Estimate the **median consensus percentile rank** (of the 7 alleles)
- Select all peptides with median consensus percentile rank  $\leq$  **20.0**
- This set of peptides can capture  $\approx$  50% of the response
- These 7 alleles can be selected as a set
- This is implemented in **CD4Episcore** tool

# Self-practice (or mini-break): 10 min

## Question:

Predict the alleles from the given set of 6 MHC class II alleles to which the peptide **VRLRAYMNTPLPVC** may bind.

Locus	Alleles
DPA1/DPB1	DPA1*01/DPB1*04:01 DPA1*01:03/DPB1*02:01
DQA1/DQB1	DQA1*05:01/DQB1*03:01 DQA1*03:01/DQB1*03:02
DRB1	DRB1*03:01 DRB1*07:01

# Self-practice

## Steps:

1. Predict the binding affinity of the peptide for the given alleles

Peptide: **VRLRAYMNTPLPVC**

Alleles:

Locus	Alleles
DPA1/DPB1	DPA1*01/DPB1*04:01 DPA1*01:03/DPB1*02:01
DQA1/DQB1	DQA1*05:01/DQB1*03:01 DQA1*03:01/DQB1*03:02
DRB1	DRB1*03:01 DRB1*07:01

2. Identify alleles with consensus percentile rank  $\leq 10.0$

# Self-practice: input

## MHC-II Binding Predictions

### Specify Sequence(s)

Enter protein sequence(s) in FASTA format  
([Browse for sequences in NCBI](#))

VRLRAYMNTPLPVC

Space\_separated format detected.

Or select file containing sequence(s)  No file chosen

### Choose a Prediction Method

Prediction Method [?](#)  
Show all the method versions:  IEDB recommended 2.22 [Help on prediction method selections](#)

### Specify what to make binding predictions for

Select species/locus: Human, HLA-DR [?](#)

Select MHC allele(s)  
Select  $\alpha$  &  $\beta$  chains separately if applicable:  [?](#)

[Select full HLA reference set:](#)  [?](#)  
[Select 7-allele HLA reference set:](#)  [?](#)

Allele

DPA1\*01/DPB1\*04:01

DPA1\*01:03/DPB1\*02:01

DQA1\*05:01/DQB1\*03:01

DQA1\*03:01/DQB1\*03:02

DRB3\*01:01

DRB1\*07:01

[?](#)

Select length(s) [?](#)

default 12-18 as is

11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

### Specify Output

Sort peptides by: Adjusted Rank [?](#)

Output format: XHTML table [?](#)

Email address (optional):  [?](#)


# Self-practice: output

## MHC-II Binding Prediction Results

### Input Sequences

#	Name	Sequence
1	sequence 1	VRLRAYMNTPLPVC

Prediction method: IEDB recommended | Low adjusted\_rank = good binders

[Download result](#) 

### Citations

Check to expand the result:

Allele	#	Start	End	Length	Method used	Peptide	Percentile Rank	Adjusted rank
HLA-DRB1*07:01	1	1	15	15	Consensus (comb.lib./smm/nn)	VRLRAYMNTPLPVC	0.64	0.64
HLA-DPA1*01/DPB1*04:01	1	1	15	15	Consensus (comb.lib./smm)	VRLRAYMNTPLPVC	10.75	10.75
HLA-DRB3*01:01	1	1	15	15	Consensus (comb.lib./smm/nn)	VRLRAYMNTPLPVC	14.00	14.00
HLA-DPA1*01:03/DPB1*02:01	1	1	15	15	Consensus (comb.lib./smm)	VRLRAYMNTPLPVC	37.50	37.50
HLA-DQA1*05:01/DQB1*03:01	1	1	15	15	Consensus (comb.lib./smm)	VRLRAYMNTPLPVC	45.00	45.00
HLA-DQA1*03:01/DQB1*03:02	1	1	15	15	Consensus (comb.lib./smm)	VRLRAYMNTPLPVC	74.00	74.00

[Download result](#) 

### Citations:

If you use these predictions in a manuscript, please include the following in the method section:

The MHCII binding predictions were made on 11/7/2019 using the IEDB analysis resource Consensus tool [1]

# TepiTool

- New interface to prediction of class I and class II epitope candidates
- Motivation:
  - Make tools more user friendly
  - Provide recommendations as default
  - Provide a set of top peptides as concise results
- In the form of a step-by-step wizard (6 steps)
- Provides recommendations as default values
- Input parameters can be adjusted as desired
- New methods incorporated

# Step 1: Sequence data

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

[Home](#) [Help](#) [Reference](#) [Download](#) [Contact](#)

### TepiTool

Steps **1** 2 3 4 5 6

**SEQUENCE - Provide sequence data:**

Enter sequence(s) in FASTA or PLAIN format.

```
>Seq_1
MKALIVLGLVLLSVTVQGKVFCEARTLKRLGMDGYRGISLANWMCLAKW
>Seq_2
MLLALVCLLSCLANSDF
>Seq_3
MKALIVLGLVLLSVTVQGKVFERCELAR
```

FASTA format detected.

Or upload file containing sequence(s)  No file chosen

# Step 2: Species & allele class

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

The screenshot shows the IEDB Analysis Resource TepiTool interface. At the top, there is a blue header with the text "IEDB Analysis Resource". Below the header is a navigation bar with buttons for "Home", "Help", "Reference", "Download", and "Contact". The main content area is titled "TepiTool" and features a progress indicator with steps 1 through 6, where step 2 is highlighted. The current step is "SPECIES & ALLELE CLASS - Select the host species and MHC allele class:". The form contains two dropdown menus: "Host species" (set to "Human") and "Allele class" (with "Class I" and "Class II" options). A red box highlights the "Allele class" dropdown, and another red box highlights the species dropdown menu which is open, showing options: Chimpanzee, Cow, Gorilla, Human, Macaque, Mouse, and Pig. A red arrow points from the "Current selections:" box to the species dropdown menu. The "Current selections:" box shows "No. of sequences" as 3. At the bottom of the form are "Start Over" and "Back" buttons.



# Step 3: Alleles - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

**IEDB Analysis Resource**

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## TepiTool

Steps 1 2 **3** 4 5 6

**ALLELES - Specify alleles:**

Human - Class I

- Select from list of frequently occurring alleles (Frequency > 1%)
- Select from list of all available alleles
- Select from list of representative alleles from different HLA supertypes
- Use panel of 27 most frequent A & B alleles
- Upload allele file

Alleles

- A\*01:01
- A\*02:01
- A\*02:06
- A\*03:01
- A\*11:01
- A\*23:01
- A\*24:02
- A\*25:01
- A\*26:01
- A\*29:02
- A\*30:01
- A\*30:02

**Current selections:**

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

[Reset alleles](#)

Start Over Back Next

# Step 4: Peptides - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps 1 2 3 **4** 5 6

#### PEPTIDES - Select peptides to be included in prediction:

Peptides to be included in prediction	<input checked="" type="radio"/> Apply default settings for low number of peptides <input type="radio"/> Apply default settings for moderate number of peptides <input type="radio"/> Apply default settings for high number of peptides <input type="radio"/> Custom selection - Select your own settings
	Handling of duplicate peptides: - Duplicate peptides will be removed.
	Peptide lengths to be considered in prediction: - Only peptide length 9 will be included 9mers = 58
Conservancy analysis (Uses only peptides conserved in specified % of sequences)	<input checked="" type="radio"/> No <input type="radio"/> Yes

Start Over Back Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

# Step 4: Peptides - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps 1 2 3 **4** 5 6

#### PEPTIDES - Select peptides to be included in prediction:

Peptides to be included in prediction	<input type="radio"/> Apply default settings for low number of peptides <input checked="" type="radio"/> Apply default settings for moderate number of peptides <input type="radio"/> Apply default settings for high number of peptides <input type="radio"/> Custom selection - Select your own settings
	Handling of duplicate peptides: - Duplicate peptides will be removed.
	Peptide lengths to be considered in prediction: - Only peptide lengths 8-11 will be included 8mers = 60 9mers = 58 10mers = 56 11mers = 54
Conservancy analysis (Uses only peptides conserved in specified % of sequences)	<input checked="" type="radio"/> No <input type="radio"/> Yes

[Start Over](#) [Back](#) [Next](#)

#### Current selections:

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

# Step 4: Peptides - Class I

[tools.iedb.org/tepitool/](http://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps 1 2 3 **4** 5 6

#### PEPTIDES - Select peptides to be included in prediction:

Peptides to be included in prediction

- Apply default settings for low number of peptides
- Apply default settings for moderate number of peptides
- Apply default settings for high number of peptides
- Custom selection - Select your own settings

---

Handling of duplicate peptides:

- Duplicate peptides will not be removed.

---

Peptide lengths to be considered in prediction:

- All peptide lengths (8-14) will be included
- 8mers = 74
- 9mers = 71
- 10mers = 68
- 11mers = 65
- 12mers = 62
- 13mers = 59
- 14mers = 56

Conservancy analysis  
(Uses only peptides conserved in specified % of sequences)

- No
- Yes

Start Over Back Next

#### Current selections:

No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

# Step 4: Peptides - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps 1 2 3 **4** 5 6

#### PEPTIDES - Select peptides to be included in prediction:

Peptides to be included in prediction

- Apply default settings for low number of peptides
- Apply default settings for moderate number of peptides
- Apply default settings for high number of peptides
- Custom selection - Select your own settings

Handling of duplicate peptides:

- Remove duplicate peptides
- Keep duplicate peptides

Peptide lengths to be considered in prediction:

- 8mers = 60
- 9mers = 58
- 10mers = 56
- 11mers = 54
- 12mers = 52
- 13mers = 50
- 14mers = 48

Conservancy analysis  
(Uses only peptides conserved in specified % of sequences)

- No
- Yes

Use peptides conserved in

Start Over Back Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01

1 sequence

10% sequences

20% sequences

30% sequences

40% sequences

50% sequences

60% sequences

70% sequences

80% sequences

90% sequences

100% sequences

# Step 5: Method - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps 1 2 3 4 5 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use	IEDB recommended
Selection of predicted peptides	Select peptides based on predicted percentile rank Select peptides with predicted consensus percentile rank $\leq$ 1

[Start Over](#) [Back](#) [Next](#)

- IEDB recommended
- Consensus
- NetMHCpan
- ANN
- SMMPMBEC
- SMM
- CombLib\_Sidney\_2008

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class I
Selected alleles	1.A*02:01 2.A*02:06 3.A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
No. of peptides included (Not considering conservancy analysis)	114
Conservancy analysis	Peptides conserved in at least 50% sequences

- Select peptides based on predicted percentile rank
- Select peptides based on predicted IC50
- Select peptides based on MHC specific predicted binding threshold\*
- Select top x% of predicted peptides\*\*
- Select top x number of predicted peptides\*\*

# Step 5: Method - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

Steps 1 2 3 4 **5** 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use: IEDB recommended

Selection of predicted peptides: **Select peptides based on predicted IC50**

Select peptides with predicted IC50  $\leq$  500 nM

Start Over Back Next

Steps 1 2 3 4 **5** 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use: IEDB recommended

Selection of predicted peptides: **Select top x% of predicted peptides\*\***

Select top 2% of 114 peptides = 2 peptide(s) per allele x 3 allele(s) = 6 peptides

(\*\*Fi

Start Over Back Next

Steps 1 2 3 4 **5** 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use: IEDB recommended

Selection of predicted peptides: **Select top x number of predicted peptides\*\***

Select top 5 peptides per allele (Maximum possible = 114)

(\*Peptide selection done based on percentile rank)

Start Over Back Next

# Step 5: Method - Class I

Steps 1 2 3 4 **5** 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use: IEDB recommended ▼

Select peptides based on MHC specific predicted binding threshold ▼

(\*Each MHC allele has its own IC50 threshold.  
Predicted peptides will correspond to 75% of immune response.  
Prediction method is **SMM**)

As of now, only the following alleles are covered by this method:

- A\*01:01
- A\*02:01
- A\*02:03
- A\*02:06
- A\*03:01
- A\*11:01
- A\*23:01
- A\*24:02
- A\*25:01
- A\*26:01
- A\*29:02
- A\*30:01
- A\*30:02
- A\*31:01
- A\*32:01
- A\*33:01
- A\*68:01
- A\*68:02
- B\*07:02
- B\*08:01
- B\*14:02
- B\*15:01
- B\*18:01
- B\*27:05
- B\*35:01
- B\*35:03
- B\*38:01
- B\*39:01
- B\*40:01
- B\*40:02
- B\*44:02
- B\*44:03
- B\*46:01
- B\*48:01
- B\*51:01
- B\*53:01
- B\*57:01
- B\*58:01

Selection of predicted peptides

Please refer this paper for more details: [Paul et al., \(2013\)](#) J of Immunol. 191(12): 5831-5839.

Start Over Back Next



# Step 5: Method - Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

## IEDB Analysis Resource

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### TepiTool

Steps [1](#) [2](#) [3](#) [4](#) [5](#) **[6](#)**

**REVIEW: Review selections, enter job details & submit data:**

**Summary:**

No. of sequences	3
Host species	Human
Allele class	Class I
Alleles	1.A*02:01 2.A*02:06 3.A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
Approx no. of peptides included	114
Peptide overlap	N/A (all possible nmers are included in class I)
Conservancy analysis	Peptides conserved in at least 50% sequences
Prediction method	IEDB recommended
Peptide selection criterion	Based on predicted consensus percentile rank (Cutoff selected = 1)

**Job details:**

Job name (optional)	<input type="text"/>
Email (optional - will notify when job is finished)	<input type="text" value="spaul@lji.org"/>

[Start Over](#) [Back](#) [Submit](#)


(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)

# Results: Class I

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)



[Home](#) [Help](#) [Reference](#) [Download](#) [Contact](#)

## TepiTool

Prediction results - concise ([Download table](#) ):

Seq #	Peptide start	Peptide end	Peptide	Percentile rank	Allele	Conservancy
1	5	14	IVLGI VLLSV	0.3	HLA-A*02:06	67%
1	10	19	VLLSVTVQ GK	0.36	HLA-A*03:01	67%
1	5	14	IVLGI VLLSV	0.77	HLA-A*02:01	67%
1	6	14	VLGLVLLSV	0.84	HLA-A*02:01	67%
1	11	19	LLSVTVQ GK	0.89	HLA-A*03:01	67%

Download results details:

<a href="#">Complete results</a> 	Prediction results of all peptides
<a href="#">Conservancy of peptides</a> 	Conservancy of peptides in the sequences

Citation information:

If you use these predictions in a manuscript, please include the following in the method section:

The MHC I binding predictions were done with IEDB analysis resource (TepiTool [1]) using Consensus method [2] which employs SMM, ANN and Combinatorial library methods.

1. Paul, S., Sidney, J., Sette, A., and Peters, B. 2016. TepiTool: A pipeline for computational prediction of T cell epitope candidates. *Curr. Protoc. Immunol.* 114:18.19.1-18.19.24.
2. Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M, Peters B. 2010. Peptide binding predictions for HLA DR, DP and DQ molecules. *BMC Bioinformatics.* 11:568.
3. Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLoS Comput Biol.* 4(4): e1000048.

For complete list of references please click here: [References](#)

**Differences in TepiTool workflow if Class II?**

# Step 3: Alleles - Class II

**IEDB Analysis Resource**

Home Help Reference Download Contact

### TepiTool

Steps 1 2 **3** 4 5 6

#### ALLELES - Specify alleles:

**Human - Class II**

Predict for custom allele set  
 Predict for pre-selected panel of alleles  
 Predict using pre-selected allele sets & methods

Options:

Select from list of alleles  
 Upload allele file

Alleles

Select  $\alpha$  and  $\beta$  chains separately when applicable

DQ

- DQA1\*01:01/DQB1\*05:01
- DQA1\*01:02/DQB1\*06:02
- DQA1\*03:01/DQB1\*03:02
- DQA1\*04:01/DQB1\*04:02
- DQA1\*05:01/DQB1\*02:01
- DQA1\*05:01/DQB1\*03:01

Start Over Back Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Selected alleles	
<a href="#">Reset alleles</a>	

# Step 4: Peptides - Class II

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

**IEDB Analysis Resource**

Home Help Reference Download Contact

**TepiTool**

Steps 1 2 3 **4** 5 6

**PEPTIDES - Select peptides to be included in prediction:**

Peptides to be included in prediction

- Apply default settings for low number of peptides
- Apply default settings for moderate number of peptides
- Apply default settings for high number of peptides
- Custom selection - Select your own settings

Handling of duplicate peptides  
- Duplicate peptides will be removed.

Desired no. of overlapping residues for 15mers  
- No. of overlapping residues fixed at 10.

Approximate no. of peptides to be considered for prediction =  
12

Conservancy analysis  
(Uses only peptides conserved in specified % of sequences)

No  
 Yes

Use peptides conserved in

Start Over Back Next

*settings  
summary* →

	Low	Moderate	High	Custom
<b>Duplicates</b>	removed	removed	not removed	user selects
<b>Overlapping residues</b>	8	10	10	user selects
<b>Approx. # peptides</b>	10	12	14	12

**Current selections:**

No. of sequences	3
Host species	Human
Allele class	Class II
Selected alleles	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03

# Step 5: Method - Class II

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

**IEDB Analysis Resource**

Home Help Reference Download Contact

### TepiTool

Steps 1 2 3 4 **5** 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use: IEDB recommended

Selection of predicted peptides: Select peptides based on predicted percentile rank

Select peptides with predicted consensus percentile rank  $\leq$  10

Start Over Back Next

**Current selections:**

No. of sequences	3
Host species	Human
Allele class	Class II
Alleles selected	1.DPA1*01:03/DPB1*02:01 2.DQA1*03:01/DQB1*03:02 3.DRB1*01:01 4.DRB1*01:02 5.DRB1*01:03
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx. no. of peptides included (Not considering conservancy analysis)	12
Conservancy analysis	Peptides conserved in at least 50% sequences

- IEDB recommended
- Consensus
- NetMHCIIpan
- NN\_align
- SMM\_align
- Combinatorial library
- Sturniolo

- Select peptides based on predicted percentile rank
- Select peptides based on predicted IC50
- Select peptides based on predicted no. of alleles binding
- Select top x% of peptides\*
- Select top x number of predicted peptides\*

*exclusive to class II*

Select peptides based on predicted no. of alleles binding

Select peptides that bind to at least 50% alleles

(binding determined by IEDB consensus percentile rank  $\leq$  20.0)

# Results – Class II

## IEDB Analysis Resource




[Home](#) [Help](#) [Reference](#) [Download](#) [Contact](#)

### TepiTool

Prediction results - concise ([Download table](#) ):

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele	Conservancy
1	2	16	KALIVLGLVLLSVTV	2.30	HLA-DRB1*01:01	67.0%
1	7	21	LGLVLLSVTVQGVKVF	8.70	HLA-DRB1*01:01	67.0%
1	3	17	ALIVLGLVLLSVTVQ	7.60	HLA-DRB1*01:02	67.0%

Download results details:

<a href="#">Non-redundant results</a> 	Prediction results with redundant peptides within each sequence removed - Includes positives and negatives
<a href="#">Complete results</a> 	Prediction results of all peptides
<a href="#">Conservancy of peptides</a> 	Conservancy of peptides in the sequences

Citation information:

If you use these predictions in a manuscript, please include the following in the method section:

The MHC II binding predictions were done with IEDB analysis resource (TepiTool [1]) using Consensus method [2,3] which employs SMM\_align, NN\_align, Combinatorial library, Sturniolo methods and NetMHCIIpan [4,5].

1. Paul, S., Sidney, J., Sette, A., and Peters, B. 2016. TepiTool: A pipeline for computational prediction of T cell epitope candidates. *Curr. Protoc. Immunol.* 114:18.19.1-18.19.24.
2. Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M, Peters B. 2010. Peptide binding predictions for HLA DR, DP and DQ molecules. *BMC Bioinformatics.* 11:568.
3. Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. 2008. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLoS Comput Biol.* 4(4): e1000048.
4. Karosiene E1, Rasmussen M, Blicher T, Lund O, Buus S, Nielsen M. 2013. NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. *Immunogenetics.* 65(10): 711.
5. Nielsen M, Lundegaard C, Blicher T, Peters B, Sette A, Justesen S, Buus S, and Lund O. 2008. Quantitative predictions of peptide binding to any HLA-DR molecule of known sequence: NetMHCIIpan. *PLoS Comput Biol.* 4(7): e1000107.

For complete list of references please click here: [References](#)

**Differences if 7 allele method or promiscuous?**

# Step 3-5: Class II -7 allele method

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

Steps 1 2 **3** 4 5 6

### ALLELES - Specify alleles:

Human - Class II

Predict for custom allele set  
 Predict for pre-selected panel of alleles  
 Predict using pre-selected allele sets & methods

Alleles

Options:

Use the "7-allele method"  
 Use panel of 26 most frequent alleles for promiscuous binding

- Selection criterion is median of percentile ranks from the 7 alleles involved.

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Selected alleles	<ul style="list-style-type: none"><li>1. DRB1*03:01</li><li>2. DRB1*07:01</li><li>3. DRB1*15:01</li><li>4. DRB3*01:01</li><li>5. DRB3*02:02</li><li>6. DRB4*01:01</li><li>7. DRB5*01:01</li></ul>

[Reset alleles](#)

Start Over

Steps 1 2 3 **4** 5 6

### PEPTIDES - Select peptides to be included in prediction:

Handling of duplicate peptides	Duplicate peptides will be removed
No. of overlapping residues for 15mer peptides to be generated (Peptide length is fixed at 15 for class II)	10
Approximate no. of peptides to be considered for prediction	12

Start Over Back Next

Steps 1 2 3 4 **5** 6

### METHOD - Select prediction & peptide selection methods and cutoff values:

Prediction method to use	IEDB recommended
Selection of predicted peptides	Promiscuity based on "7-allele method" - Peptides considered as binders if median consensus percentile $\leq$ <input type="text" value="20"/>

Start Over Back Next

# Step 3-5: Class II – promiscuous

[tools.iedb.org/tepitool/](https://tools.iedb.org/tepitool/)

Steps 1 2 3 4 5 6

**ALLELES - Specify alleles:**

Human - Class II

Predict for custom allele set

Predict for pre-selected panel of alleles

Predict using pre-selected allele sets & methods

Options:

Use the "7-allele method"

Use panel of 26 most frequent alleles for promiscuous binding

Alleles

Current selections:

No. of sequences	3
Host species	Human
Allele class	Class II

Selected alleles  
[Reset alleles](#)

1. HLA-DPA1\*01/DPB1\*04:01
2. HLA-DPA1\*01:03/DPB1\*02:01
3. HLA-DPA1\*02:01/DPB1\*01:01
4. HLA-DPA1\*02:01/DPB1\*05:01
5. HLA-DPA1\*03:01/DPB1\*04:02
6. HLA-DQA1\*01:01/DQB1\*05:01
7. HLA-DQA1\*01:02/DQB1\*06:02
8. HLA-DQA1\*03:01/DQB1\*03:02
9. HLA-DQA1\*04:01/DQB1\*04:02
10. HLA-DQA1\*05:01/DQB1\*02:01
11. HLA-DQA1\*05:01/DQB1\*03:01
12. HLA-DRB1\*01:01
13. HLA-DRB1\*03:01
14. HLA-DRB1\*04:01
15. HLA-DRB1\*04:05
16. HLA-DRB1\*07:01
17. HLA-DRB1\*08:02
18. HLA-DRB1\*09:01
19. HLA-DRB1\*11:01
20. HLA-DRB1\*12:01
21. HLA-DRB1\*13:02
22. HLA-DRB1\*15:01
23. HLA-DRB3\*01:01
24. HLA-DRB3\*02:02
25. HLA-DRB4\*01:01
26. HLA-DRB5\*01:01

Steps 1 2 3 4 5 6

**PEPTIDES - Select peptides to be included in prediction:**

Handling of duplicate peptides	Duplicate peptides will be removed
No. of overlapping residues for 15mer peptides to be generated (Peptide length is fixed at 15 for class II)	10
Approximate no. of peptides to be considered for prediction	

Start Over Back Next

Steps 1 2 3 4 5 6

**METHOD - Select prediction & peptide selection methods and cutoff values:**

Prediction method to use	IEDB recommended
Selection of predicted peptides	Promiscuity based on no. of alleles binding (Peptide considered as binder if it binds to at least 50% of the 26 most frequent alleles)

Start Over Back Next



# Datasets

the paper, different cross-validation strategies (i.e. `cv_rnd`, `cv_sr`, and `cv_gs`) were tested. Please see the Methods section for details of the cross-validation strategies.

- **Data format:** Text file format.
- **Dataset availability:** [benchmark\\_reliability.tar.gz](#)
- Dataset used for retraining the IEDB class I binding prediction tools.
  - **Description of the dataset:** The dataset is largely identical to that of Kim et al (2014), described above, but includes additional data that was not publicly available at the time.
  - **Date of the dataset generation:** 2013
  - **Details on the dataset generation:** The dataset was compiled from three sources: the IEDB, the Sette lab, and the Buus lab. If a peptide/allele combination had more than 1 measurement among the three sources, its geometric mean was taken.
  - **Data format:** Compressed text file containing binding data.
  - **Dataset availability:** [binding\\_data\\_2013.zip](#)
- Derivation of an amino acid similarity matrix for peptide: MHC binding and its application as a Bayesian prior.  
*Kim Y, Sidney J, Pinilla C, Sette A, Peters B.*  
BMC Bioinformatics, 2009.
  - **Description of the dataset:** Cross-validated predictive performances for SMMPMBEC using the same binding data set as in [Peters et al. PLOS Comput Biol 2006].
  - **Date of the dataset generation:** 2009
  - **Details on the dataset generation:** Using the same cross-validation data partitions as was done for ANN and ARB in 2006, cross-validated predictions using SMMPMBEC were made.
  - **Data format:** A table in Excel file format.
  - **Dataset availability:** <http://www.biomedcentral.com/1471-2105/10/394/additional>
- A Community Resource Benchmarking Predictions of Peptide Binding to MHC-I Molecules.  
*Peters B, Bui HH, Frankild S, Nielsen M, Lundegaard C, Kostem E, Basch D, Lamberth K, Harndahl M, Fleri W, Wilson SS, Sidney J, Lund O, Buus S, Sette A.*  
PLOS Computational Biology, 2006.
  - **Description of the dataset:** Experimentally measured peptide binding affinities for MHC class I molecules from two sources: the Alessandro Sette lab at the La Jolla Institute and the Soren Buus lab at the University of Copenhagen. The dataset contains 48,828 affinities and covers a total of 48 mouse, human, macaque and chimpanzee MHC class I alleles.
  - **Date of the dataset generation:** 2006
  - **Details on the dataset generation:** Used two different assays to generate the binding data.
  - **Data format:** Compressed text files containing experimental binding data as well as cross-validated predicted affinities.
  - **Dataset availability:** [ANN](#), [ARB](#), [SMM](#)

# Benchmarking of class I epitope prediction methods

- List of 44 methods

- Freely available

- Selected 15 methods

- Trained models for H-2D<sup>b</sup> & H-2K<sup>b</sup>

- 2 variants

- NetMHCpan-4.0-B & L
- MHCflurry-B & L
- Total 17 methods

#	Method	Year first published*	URLs to method	Reference	URL to manuscript**	Included/Excluded (re
1	BIMAS	1994	<a href="https://www.bimas.cit.nih.gov/">https://www.bimas.cit.nih.gov/</a>	Parker et al., 1994	<a href="http://www.jimmunol.org/">http://www.jimmunol.org/</a>	Included
2	PREDEP	1995	<a href="http://margalit.huji.ac.il/Tal/">http://margalit.huji.ac.il/Tal/</a>	Altuvia et al., 1995	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Included
3	SYFPEITHI	1997	<a href="http://www.syfpeithi.de/">http://www.syfpeithi.de/</a>	Rammensee et al., 1997	<a href="https://www.springer.com/">https://www.springer.com/</a>	Included
4	Rankpep	2002	<a href="http://imed.med.ucm.es/Tools/rankpep/">http://imed.med.ucm.es/Tools/rankpep/</a>	Reche et al., 2002	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Included
5	ProPred1	2003	<a href="http://crdd.osdd.net/raghava/propred1/">http://crdd.osdd.net/raghava/propred1/</a>	Singh and Raghava, 2003	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Included
6	SMM	2005	<a href="http://tools.iedb.org/mhci/">http://tools.iedb.org/mhci/</a>	Peters and Sette, 2005	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Included
7	ARB	2005	<a href="http://tools.iedb.org/mhci/">http://tools.iedb.org/mhci/</a>	Bui et al., 2005	<a href="https://link.springer.com/">https://link.springer.com/</a>	Included
8	IEDB Consensus	2006	<a href="http://tools.iedb.org/mhci/">http://tools.iedb.org/mhci/</a>	Moutaftsi et al., 2006	<a href="https://www.nature.com/">https://www.nature.com/</a>	Included
9	SMPMBEC	2009	<a href="http://tools.iedb.org/mhci/">http://tools.iedb.org/mhci/</a>	Kim et al., 2009	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Included
10	PACComplex	2011	<a href="http://pacocomplex.life.nctu.edu.tw/">http://pacocomplex.life.nctu.edu.tw/</a>	Liu et al., 2011	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Included
11	NetMHCpan-3.0	2016	<a href="http://www.cbs.dtu.dk/services/NetMHCpan-3.0/">http://www.cbs.dtu.dk/services/NetMHCpan-3.0/</a>	Nielsen and Andreatta, 2016	<a href="https://genomemedicine.com/">https://genomemedicine.com/</a>	Included
12	NetMHC-4.0	2016	<a href="http://www.cbs.dtu.dk/services/NetMHC-4.0/">http://www.cbs.dtu.dk/services/NetMHC-4.0/</a>	Andreatta and Nielsen, 2016	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Included
13	NetMHCpan-4.0***	2017	<a href="http://www.cbs.dtu.dk/services/NetMHCpan-4.0.3/">http://www.cbs.dtu.dk/services/NetMHCpan-4.0.3/</a>	Jurtz et al., 2017	<a href="http://www.jimmunol.org/">http://www.jimmunol.org/</a>	Included
14	MHCflurry***	2018	<a href="https://openvax.github.io/">https://openvax.github.io/</a>	O'Donnell et al., 2018	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Included
15	MHCLovac	Not published yet	<a href="https://github.com/stef333/mhclovac">https://github.com/stef333/mhclovac</a>	Stojanovic, S.	-	Included
16	CTLPred	2004	<a href="http://crdd.osdd.net/raghava/ctlpred/">http://crdd.osdd.net/raghava/ctlpred/</a>	Bhasin and Raghava, 2004	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Excluded
17	EpiJen	2006	<a href="http://www.dgg-pharmfac.com/epijen/">http://www.dgg-pharmfac.com/epijen/</a>	Doytchinova et al., 2006	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Excluded (Trained model)
18	nHLAPred (ANNPred, ComPred)	2006	<a href="http://crdd.osdd.net/raghava/nhlapred/">http://crdd.osdd.net/raghava/nhlapred/</a>	Bhasin and Raghava, 2006	<a href="https://www.ias.ac.in/">https://www.ias.ac.in/</a>	Excluded
19	SVMHC	2006	<a href="http://abi.inf.uni-tuebingen.de/svmhc/">http://abi.inf.uni-tuebingen.de/svmhc/</a>	Donnes and Kohlbach, 2006	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Method not available)
20	SVRMHC	2006	<a href="http://c1.accurascience.com/svmhc/">http://c1.accurascience.com/svmhc/</a>	Wan et al., 2006	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Excluded (Not working)
21	KISS	2007	<a href="http://cbio.enscm.fr/kiss/">http://cbio.enscm.fr/kiss/</a>	Jacob and Vert, 2007	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Not working)
22	PickPocket	2009	<a href="http://www.cbs.dtu.dk/services/PickPocket/">http://www.cbs.dtu.dk/services/PickPocket/</a>	Zhang et al., 2009	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Author suggested)
23	Multipred2	2011	<a href="http://cvc.dfci.harvard.edu/multipred2/">http://cvc.dfci.harvard.edu/multipred2/</a>	Zhang et al., 2011	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Excluded (Trained model)
24	NetMHCcons	2011	<a href="http://www.cbs.dtu.dk/services/NetMHCcons/">http://www.cbs.dtu.dk/services/NetMHCcons/</a>	Karosiene et al., 2011	<a href="https://link.springer.com/">https://link.springer.com/</a>	Excluded (Author suggested)
25	KernelIRLSpanl	2014	<a href="https://github.com/guoxinshen/kernelirspanl">https://github.com/guoxinshen/kernelirspanl</a>	Shen et al., 2014	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Excluded (Trained model)
26	NIELuter	2015	<a href="http://immunet.cn/nie/cgi-bin/nie.cgi">http://immunet.cn/nie/cgi-bin/nie.cgi</a>	Tang et al., 2015	<a href="https://www.sciencedirect.com/">https://www.sciencedirect.com/</a>	Excluded (Trained model)
27	HONN	2015	Method not available	Kuksa et al., 2015	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Trained model)
28	ESMACS	2015	Method not available	Wan et al., 2015	<a href="https://pubs.acs.org/">https://pubs.acs.org/</a>	Excluded (Too resource intensive)
29	sNeBula	2016	Method not available	Luo et al., 2016	<a href="https://www.nature.com/">https://www.nature.com/</a>	Excluded (Trained model)
30	HLaffy	2016	<a href="http://proline.biochem.iisc.ernet.in/hlaffy/">http://proline.biochem.iisc.ernet.in/hlaffy/</a>	Mukherjee et al., 2016	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Trained model)
31	ConvMHC	2017	<a href="http://jumong.kaist.ac.kr/convmhc/">http://jumong.kaist.ac.kr/convmhc/</a>	Han and Kim, 2017	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Excluded (Trained model)
32	PSSMHCpan	2017	<a href="https://github.com/BGI2017/pssmhcpan">https://github.com/BGI2017/pssmhcpan</a>	Liu et al., 2017	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Trained model)
33	MixMHCpred	2017	<a href="https://github.com/Gfeller/mixmhc">https://github.com/Gfeller/mixmhc</a>	Bassani-Sternberg et al., 2017	<a href="https://journals.plos.org/">https://journals.plos.org/</a>	Excluded (Trained model)
34	HLA-CNN (HLA-bind)	2017	<a href="https://github.com/uci-cbd/hla-cnn">https://github.com/uci-cbd/hla-cnn</a>	Yang and Xie, 2017	<a href="https://academic.oup.com/">https://academic.oup.com/</a>	Excluded (Trained model)
35	EDGE	2018	Model provided as part of	Bulik-Sullivan et al.	<a href="https://www.nature.com/">https://www.nature.com/</a>	Excluded (Not working)
36	MAM	2018	<a href="http://mhc.deepomics.org/">http://mhc.deepomics.org/</a>	Xiao et al., 2018	<a href="https://bmcbioinformatics.org/">https://bmcbioinformatics.org/</a>	Excluded (Trained model)
37	DeepSeqPan	2019	<a href="https://github.com/ppcliu/deepseqpan">https://github.com/ppcliu/deepseqpan</a>	Liu et al., 2019	<a href="https://www.nature.com/">https://www.nature.com/</a>	Excluded (Trained model)
38	ForestMHC	2019	<a href="https://github.com/kmboe/forestmhc">https://github.com/kmboe/forestmhc</a>	Boehm et al., 2019	<a href="https://link.springer.com/">https://link.springer.com/</a>	Excluded (Trained model)
39	DeepMHC	Not published yet	<a href="http://mleg.cse.sc.edu/deepmhc/">http://mleg.cse.sc.edu/deepmhc/</a>	Hu and Liu	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Trained model)
40	AI-MHC	Not published yet	<a href="https://baras.pathology.jhu.edu/ai-mhc/">https://baras.pathology.jhu.edu/ai-mhc/</a>	Sidhom et al.	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Trained model)
41	MHCSeqNet	Not published yet	<a href="https://github.com/cmbcu/mhcseqnet">https://github.com/cmbcu/mhcseqnet</a>	Phloyphisut et al.	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Trained model)
42	ACME	Not published yet	<a href="https://github.com/HYSxe/acme">https://github.com/HYSxe/acme</a>	Hu et al.	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Trained model)
43	Deep-Learning-MHCI	Not published yet	<a href="https://github.com/altayg/deep-learning-mhci">https://github.com/altayg/deep-learning-mhci</a>	Altay, G.	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Trained model)
44	MHCnuggets	Not published yet	<a href="https://karchinlab.org/app/mhc-nuggets/">https://karchinlab.org/app/mhc-nuggets/</a>	Bhattacharya et al.	<a href="https://www.biorxiv.org/">https://www.biorxiv.org/</a>	Excluded (Author suggested)

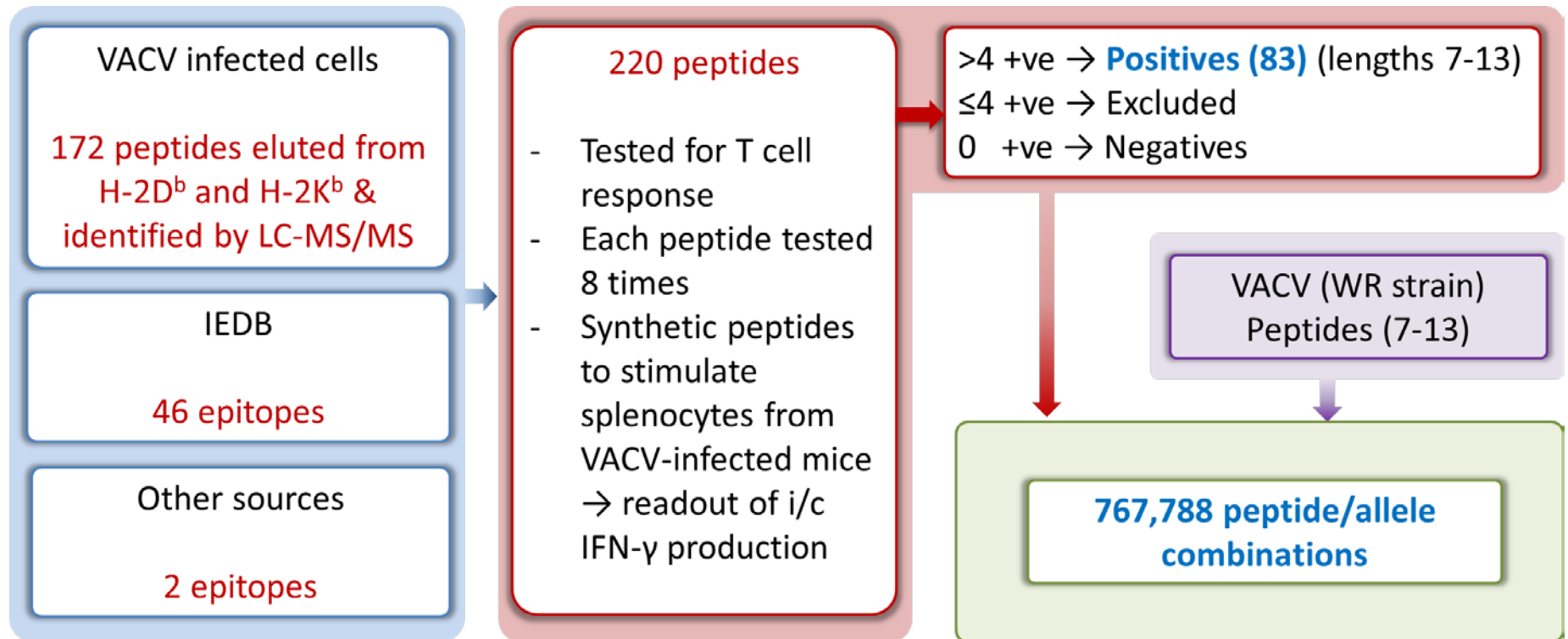
# Dataset

- Comprehensive epitope dataset from Vaccinia virus

## Most viral peptides displayed by class I MHC on infected cells are immunogenic

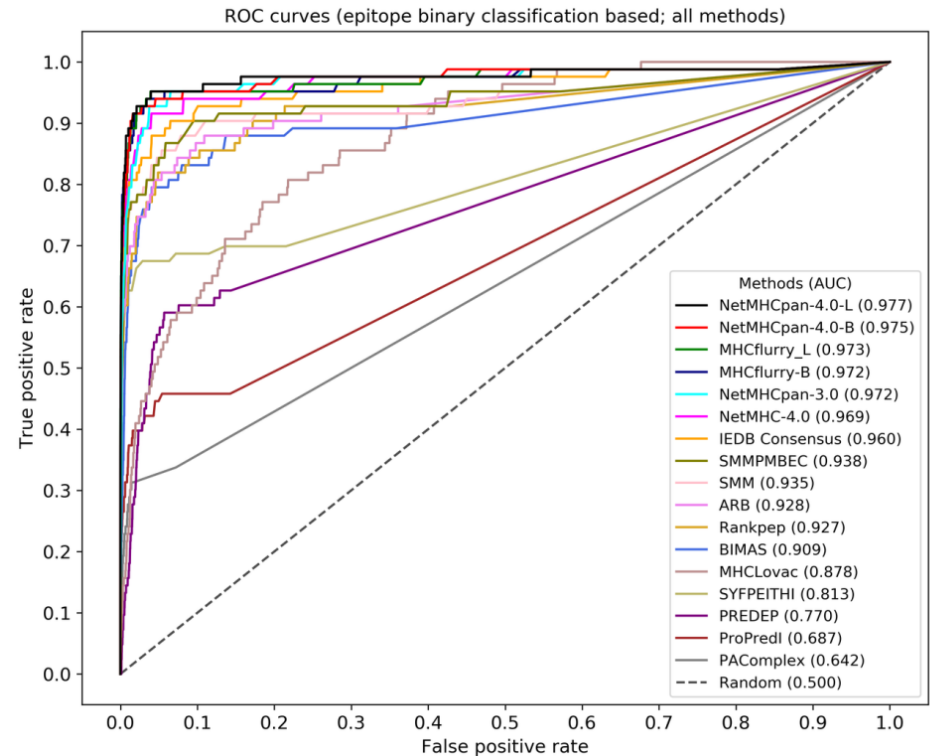
Nathan P. Croft<sup>a,b,1</sup>, Stewart A. Smith<sup>c</sup>, Jana Pickering<sup>c</sup>, John Sidney<sup>d</sup>, Bjoern Peters<sup>d,e</sup>, Pouya Faridi<sup>a,b</sup>, Matthew J. Witney<sup>c</sup>, Prince Sebastian<sup>c</sup>, Inge E. A. Flesch<sup>c</sup>, Sally L. Heading<sup>c</sup>, Alessandro Sette<sup>d,e</sup>, Nicole L. La Gruta<sup>a,b,f</sup>, Anthony W. Purcell<sup>a,b,1,2</sup>, and David C. Tschirke<sup>c,1,2</sup>

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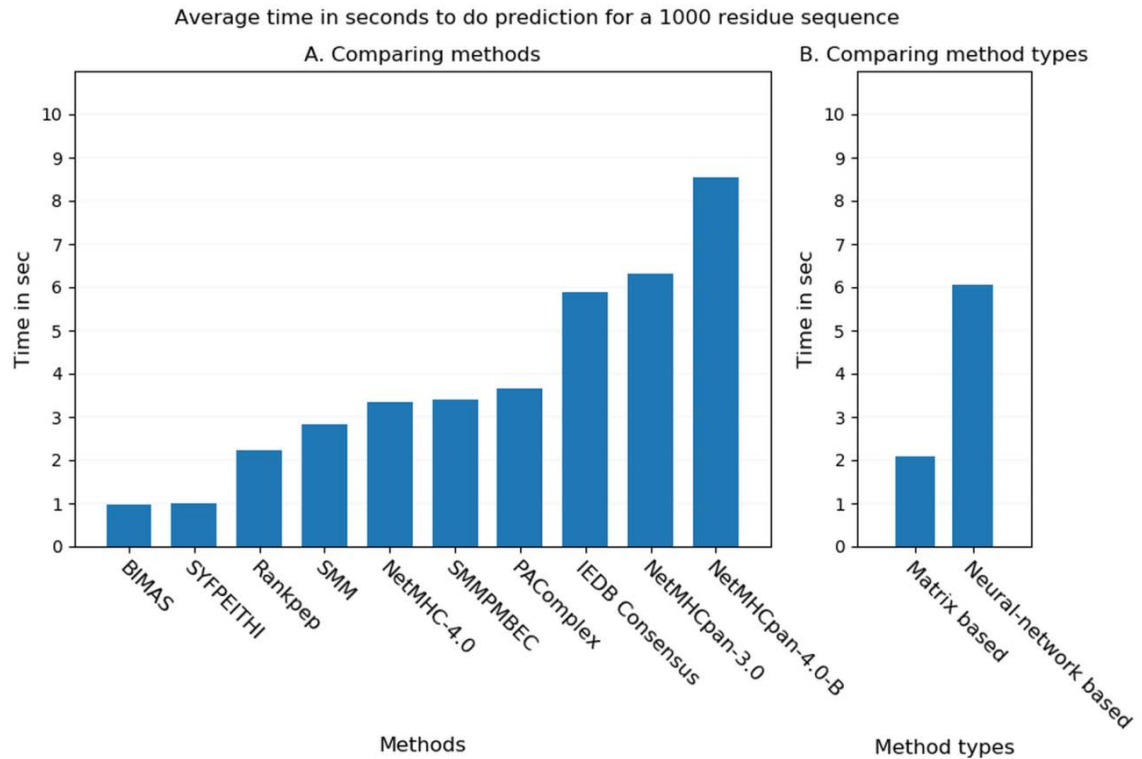
# Results

- Binary classification (epitope/non-epitope) & T cell response based
- In terms of AUC (Area under curve of ROC curves)



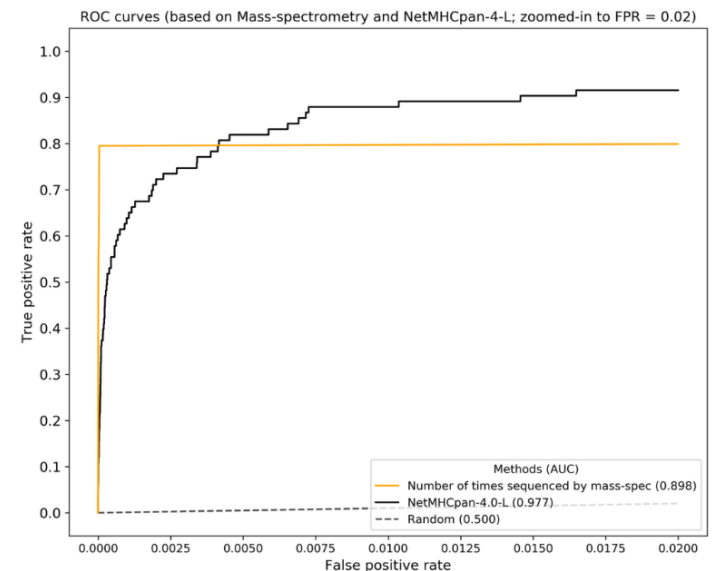
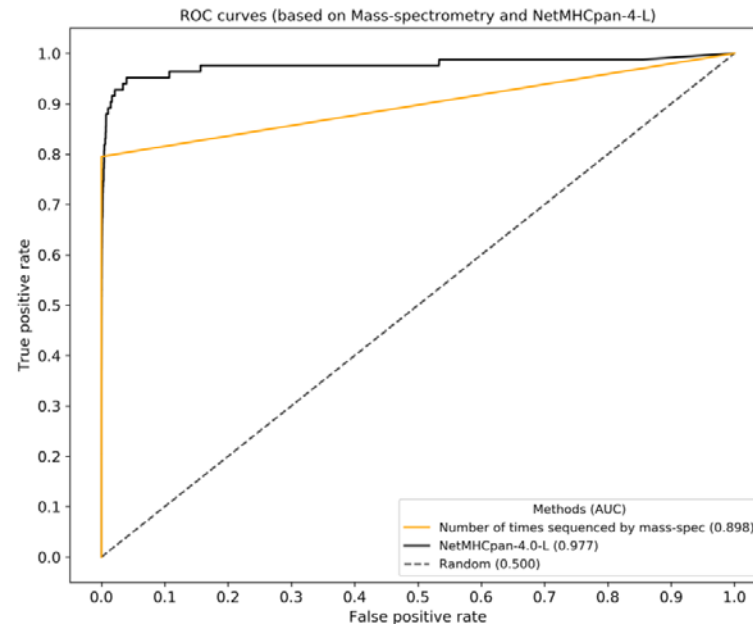
# Prediction speed comparison

- 5 random sequences of 1000 residues each
- Matrix-based methods faster compared to Neural network-based methods



# Results – peptide selection by MS vs. prediction

- Number of times peptides were identified by MS used as “MS score”
- Compared with NetMHCpan-4.0-L
- MS needs much less peptides to capture 50% epitopes (0.01%, N=48 vs. 0.04%, N = 277 for NetMHCpan-4.0-L)
- NetMHCpan-4.0-L better when considering all epitopes



# Contribute tools to IEDB

[Overview](#)[T Cell Tools](#)[B Cell Tools](#)[Analysis Tools](#)[Tools-API](#)[Usage](#)[Download](#)[Datasets](#)[Contribute Tools](#)[References](#)

## Contribute tools to the IEDB-AR

One of the overarching goals of the IEDB is to be the central repository for tools that are of general use to the Immunology and Immunoinformatics communities. As such, we encourage developers of such tools to contact us to inquire about hosting your tool at the IEDB. The IEDB team would work with the developers to create a web portal and keep it up and running indefinitely. We believe this arrangement benefits all parties involved and the Immunology community as a whole. The process for submitting your tool for inclusion at the IEDB-AR is outlined below

### Tool contribution process

1. Send an email to [help@iedb.org](mailto:help@iedb.org) and include the following information:
  - A summary of the problem that is addressed by your tool and why it is of general interest.
  - The publication status of your tool.
  - If there is a web server that currently hosts your tool, please provide the URL.
  - The time frame in which you will be ready to hand off your tool to IEDB developers.
2. Submissions will be evaluated by IEDB staff to determine whether the tool fits within the scope of the IEDB and we have the capability (hardware, personnel, etc.) to implement it.
3. You will receive a reply within 2 weeks with either a decision or a request for further information.
4. Once your tool is approved for inclusion, you will work with IEDB developers to hand off code and create a web portal at the IEDB.
5. The tool will be thoroughly tested for bugs and the load it exerts on the IEDB servers.
6. After you give the go-ahead, links will be made public and it will be officially announced in the IEDB Newsletter as well as the IEDB-AR release notes. It will also be referenced in any future publication on the general capabilities of the IEDB-AR, (e.g., the annual NAR webserver issue).
7. Finally, any updates you make to the tool can be applied, tested, and released in our 6-month development cycle.

# Summary

- MHC class I binding prediction
- MHC class II binding prediction
- TepiTool
- Datasets
- Benchmarking of class I epitope prediction tools
- Contributing tools to IEDB