La Jolla Institute FOR ALLERGY AND IMMUNOLOGY | Life Without Disease.

The Immune Epitope Database Analysis Resource:

MHC class II peptide binding predictions

Sinu Paul

spaul@lji.org

IEDB user workshop 2018

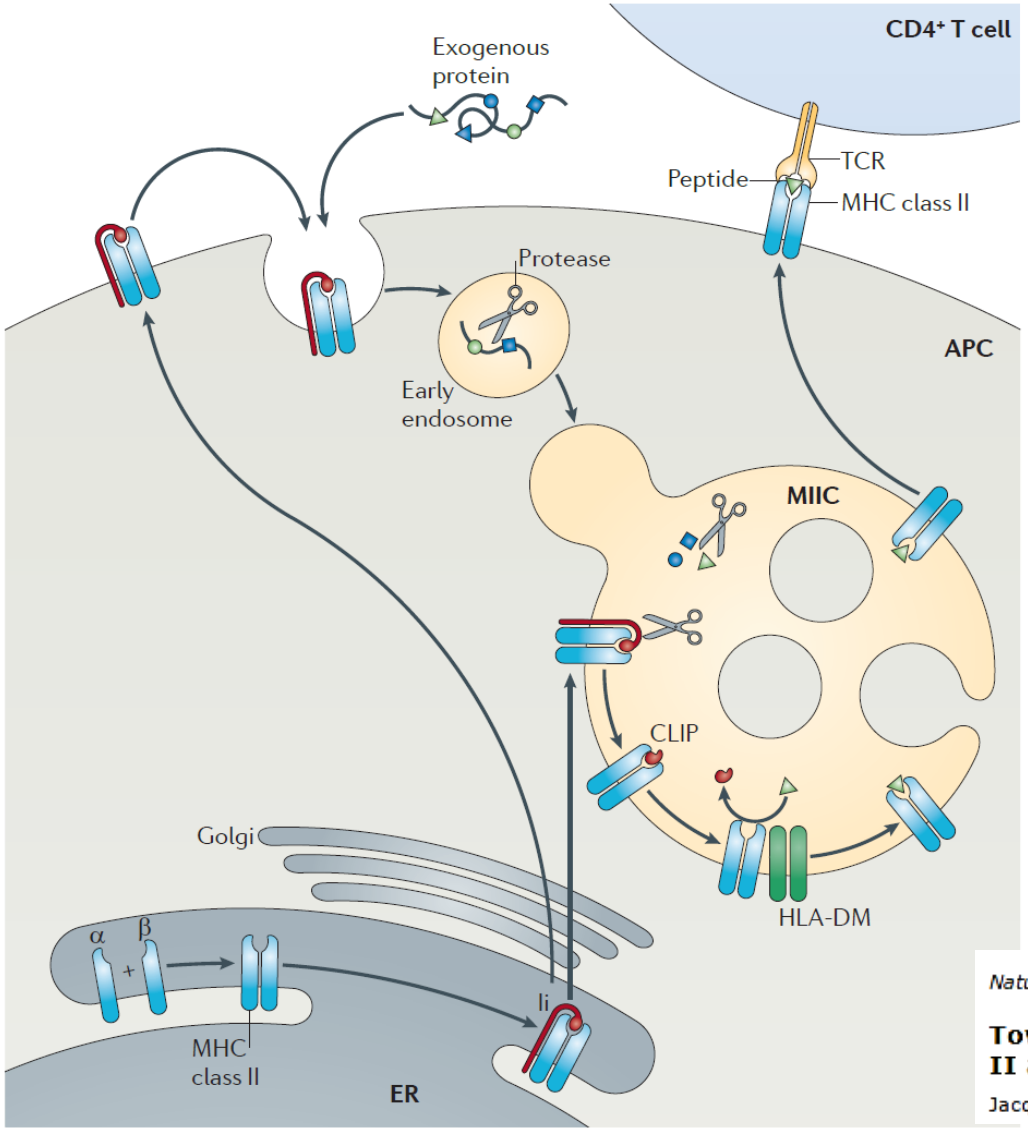
Oct 22-23, 2018

Outline

- Introduction
- Class II binding prediction tool – Web version
- IEDB recommendations & guidelines
- Prediction of promiscuous binders
- Exercise
- TepiTool - New interface for binding predictions
- Other versions
  - API/RESTful interface
  - Standalone

Introduction

Exogenous antigen processing pathway (class II)



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

Nature Reviews Immunology **11**, 823-836 (December 2011) | doi:10.1038/nri3084

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

Jacques Neefjes[1], Marlieke L. M. Jongsma[1], Petra Paul[1] & Oddmund Bakke[2]

MHC class II binding prediction tool

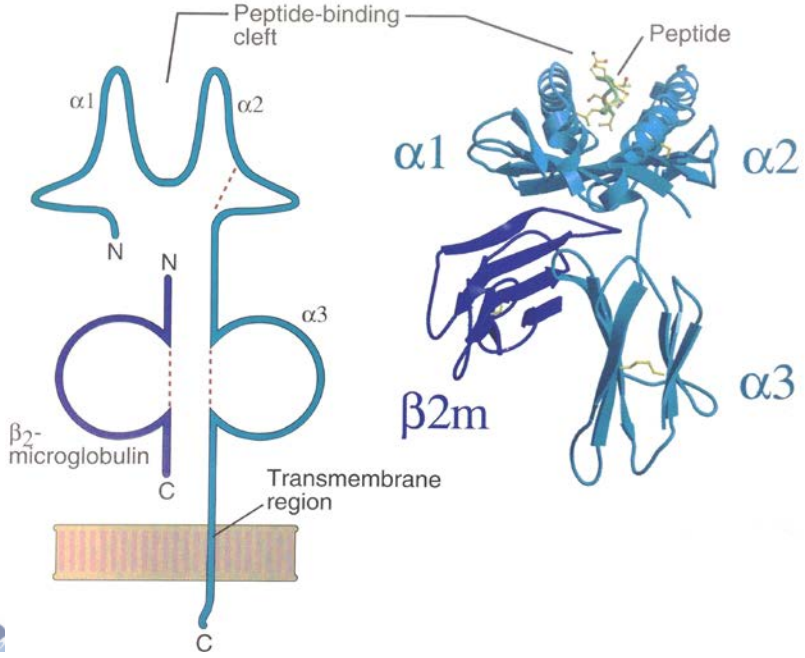
- Basic structure and principles same as class I binding prediction tool.

- Some differences.

MHC Class I & II molecules

Class I:

- Present in all nucleated cells
- One MHC encoded polymorphic chain (α) (2nd chain – β_2-microglobulin).
- Only one chain (α) impacts binding.
- Binding groove is closed.
- Can bind only shorter peptides (8-14 AA).
- Presents antigen to $CD8^+$ T cells



Class II:

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

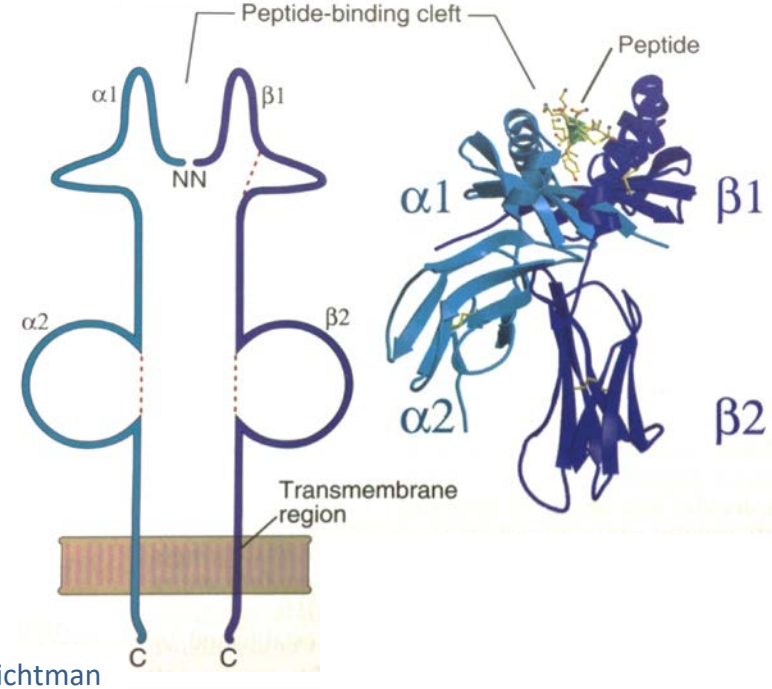


Figure source: Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman

HLA Nomenclature

- Class I:
  - Only α chain is variable
    - HLA-B*07:02
- Class II:
  - Both α and β chains are variable for DP & DQ loci
    - HLA-DPA1*01:03/DPB1*02:01
    - HLA-DQA1*01:01/DQB1*05:01
  - Only β chain is variable for DR locus
    - HLA-DRB1*01:01

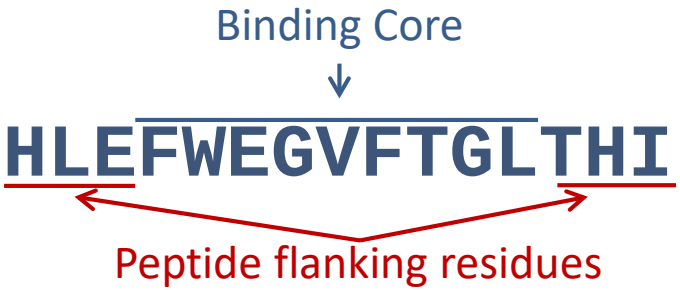
Class II binding peptide “Binding core”

- 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

- Peptide length = 15 (for the tool)
- Lesser accuracy compared to class-I tool

Class I		Class II	
Method	**AUC***	**Method**	**AUC***
NetMHCpan	0.900[1]	NetMHCIIpan	0.781[2]
SMM	0.894[3]	SMM-align	0.763[4]

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

- Higher threshold for selecting binders than class-I.

1. Andreatta & Nielsen, 2016, Bioinformatics
2. Jensen et al. 2018, Immunology
3. Kim et al. 2009, BMC Bioinformatics
4. Wang et al. 2010, BMC Bioinformatics

MHC class II binding prediction methods available

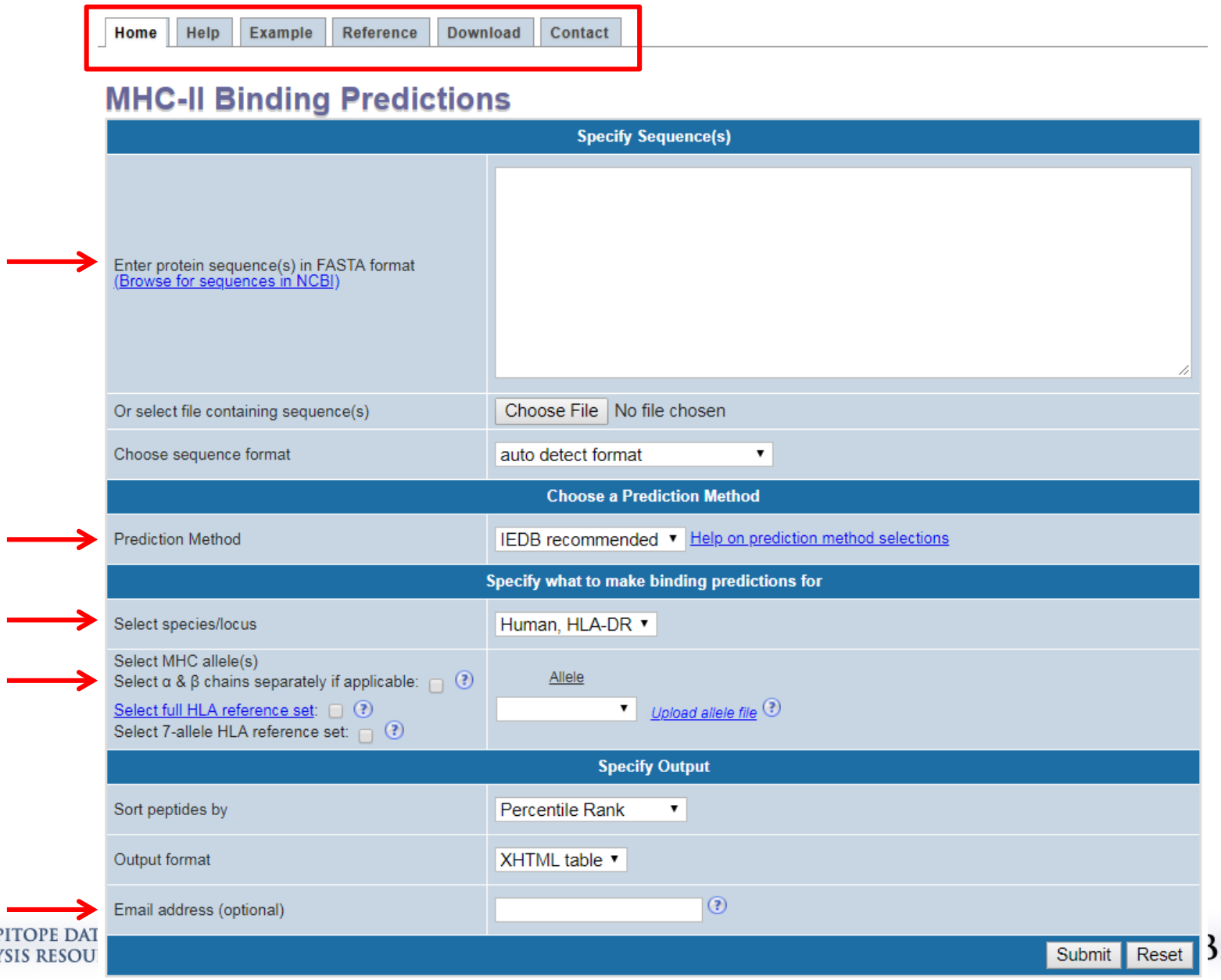
Methods	Prediction based on	Reference	Performance reported*
Consensus	Combination of SMM-align, NN-align & CombLib/Sturniolo	Wang et al., 2008	0.783 AUC
NetMHCIIpan-3.1	Artificial Neural Network	Andreatta et al., 2015	0.870 AUC
NN_align-2.2	Artificial Neural Network	Nielsen & Lund, 2009	0.782 AUC
SMM_align-1.1	Stabilization Matrix Alignment	Nielsen et al., 2007	0.763 AUC
Combinatorial Library	Position scanning combinatorial libraries	Sidney et al., 2008	0.691 AUC
Sturniolo	Scoring matrix based	Sturniolo et al., 1999	

* All AUCs are averaged across several MHC molecules and obtained from Wang (2010) BMC Bioinformatics (Table-4) with similarity reduced data set, except NetMHCIIpan-3.1 (from Andreatta et al., 2015).

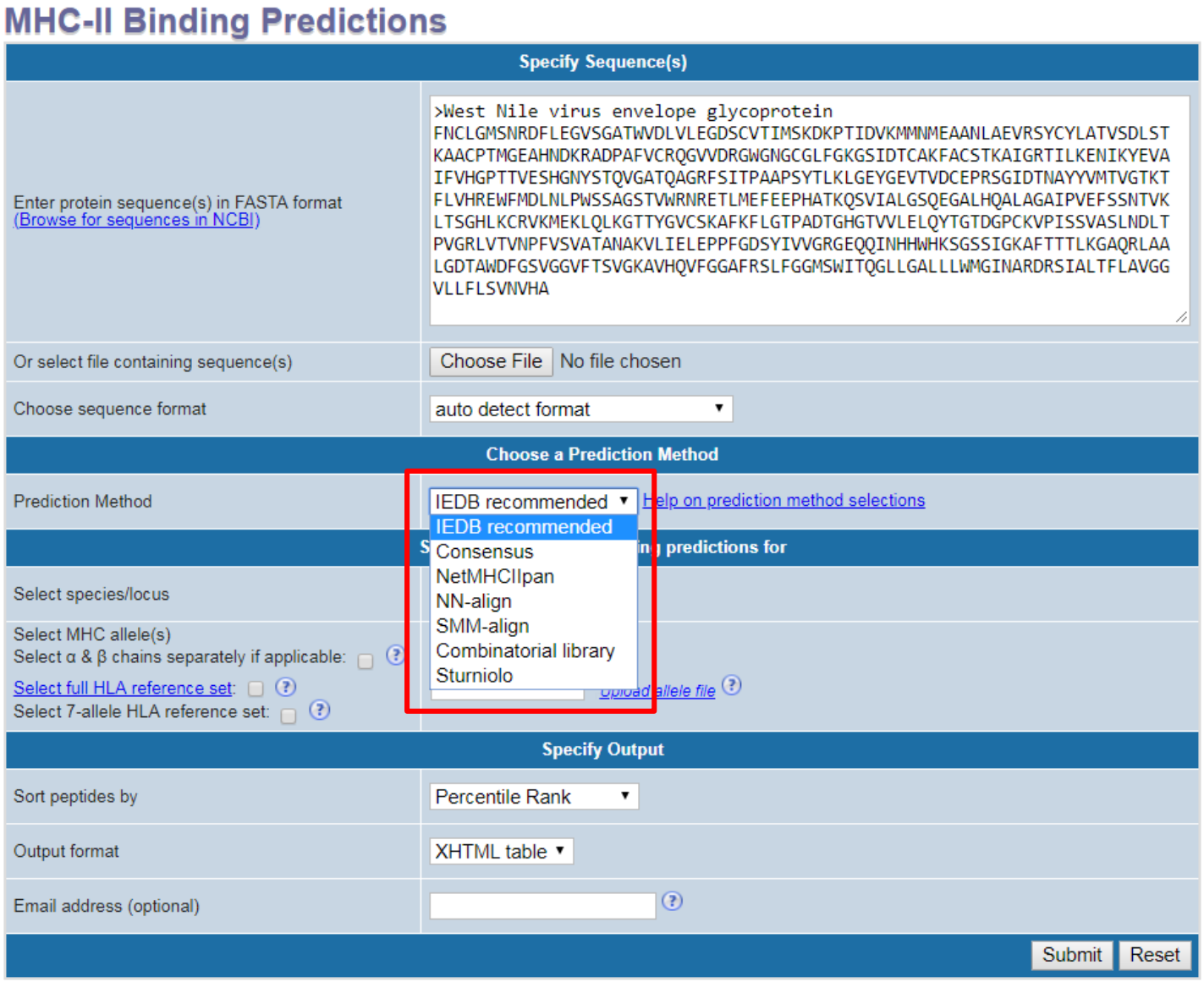
Class II tool – Web version

http://tools.iedb.org/mhcii

Web interface - http://tools.iedb.org/mhcii



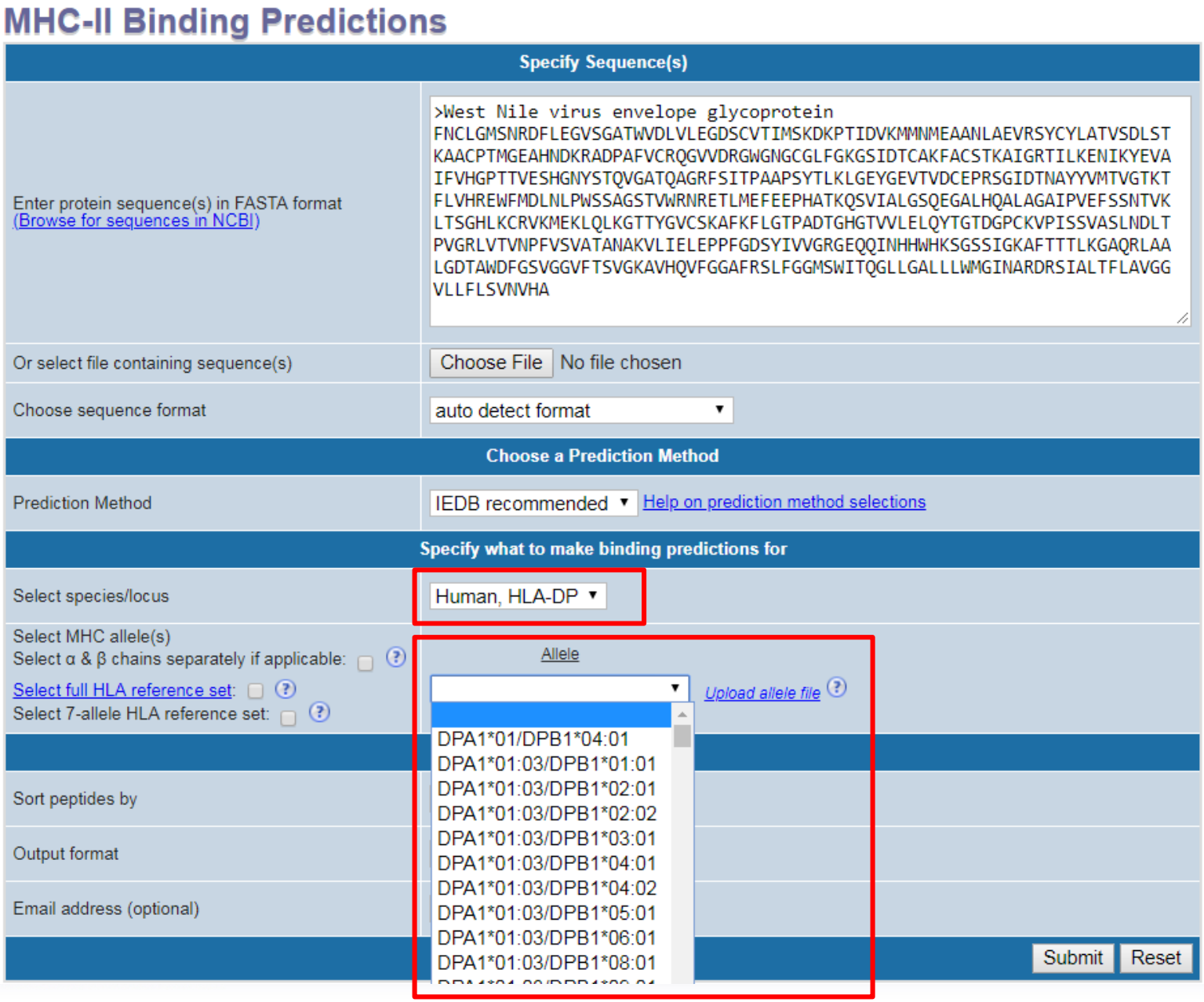
Guidelines: Choosing the method



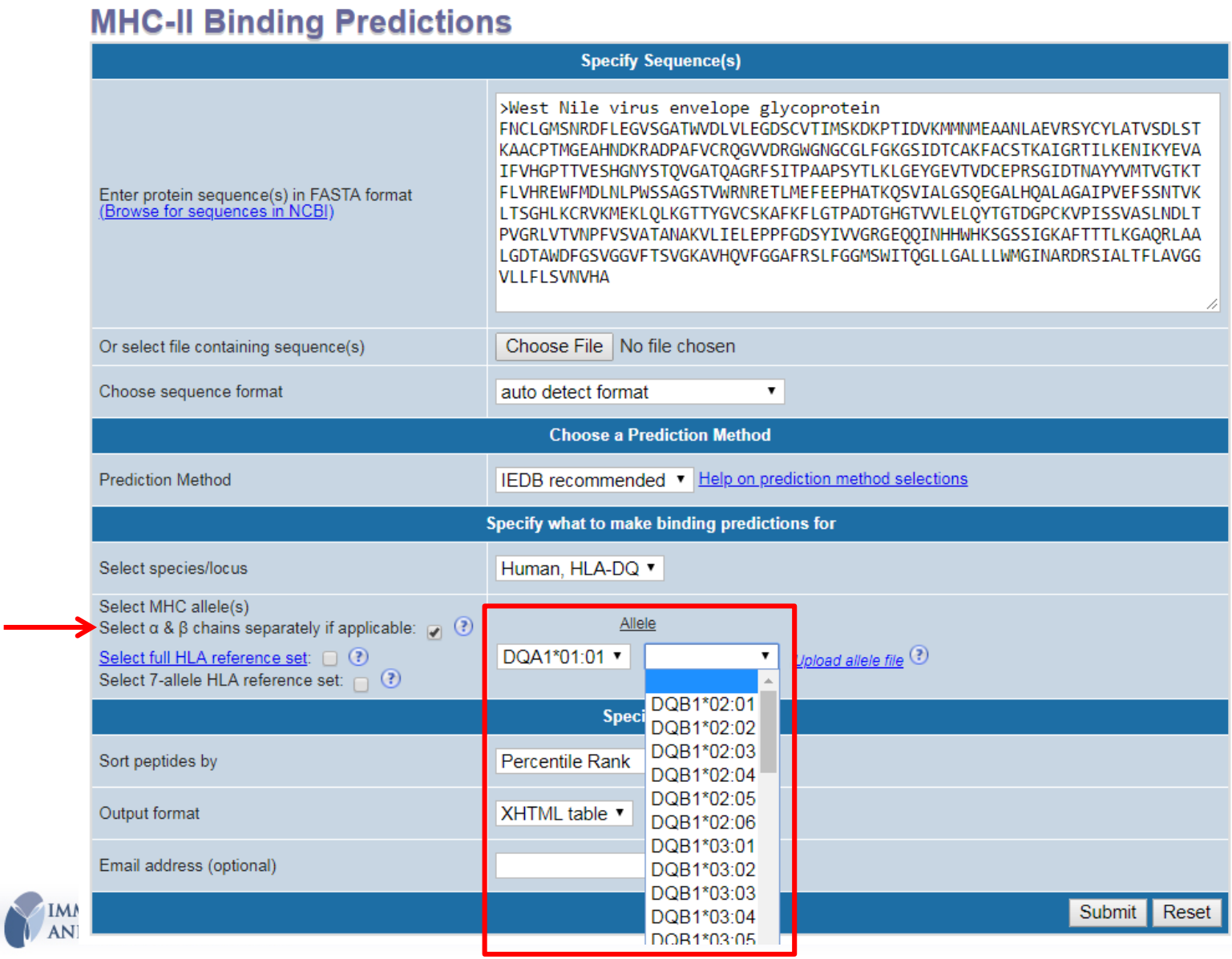
Guidelines: Choosing the method

- Method to use: **IEDB recommended method** - employs Consensus (Combination of NN-align, SMM-align & CombLib/Sturniolo) or NetMHCIIpan depending on the allele.
- Advantages:
  - Best available methods.
  - Gives a consensus percentile rank.
  - Gives binding affinity & percentile rank for each method separately as well.

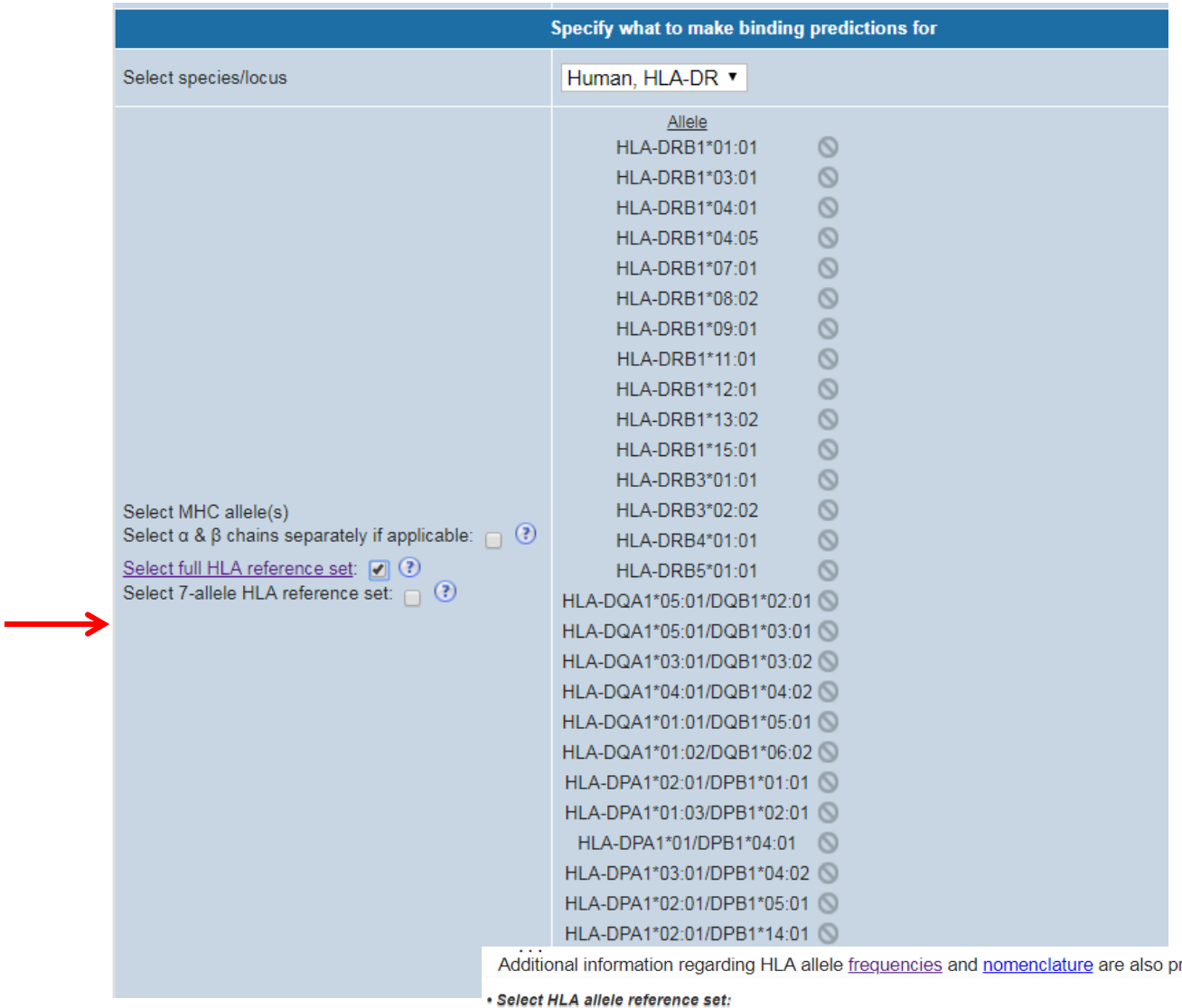
Allele selection



Allele selection - α and β chains separately



Allele selection – 27 allele reference set

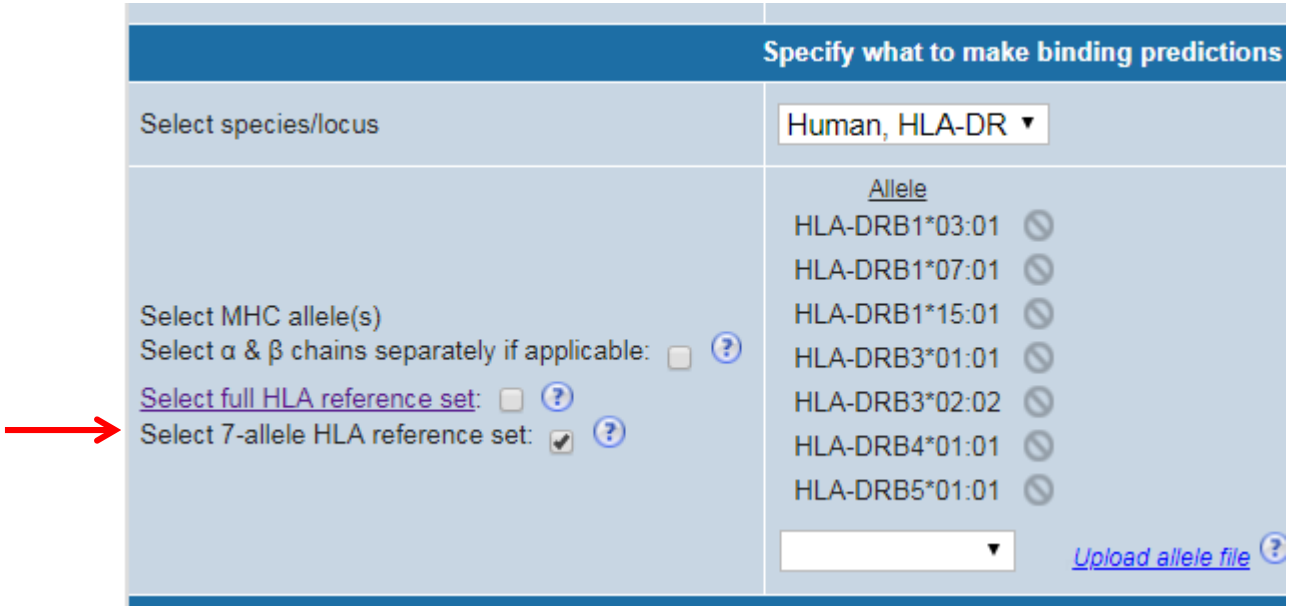


Help page

Additional information regarding HLA allele frequencies and nomenclature are also provided.

- ***Select HLA allele reference set:***
  When the IEDB recommended option is selected, this box can be checked to select a reference panel of 27 alleles, as described here.
- ***Select "7-allele" reference set:***
  When the IEDB recommended option is selected, this box can be checked to select a reference panel of 7 alleles, as described in Paul et al, 2015.

Allele selection – 7 allele set



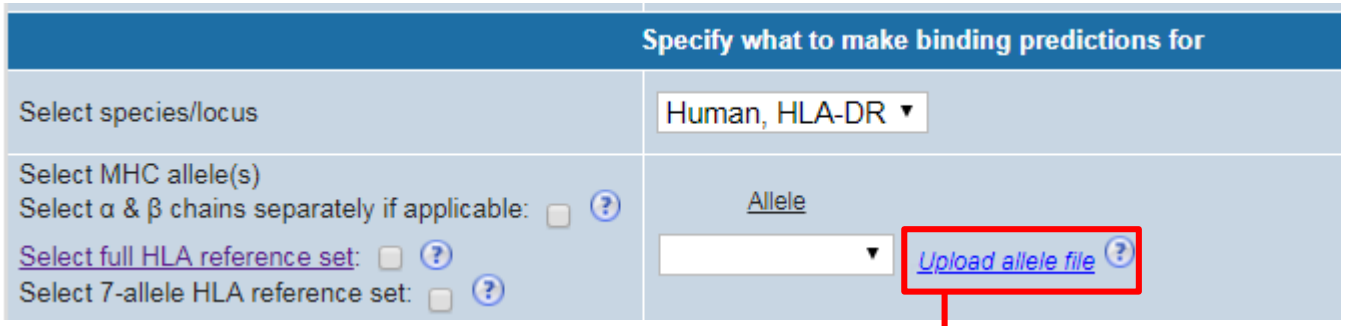
. . .

Additional information regarding HLA allele frequencies and nomenclature are also provided.

Help page

- ***Select HLA allele reference set:***
  When the IEDB recommended option is selected, this box can be checked to select a reference panel of 27 alleles, as described here.
- ***Select "7-allele" reference set:***
  When the IEDB recommended option is selected, this box can be checked to select a reference panel of 7 alleles, as described in Paul et al, 2015.

Allele selection – upload file



Help page

3. Specify what to make predictions for:

Predictions are limited to alleles that are currently covered by specific prediction methods. Selection of a particular pred

- ***Format for the upload allele file:***

File should be in simple text format containing an allele in each line (example given below).
Example:

```
H2-IAb
HLA-DPA1*01/DPB1*04:01
HLA-DRB1*01:01
...
```

Additional information regarding HLA allele frequencies and nomenclature are also provided.

- Only available alleles
- No allele sequence

How the tool works

1. Breaks sequence into all possible **15-mer peptides**.
2. Predicts the binding affinity for each peptide based on the method.
3. Compares the predicted affinity to that of a large set of randomly selected peptides.
4. Assigns a percentile rank depending on individual predicted affinity.
5. Consensus picks median rank of the methods used – consensus percentile rank

Percentile rank

- Generated by comparing the selected peptide's predicted binding affinity against that of a large set of peptides.
- Provides a uniform scale allowing comparisons across different predictors.
- A lower percentile rank indicates higher affinity.
- In case of consensus method, median of the percentile ranks of the three methods involved is consensus percentile rank.

Input

MHC-II Binding Predictions

Specify Sequence(s)

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

```
>HCV_NS3
APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGP
VIQMYTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSG
GPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGK
STKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFLADGGCS
GGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFY
GKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTG
DFDSVIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYRFVAPGERPSGMFDSSV
LCECYDAGCAWYELTPAETTVRLRAYMNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYL
VAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMSADLEVV
```

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

Choose sequence format — auto detect format

Choose a Prediction Method

Prediction Method — IEDB recommended — Help on prediction method selections

Specify what to make binding predictions for

Select species/locus — Human, HLA-DP

Select MHC allele(s)
Select α & β chains separately if applicable:
Select full HLA reference set:
Select 7-allele HLA reference set:

Allele
DPA1*01/DPB1*04:01

Upload allele file

Specify Output

MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	HCV_NS3	APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATCING VCWTVYHGAGTRTIASPKGPVIQMYTNVDQDLVGWPAPQGSRSLTPCTCG SSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLCPAGHA VGIFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVA HLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDP NIRTGVRTITTGSPITYSTYGKFLADGGCSGGAYDIIICDECHSTDATSI LGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFY GKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSVI PTSGDVVVVATDALMTGYTGDFDSVIDCNTCVTQTVDFSLDPTFTIETIT LPQDAVSRTQRRGRTGRGKPGIYRFVAPGERPSGMFDSSVLCECYDAGCA WYELTPAETTVRLRAYMNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQT KQSGENLPYLVAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLY RLGAVQNEITLTHPVTKYIMTCMSADLEVVT

Prediction method: IEDB recommended | Low percentile_rank = good binders

Download result

Citations

Check to expand the result:

Allele	#	Start	End	Peptide	Method used	Percentile rank
HLA-DPA1*01/DPB1*04:01	1	527	541	DHLEFWEGVFTGLTH	Consensus (comb.lib./smm/nn)	2.52
HLA-DPA1*01/DPB1*04:01	1	528	542	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	2.57
HLA-DPA1*01/DPB1*04:01	1	526	540	QDHLEFWEGVFTGLT	Consensus (comb.lib./smm/nn)	2.62
HLA-DPA1*01/DPB1*04:01	1	529	543	LEFWEGVFTGLTHID	Consensus (comb.lib./smm/nn)	3.13
HLA-DPA1*01/DPB1*04:01	1	525	539	CQDHLEFWEGVFTGL	Consensus (comb.lib./smm/nn)	3.26
HLA-DPA1*01/DPB1*04:01	1	39	53	AAQTFLATCINGVCW	Consensus (comb.lib./smm/nn)	3.80
HLA-DPA1*01/DPB1*04:01	1	262	276	GSPITYSTYGKFLAD	Consensus (comb.lib./smm/nn)	4.07
HLA-DPA1*01/DPB1*04:01	1	40	54	AQTFLATCINGVCWT	Consensus (comb.lib./smm/nn)	4.08
HLA-DPA1*01/DPB1*04:01	1	263	277	SPITYSTYGKFLADG	Consensus (comb.lib./smm/nn)	4.08
HLA-DPA1*01/DPB1*04:01	1	38	52	TAAQTFLATCINGVC	Consensus (comb.lib./smm/nn)	4.13
HLA-DPA1*01/DPB1*04:01	1	37	51	STAAQTFLATCINGV	Consensus (comb.lib./smm/nn)	4.56
HLA-DPA1*01/DPB1*04:01	1	261	275	TGSPITYSTYGKFLA	Consensus (comb.lib./smm/nn)	4.78
HLA-DPA1*01/DPB1*04:01	1	530	544	EFWEGVFTGLTHIDA	Consensus (comb.lib./smm/nn)	5
HLA-DPA1*01/DPB1*04:01	1	102	116	SDLYLVTRHADVIPV	Consensus (comb.lib./smm/nn)	7.45
HLA-DPA1*01/DPB1*04:01	1	41	55	QTFLATCINGVCWTV	Consensus (comb.lib./smm/nn)	7.57
HLA-DPA1*01/DPB1*04:01	1	101	115	SSDLYLVTRHADVIP	Consensus (comb.lib./smm/nn)	7.57
HLA-DPA1*01/DPB1*04:01	1	260	274	TTGSPITYSTYGKFL	Consensus (comb.lib./smm/nn)	7.71
HLA-DPA1*01/DPB1*04:01	1	100	114	GSSDLYLVTRHADVI	Consensus (comb.lib./smm/nn)	7.85
HLA-DPA1*01/DPB1*04:01	1	531	545	FWEGVFTGLTHIDAH	Consensus (comb.lib./smm/nn)	7.97

Result

Expanded Result

Check to expand the result: ☑

Allele	#	Start	End	Pepide	Method used	Percentile rank	Comblib. core	Comblib. score	Comblib. rank	SMM align core	SMM align IC50(nM)	SMM align rank	NN align core	NN align IC50(nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	1	527	541	DHLEFWEGVFTGLTH	Consensus (comb.lib./smm/nn)	2.52	FWEGVFTGL	6.90	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	39.40	2.52
HLA-DPA1*01/DPB1*04:01	1	528	542	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	2.57	FWEGVFTGL	6.90	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	40.30	2.57
HLA-DPA1*01/DPB1*04:01	1	526	540	QDHLEFWEGVFTGLT	Consensus (comb.lib./smm/nn)	2.62	FWEGVFTGL	6.90	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	41.30	2.62
HLA-DPA1*01/DPB1*04:01	1	529	543	LEFWEGVFTGLTHID	Consensus (comb.lib./smm/nn)	3.13	FWEGVFTGL	6.90	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	51.10	3.13
HLA-DPA1*01/DPB1*04:01	1	525	539	CQDHLEFWEGVFTGL	Consensus (comb.lib./smm/nn)	3.26	FWEGVFTGL	6.90	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	53.50	3.26
HLA-DPA1*01/DPB1*04:01	1	39	53	AAQTFLATCINGVCW	Consensus (comb.lib./smm/nn)	3.80	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	64.70	3.80
HLA-DPA1*01/DPB1*04:01	1	262	276	GSPITYSTYGKFLAD	Consensus (comb.lib./smm/nn)	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	54	3.29
HLA-DPA1*01/DPB1*04:01	1	40	54	AQTFLATCINGVCWT	Consensus (comb.lib./smm/nn)	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	71.10	4.08
HLA-DPA1*01/DPB1*04:01	1	263	277	SPITYSTYGKFLADG	Consensus (comb.lib./smm/nn)	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	54.10	3.29
HLA-DPA1*01/DPB1*04:01	1	38	52	TAAQTFLATCINGVC	Consensus (comb.lib./smm/nn)	4.13	TAAQTFLAT	52.52	29.77	FLATCINGV	478	1.49	FLATCINGV	72.20	4.13
HLA-DPA1*01/DPB1*04:01	1	37	51	STAAQTFLATCINGV	Consensus (comb.lib./smm/nn)	4.56	TAAQTFLAT	52.52	29.77	TAAQTFLAT	464	1.41	FLATCINGV	81.80	4.56
HLA-DPA1*01/DPB1*04:01	1	261	275	TGSPITYSTYGKFLA	Consensus (comb.lib./smm/nn)	4.78	TYSTYGKFL	2.38	15.24	ITYSTYGKF	908	4.78	ITYSTYGKF	61	3.61
HLA-DPA1*01/DPB1*04:01	1	530	544	EFWEGVFTGLTHIDA	Consensus (comb.lib./smm/nn)	5	FWEGVFTGL	6.90	19.59	FWEGVFTGL	664	2.75	FWEGVFTGL	92.60	5
HLA-DPA1*01/DPB1*04:01	1	102	116	SDLYLVTRHADVIPV	Consensus (comb.lib./smm/nn)	7.45	LVTRHADVI	23.49	25.43	YLVTRHADV	1194	7.45	YLVTRHADV	149.80	7.04
HLA-DPA1*01/DPB1*04:01	1	41	55	QTFLATCINGVCWTV	Consensus (comb.lib./smm/nn)	7.57	QTFLATCIN	728.23	45.84	FLATCINGV	829	4.09	FLATCINGV	166.90	7.57
HLA-DPA1*01/DPB1*04:01	1	101	115	SSDLYLVTRHADVIP	Consensus (comb.lib./smm/nn)	7.57	LVTRHADVI	23.49	25.43	YLVTRHADV	1206	7.57	YLVTRHADV	160.40	7.37
HLA-DPA1*01/DPB1*04:01	1	260	274	TTGSPITYSTYGKFL	Consensus (comb.lib./smm/nn)	7.71	TYSTYGKFL	2.38	15.24	ITYSTYGKF	1221	7.71	ITYSTYGKF	100.10	5.31
HLA-DPA1*01/DPB1*04:01	1	100	114	GSSDLYLVTRHADVI	Consensus (comb.lib./smm/nn)	7.85	GSSDLYLVT	0.74	11.33	YLVTRHADV	1183	7.34	YLVTRHADV	176.10	7.85
HLA-DPA1*01/DPB1*04:01	1	531	545	FWEGVFTGLTHIDAH	Consensus (comb.lib./smm/nn)	7.97	FWEGVFTGL	6.90	19.59	FWEGVFTGL	728	3.24	FWEGVFTGL	179.70	7.97
HLA-DPA1*01/DPB1*04:01	1	103	117	DLYLVTRHADVIPVR	Consensus (comb.lib./smm/nn)	8.57	LVTRHADVI	23.49	25.43	YLVTRHADV	1307	8.57	YLVTRHADV	169.50	7.65
HLA-DPA1*01/DPB1*04:01	1	36	50	VSTAAQTFLATCING	Consensus (comb.lib./smm/nn)	9.33	TAAQTFLAT	52.52	29.77	TAAQTFLAT	374	0.93	AAQTFLATC	230.40	9.33
HLA-DPA1*01/DPB1*04:01	1	557	571	LPYLVAYQATVCARA	Consensus (comb.lib./smm/nn)	9.76	LVAYQATVC	19.93	24.60	YLVAYQATV	594	2.25	YLVAYQATV	247.70	9.76
HLA-DPA1*01/DPB1*04:01	1	264	278	PITYSTYGKFLADGG	Consensus (comb.lib./smm/nn)	9.80	TYSTYGKFL	2.38	15.24	STYGKFLAD	1430	9.80	ITYSTYGKF	60.60	3.60

Result – Consensus percentile rank

Method used	Percentile rank	Comblib. core	Comblib. score	Comblib. rank	SMM align core	SMM align IC50(nM)	SMM align rank	NN align core	NN align IC50(nM)	NN align rank
Consensus (comb.lib./smm/nn)	2.52	FWEGVFTGL	6.90	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	39.40	2.52
Consensus (comb.lib./smm/nn)	2.57	FWEGVFTGL	6.90	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	40.30	2.57
Consensus (comb.lib./smm/nn)	2.62	FWEGVFTGL	6.90	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	41.30	2.62
Consensus (comb.lib./smm/nn)	3.13	FWEGVFTGL	6.90	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	51.10	3.13
Consensus (comb.lib./smm/nn)	3.26	FWEGVFTGL	6.90	19.59	EFWEGVFTG	320	0.66	FWEGVFTGL	53.50	3.26
Consensus (comb.lib./smm/nn)	3.80	QTFLATCIN	728.23	45.84	FLATCINGV	742	3.36	FLATCINGV	64.70	3.80
Consensus (comb.lib./smm/nn)	4.07	TYSTYGKFL	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGKF	54	3.29
Consensus (comb.lib./smm/nn)	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	71.10	4.08
Consensus (comb.lib./smm/nn)	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	54.10	3.29
Consensus (comb.lib./smm/nn)	4.13	TAAQTFLAT	52.52	29.77	FLATCINGV	478	1.49	FLATCINGV	72.20	4.13
Consensus (comb.lib./smm/nn)	4.56	TAAQTFLAT	52.52	29.77	TAAQTFLAT	464	1.41	FLATCINGV	81.80	4.56
Consensus (comb.lib./smm/nn)	4.78	TYSTYGKFL	2.38	15.24	ITYSTYGKF	908	4.78	ITYSTYGKF	61	3.61
Consensus (comb.lib./smm/nn)	5	FWEGVFTGL	6.90	19.59	FWEGVFTGL	664	2.75	FWEGVFTGL	92.60	5
Consensus (comb.lib./smm/nn)	7.45	LVTRHADVI	23.49	25.43	YLVTRHADV	1194	7.45	YLVTRHADV	149.80	7.04
Consensus (comb.lib./smm/nn)	7.57	QTFLATCIN	728.23	45.84	FLATCINGV	829	4.09	FLATCINGV	166.90	7.57

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 9/29/2014 using the IEDB analysis resource Consensus tool [1] [2].

1. 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.
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.

Result – Downloaded file (CSV)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	allele	seq_num	start	end	peptide	method	percentile_rank	comblib_c	comblib_s	comblib_r	smm_alig	smm_alig	smm_alig	nn_align
2	HLA-DPA1*01/DPB1*0401	1	527	541	DHLEFWEGVFTGLTH	Consensus (comb.lib./smm/nn)	2.52	FWEGVFT	6.9	19.59	FWEGVFT	310	0.62	EFWEGV
3	HLA-DPA1*01/DPB1*0401	1	528	542	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	2.57	FWEGVFT	6.9	19.59	FWEGVFT	302	0.58	FWEGVF
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	Consensus (comb.lib./smm/nn)	2.62	FWEGVFT	6.9	19.59	FWEGVFT	310	0.62	EFWEGV
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	Consensus (comb.lib./smm/nn)	3.13	FWEGVFT	6.9	19.59	FWEGVFT	308	0.61	FWEGVF
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	Consensus (comb.lib./smm/nn)	3.26	FWEGVFT	6.9	19.59	EFWEGVF	320	0.66	FWEGVF
7	HLA-DPA1*01/DPB1*0401	1	39	53	AAQTFLATCINGVCW	Consensus (comb.lib./smm/nn)	3.8	QTFLATCI	728.23	45.84	FLATCING	742	3.36	FLATCIN
8	HLA-DPA1*01/DPB1*0401	1	262	276	GSPITYSTYGKFLAD	Consensus (comb.lib./smm/nn)	4.07	TYSTYGKF	2.38	15.24	ITYSTYGKF	827	4.07	ITYSTYGK
9	HLA-DPA1*01/DPB1*0401	1	40	54	AQTFLATCINGVCWT	Consensus (comb.lib./smm/nn)	4.08	QTFLATCI	728.23	45.84	FLATCING	746	3.39	FLATCIN
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	Consensus (comb.lib./smm/nn)	4.08	TYSTYGKF	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGK
11	HLA-DPA1*01/DPB1*0401	1	38	52	TAAQTFLATCINGVC	Consensus (comb.lib./smm/nn)	4.13	TAAQTFLA	52.52	29.77	FLATCING	478	1.49	FLATCIN
12	HLA-DPA1*01/DPB1*0401	1	37	51	STAAQTFLATCINGV	Consensus (comb.lib./smm/nn)	4.56	TAAQTFLA	52.52	29.77	TAAQTFLA	464	1.41	FLATCIN
13	HLA-DPA1*01/DPB1*0401	1	261	275	TGSPITYSTYGKFLA	Consensus (comb.lib./smm/nn)	4.78	TYSTYGKF	2.38	15.24	ITYSTYGKF	908	4.78	ITYSTYGK
14	HLA-DPA1*01/DPB1*0401	1	530	544	EFWEGVFTGLTHIDA	Consensus (comb.lib./smm/nn)	5	FWEGVFT	6.9	19.59	FWEGVFT	664	2.75	FWEGVF
15	HLA-DPA1*01/DPB1*0401	1	102	116	SDLYLVTRHADVIPV	Consensus (comb.lib./smm/nn)	7.45	LVTRHADV	23.49	25.43	YLVTRHAD	1194	7.45	YLVTRHA
16	HLA-DPA1*01/DPB1*0401	1	41	55	QTFLATCINGVCWTV	Consensus (comb.lib./smm/nn)	7.57	QTFLATCI	728.23	45.84	FLATCING	829	4.09	FLATCIN
17	HLA-DPA1*01/DPB1*0401	1	101	115	SSDLYLVTRHADVIP	Consensus (comb.lib./smm/nn)	7.57	LVTRHADV	23.49	25.43	YLVTRHAD	1206	7.57	YLVTRHA
18	HLA-DPA1*01/DPB1*0401	1	260	274	TTGSPITYSTYGKFL	Consensus (comb.lib./smm/nn)	7.71	TYSTYGKF	2.38	15.24	ITYSTYGKF	1221	7.71	ITYSTYGK
19	HLA-DPA1*01/DPB1*0401	1	100	114	GSSDLYLVTRHADVI	Consensus (comb.lib./smm/nn)	7.85	GSSDLYLV	0.74	11.33	YLVTRHAD	1183	7.34	YLVTRHA
20	HLA-DPA1*01/DPB1*0401	1	531	545	FWEGVFTGLTHIDAH	Consensus (comb.lib./smm/nn)	7.97	FWEGVFT	6.9	19.59	FWEGVFT	728	3.24	FWEGVF
21	HLA-DPA1*01/DPB1*0401	1	103	117	DLYLVTRHADVIPVR	Consensus (comb.lib./smm/nn)	8.57	LVTRHADV	23.49	25.43	YLVTRHAD	1307	8.57	YLVTRHA
22	HLA-DPA1*01/DPB1*0401	1	36	50	VSTAAQTFLATCING	Consensus (comb.lib./smm/nn)	9.33	TAAQTFLA	52.52	29.77	TAAQTFLA	374	0.93	AAQTFLA
23	HLA-DPA1*01/DPB1*0401	1	557	571	LPYLVAYQATVCARA	Consensus (comb.lib./smm/nn)	9.76	LVAYQATV	19.93	24.6	YLVAYQAT	594	2.25	YLVAYQA
24	HLA-DPA1*01/DPB1*0401	1	264	278	PITYSTYGKFLADGG	Consensus (comb.lib./smm/nn)	9.8	TYSTYGKF	2.38	15.24	STYGKFLA	1430	9.8	ITYSTYGK
25	HLA-DPA1*01/DPB1*0401	1	99	113	CGSSDLYLVTRHADV	Consensus (comb.lib./smm/nn)	10.02	GSSDLYLV	0.74	11.33	LYLVTRHA	1185	7.36	YLVTRHA
26	HLA-DPA1*01/DPB1*0401	1	558	572	PYLVAYQATVCARAQ	Consensus (comb.lib./smm/nn)	10.5	LVAYQATV	19.93	24.6	YLVAYQAT	1257	8.07	AYQATV
27	HLA-DPA1*01/DPB1*0401	1	35	49	IVSTAAQTFLATCIN	Consensus (comb.lib./smm/nn)	10.61	IVSTAAQT	50.69	29.58	TAAQTFLA	371	0.91	AAQTFLA
28	HLA-DPA1*01/DPB1*0401	1	559	573	YLVAYQATVCARAQA	Consensus (comb.lib./smm/nn)	10.76	LVAYQATV	19.93	24.6	YLVAYQAT	1251	8.01	AYQATV
29	HLA-DPA1*01/DPB1*0401	1	34	48	QIVSTAAQTFLATCI	Consensus (comb.lib./smm/nn)	10.89	IVSTAAQT	50.69	29.58	TAAQTFLA	367	0.89	AAQTFLA
30	HLA-DPA1*01/DPB1*0401	1	265	279	ITYSTYGKFLADGGC	Consensus (comb.lib./smm/nn)	12.18	TYSTYGKF	2.38	15.24	ITYSTYGKF	1665	12.18	YSTYGKF
31	HLA-DPA1*01/DPB1*0401	1	33	47	VQIVSTAAQTFLATC	Consensus (comb.lib./smm/nn)	13.08	IVSTAAQT	50.69	29.58	TAAQTFLA	377	0.94	AAQTFLA
32	HLA-DPA1*01/DPB1*0401	1	259	273	ITTGSPITYSTYGKF	Consensus (comb.lib./smm/nn)	13.28	ITYSTYGKF	926.52	47.4	GSPITYSTY	1339	8.88	ITYSTYGK
33	HLA-DPA1*01/DPB1*0401	1	556	570	NLPYLVAYQATVCAR	Consensus (comb.lib./smm/nn)	13.94	LVAYQATV	19.93	24.6	YLVAYQAT	658	2.71	YLVAYQA
34	HLA-DPA1*01/DPB1*0401	1	512	526	RLRAYMNTPGLPVCQ	Consensus (comb.lib./smm/nn)	15.04	RAYMNTP	16.44	23.64	YMNTPGL	310	0.62	AYMNTP
35	HLA-DPA1*01/DPB1*0401	1	266	280	TYSTYGKFLADGGCS	Consensus (comb.lib./smm/nn)	15.24	TYSTYGKF	2.38	15.24	STYGKFLA	2971	25.27	YSTYGKF
36	HLA-DPA1*01/DPB1*0401	1	513	527	LRAYMNTPGLPVCQD	Consensus (comb.lib./smm/nn)	15.66	RAYMNTP	16.44	23.64	YMNTPGL	310	0.62	YMNTPG
37	HLA-DPA1*01/DPB1*0401	1	511	525	VRLRAYMNTPGLPVC	Consensus (comb.lib./smm/nn)	16.06	VRLRAYM	4.2	17.48	YMNTPGL	311	0.62	YMNTPG
38	HLA-DPA1*01/DPB1*0401	1	42	56	TFLATCINGVCWTVY	Consensus (comb.lib./smm/nn)	16.28	TFLATCIN	27375.94	68.61	FLATCING	2062	16.28	FLATCIN
39	HLA-DPA1*01/DPB1*0401	1	483	497	SGMFDSSVLCECYDA	Consensus (comb.lib./smm/nn)	16.7	GMFDSSV	2166.88	52.9	FDSSVLCE	1664	12.17	MFDSSVL
40	HLA-DPA1*01/DPB1*0401	1	532	546	WEGVFTGLTHIDAHF	Consensus (comb.lib./smm/nn)	17.22	GVFTGLTH	164.94	36.49	GVFTGLTH	2145	17.08	GVFTGLT

Result – Email

IEDB Tools MHC class II prediction result (2018-10-03 16:28:50) Inbox ×

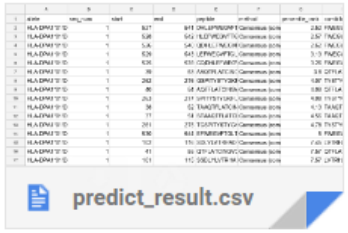
IEDB Tools <Prediction-results-noreply@tools.iedb.org>
to me

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

Input parameters
Method: recommended
Number of sequences: 1
Input sequences: attached
Alleles: DPA1*01-DPB1*04:01

Job parameters
Submission date: 2018-10-03 16:28:50
Completion date: 2018-10-03 16:28:53
Total walltime since submission: 3 seconds

2 Attachments



predict_result.csv

input_sequences.txt

Guidelines: Selecting binders

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

Alternate approaches for selecting binders

- Recommended threshold is arbitrary.
- Change cut-off 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_nu	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	YLVTRHADV	1194	7.45	YLVTRHADV	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	YLVTRHADV	1206	7.57	YLVTRHADV	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	YLVTRHADV	1183	7.34	YLVTRHADV	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	YLVTRHADV	1307	8.57	YLVTRHADV	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	528	542	HLEFWEGVFTGLTHI	2.57	FWEGVFTGL	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	2.62	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	3.13	FWEGVFTGL	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	3.26	FWEGVFTGL	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	40	54	AQTFLATCINGVCWT	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5

Prediction of promiscuous binders

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 ≤ **20.0**[1]

1. Oseroff et al. 2010, J Immunol

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
APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGP
VIQMYTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSG
GPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGK
STKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFLADGGCS
GGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFY
GKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTG
DFDSVIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYRFVAPGERPSGMFDSSV
LCECYDAGCAWYELTPAETTVRLRAYMNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYL
VAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMSADLEVV
```

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

Choose sequence format — auto detect format

Choose a Prediction Method

Prediction Method — IEDB recommended — Help on prediction method selections

Specify what to make binding predictions for

Select species/locus — Human, HLA-DR

Select MHC allele(s)

Select α & β chains separately if applicable:

Select HLA allele reference set:

Allele
DPA1*01/DPB1*04:01
DPA1*03:01/DPB1*04:02
DPA1*02:01/DPB1*05:01
DRB1*01:01

Upload allele file

Multiple alleles - Result

MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	HCV_NS3	APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATCING VCWTVYHGAGTRTIASPKGPVIQMYTNVDQDLVGWPAPQGSRSLTPCTCG SSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLCPAGHA VGIFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVA HLHAPTGSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDP NIRTGVRTITTGSPITYSTYGKFLADGGCSGGAYDIIICDECHSTDATSI LGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEVALSTTGEIPFY GKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSVI PTSGDVVVVATDALMTGYTGDFDSVIDCNTCVTQTVDFSLDPTFTIETIT LPQDAVSRTQRRGRTGRGKPGIYRFVAPGERPSGMFDSSVLCECYDAGCA WYELTPAETTVRLRAYMNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQT KQSGENLPYLVAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLY RLGAVQNEITLTHPVTKYIMTCMSADLEVVT

Prediction method: IEDB recommended | Low percentile_rank = good binders

Download result

Citations

Check to expanded the result:

Allele	#	Start	End	Peptide	Method used	Percentile rank
HLA-DRB1*01:01	1	222	236	GYKVLVLNPSVAATL	Consensus (comb.lib./smm/nn)	0.32
HLA-DRB1*01:01	1	223	237	YKVLVLNPSVAATLG	Consensus (comb.lib./smm/nn)	0.54
HLA-DRB1*01:01	1	220	234	AQGYKVLVLNPSVAA	Consensus (comb.lib./smm/nn)	1.06
HLA-DRB1*01:01	1	221	235	QGYKVLVLNPSVAAT	Consensus (comb.lib./smm/nn)	1.06
HLA-DRB1*01:01	1	224	238	KVLVLNPSVAATLGF	Consensus (comb.lib./smm/nn)	1.33
HLA-DRB1*01:01	1	219	233	AAQGYKVLVLNPSVA	Consensus (comb.lib./smm/nn)	2.18
HLA-DPA1*01/DPB1*04:01	1	527	541	DHLEFWEGVFTGLTH	Consensus (comb.lib./smm/nn)	2.52
HLA-DPA1*01/DPB1*04:01	1	528	542	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	2.57
HLA-DPA1*01/DPB1*04:01	1	526	540	QDHLEFWEGVFTGLT	Consensus (comb.lib./smm/nn)	2.62
HLA-DRB1*01:01	1	378	392	AAKLVALGINAVAYY	Consensus (comb.lib./smm/nn)	2.64
HLA-DRB1*01:01	1	225	239	VLVLNPSVAATLGFG	Consensus (comb.lib./smm/nn)	2.82
HLA-DRB1*01:01	1	379	393	AKLVALGINAVAYYR	Consensus (comb.lib./smm/nn)	3.09
HLA-DPA1*01/DPB1*04:01	1	529	543	LEFWEGVFTGLTHID	Consensus (comb.lib./smm/nn)	3.13
HLA-DPA1*01/DPB1*04:01	1	525	539	CQDHLEFWEGVFTGL	Consensus (comb.lib./smm/nn)	3.26
HLA-DPA1*01/DPB1*04:01	1	39	53	AAQTFLATCINGVCW	Consensus (comb.lib./smm/nn)	3.80
HLA-DRB1*01:01	1	377	391	LAAKLVALGINAVAY	Consensus (comb.lib./smm/nn)	3.97
HLA-DPA1*01/DPB1*04:01	1	262	276	GSPITYSTYGKFLAD	Consensus (comb.lib./smm/nn)	4.07

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
	Combined	71.1
DRB3/4/5	DRB3*01:01	26.1
	DRB3*02:02	34.3
	DRB4*01:01	41.8
	DRB5*01:01	16.0
	Combined	87.7

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
	Combined	81.6
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
	Combined	94.5

Greenbaum et al., 2011. Immunogenetics

- Set of alleles used for promiscuous binder predictions
- Link provided in the “Help” tab (section-3) (allele file can be uploaded to the tool)

Promiscuous binders

- Binders with ≥ **50% alleles** binding (consensus percentile ≤ **20.0**) considered promiscuous binders

1	#	Peptide	Start	End	Count of alleles binding	% of alleles binding (out of 27)
2	1	APITAYAQQTRGLLG	1	15	8	29.6%
3	2	YAQQTRGLLGCIITS	6	20	3	11.1%
4	3	RGLLGCIITSLTGRD	11	25	8	29.6%
5	4	CIITSLTGRDKNQVE	16	30	8	29.6%
6	5	LTGRDKNQVEGEVQI	21	35	0	0.0%
7	6	KNQVEGEVQIVSTAA	26	40	5	18.5%
8	7	GEVQIVSTAAQTFLA	31	45	21	77.8%
9	8	VSTAAQTFLATCING	36	50	4	14.8%
10	9	QTFLATCINGVCWTV	41	55	11	40.7%
11	10	TCINGVCWTVYHGAG	46	60	1	3.7%
12	11	VCWTVYHGAGTRTIA	51	65	9	33.3%
13	12	YHGAGTRTIASPKGP	56	70	7	25.9%
14	13	TRTIASPKGPVIQMY	61	75	3	11.1%
15	14	SPKGPVIQMYTNVDQ	66	80	9	33.3%
16	15	VIQMYTNVDQDLVGW	71	85	9	33.3%
17	16	TNVDQDLVGWPAPQG	76	90	3	11.1%
18	17	DLVGWPAPQGSRSLT	81	95	2	7.4%
19	18	PAPQGSRSLTPCTCG	86	100	0	0.0%

“7-allele” method

Paul et al. (2015) Development and validation of a broad scheme for prediction of HLA class II restricted T cell epitopes. *Journal of immunological methods*.

“7-allele” method

- Aim was to capture maximum immune response with minimum no. of peptides
- 6 peptide datasets with measured immune responses (SFCs/10^6 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 ≥ 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 ≈ 50% response
- The **median consensus percentile rank of the 7 alleles ≈ 20.0**
  - Universal prediction threshold

1. Paul et al. (2015) Journal of Immunological Methods 422, 28-34

“7-allele” method

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

Exercise

Exercise

- **Question:**

  Predict the alleles from the given set of 6 MHC class II alleles to which the peptide "**HLEFWEGVFTGLTHI**" 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

Exercise

- **Steps:**

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

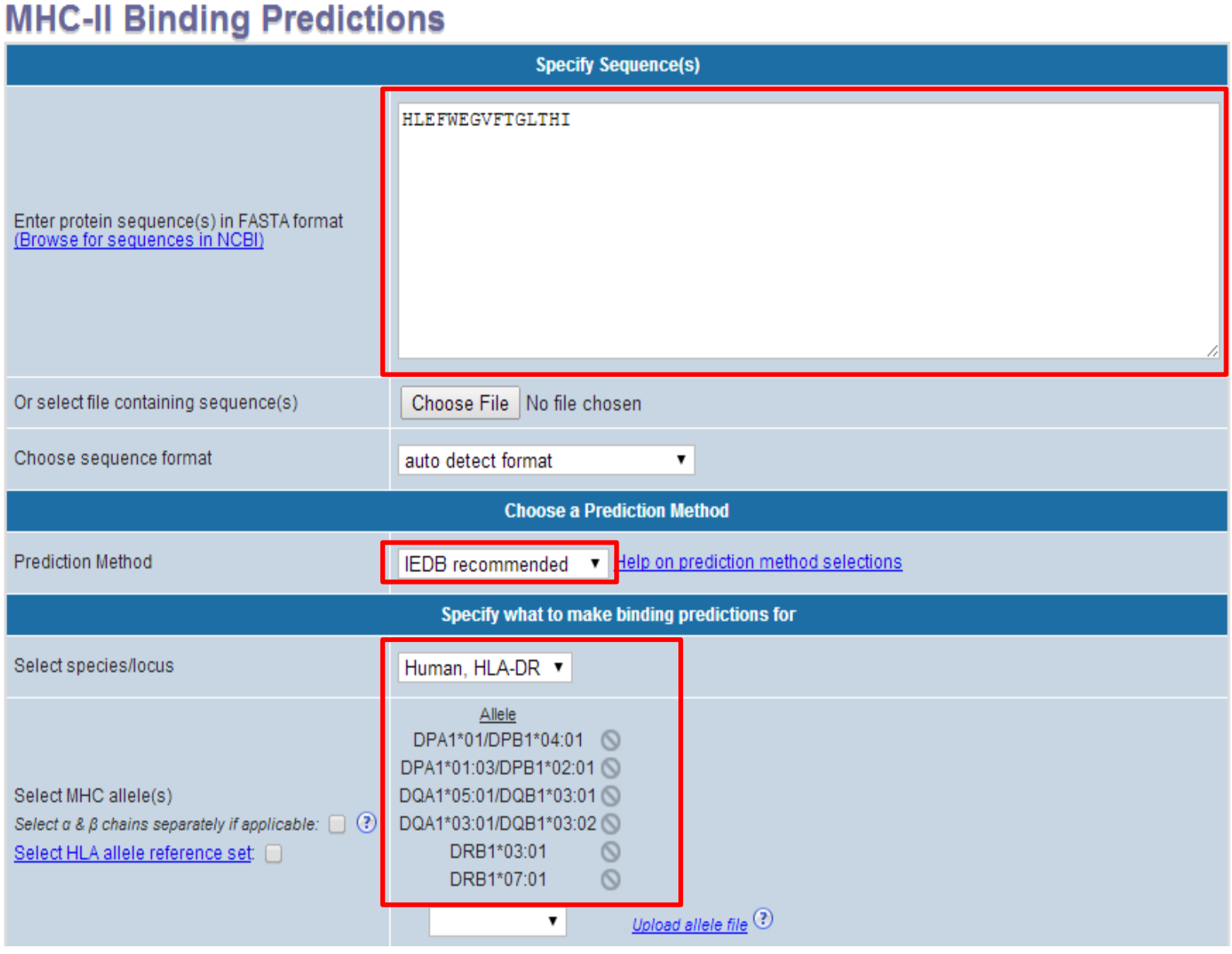
   Peptide: **HLEFWEGVFTGLTHI**

   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 ≤ 10.0

Exercise: Input



Exercise: Output

MHC-II Binding Prediction Results

Input Sequences

#	Name	Sequence
1	sequence 1	HLEFWEGVFTGLTHI

Prediction method: IEDB recommended | Low percentile_rank = good binders

Download result

Citations

Check to expanded the result:

Allele	#	Start	End	Peptide	Method used	Percentile rank
HLA-DPA1*01/DPB1*04:01	1	1	15	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	2.57
HLA-DPA1*01:03/DPB1*02:01	1	1	15	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	3.76
HLA-DRB1*07:01	1	1	15	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	19.31
HLA-DQA1*05:01/DQB1*03:01	1	1	15	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	23.49
HLA-DQA1*03:01/DQB1*03:02	1	1	15	HLEFWEGVFTGLTHI	Consensus (comb.lib./smm/nn)	31.14
HLA-DRB1*03:01	1	1	15	HLEFWEGVFTGLTHI	Consensus (smm/nn/sturniolo)	70.94

Download result

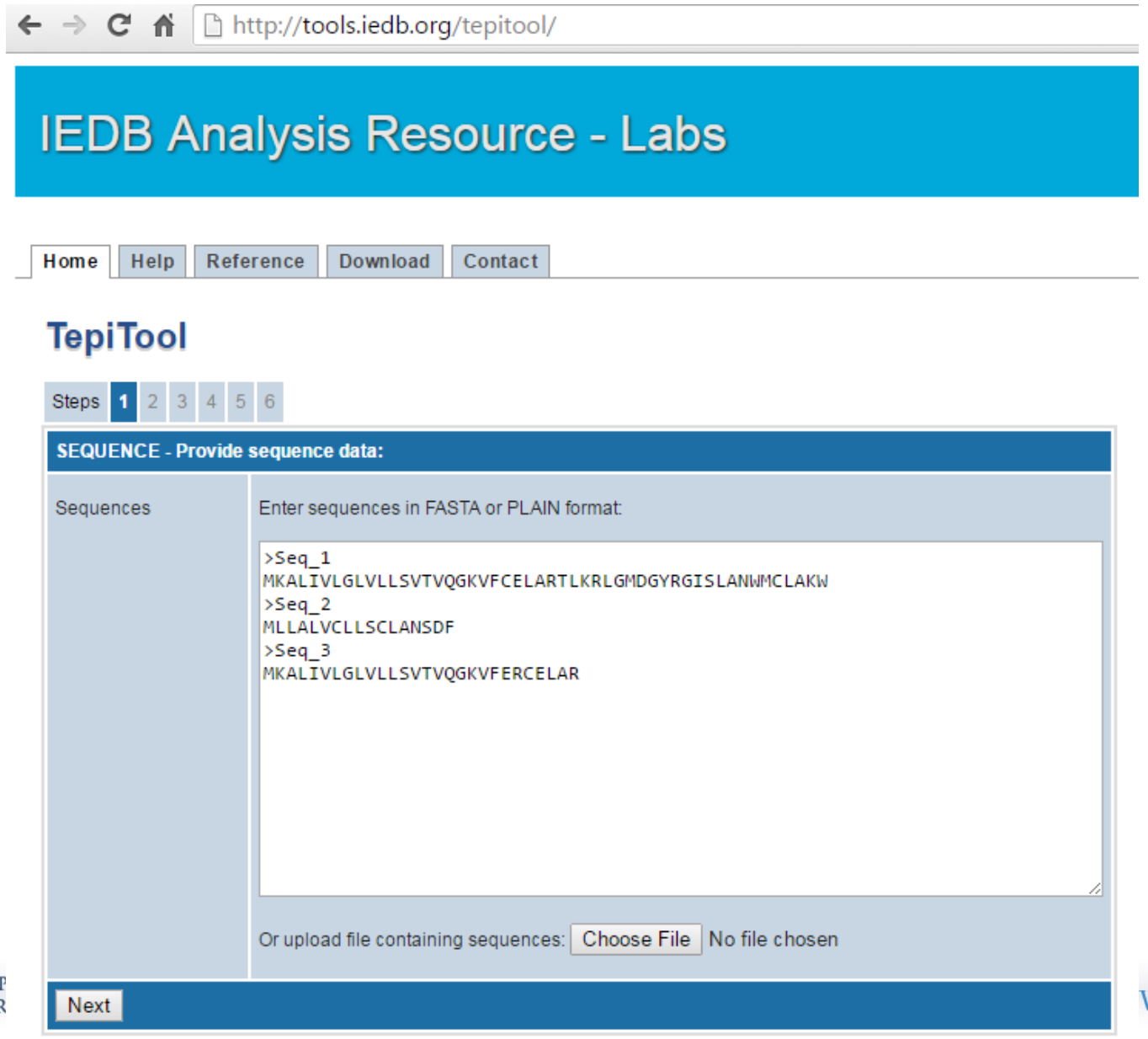
Tepitool

http://tools.iedb.org/tepitool

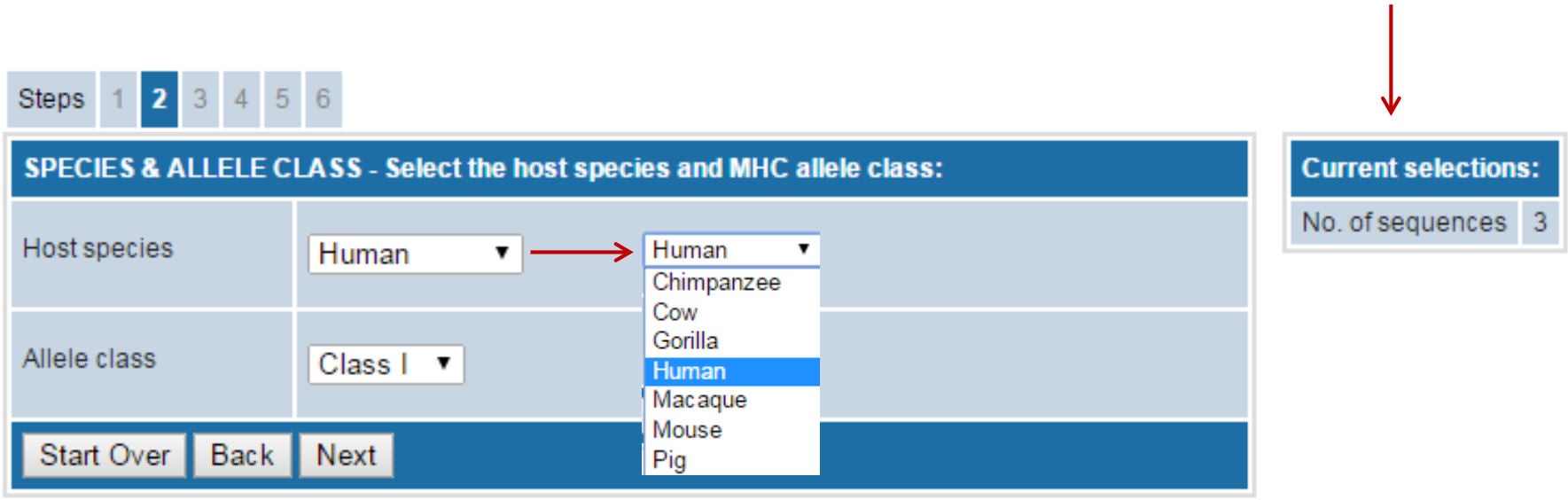
TepiTool

- 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.
- Available at **http://tools.iedb.org/tepitool**

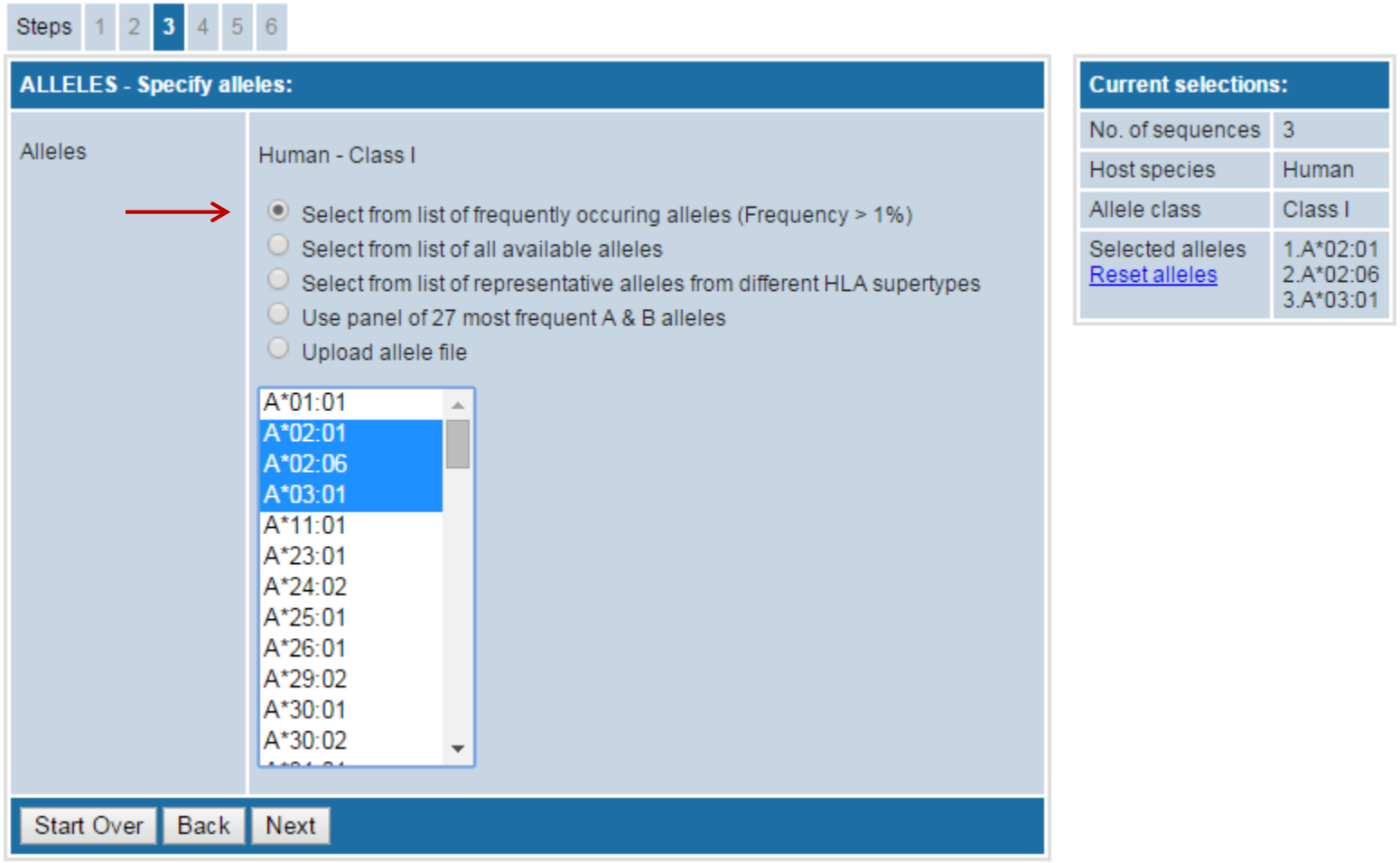
Step 1: Sequence data



Step 2: Species & Allele class



Step 3: Alleles - Class I



Step 4: Peptides - Class I

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. 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)	(•) 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

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. 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)	◉ 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

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

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

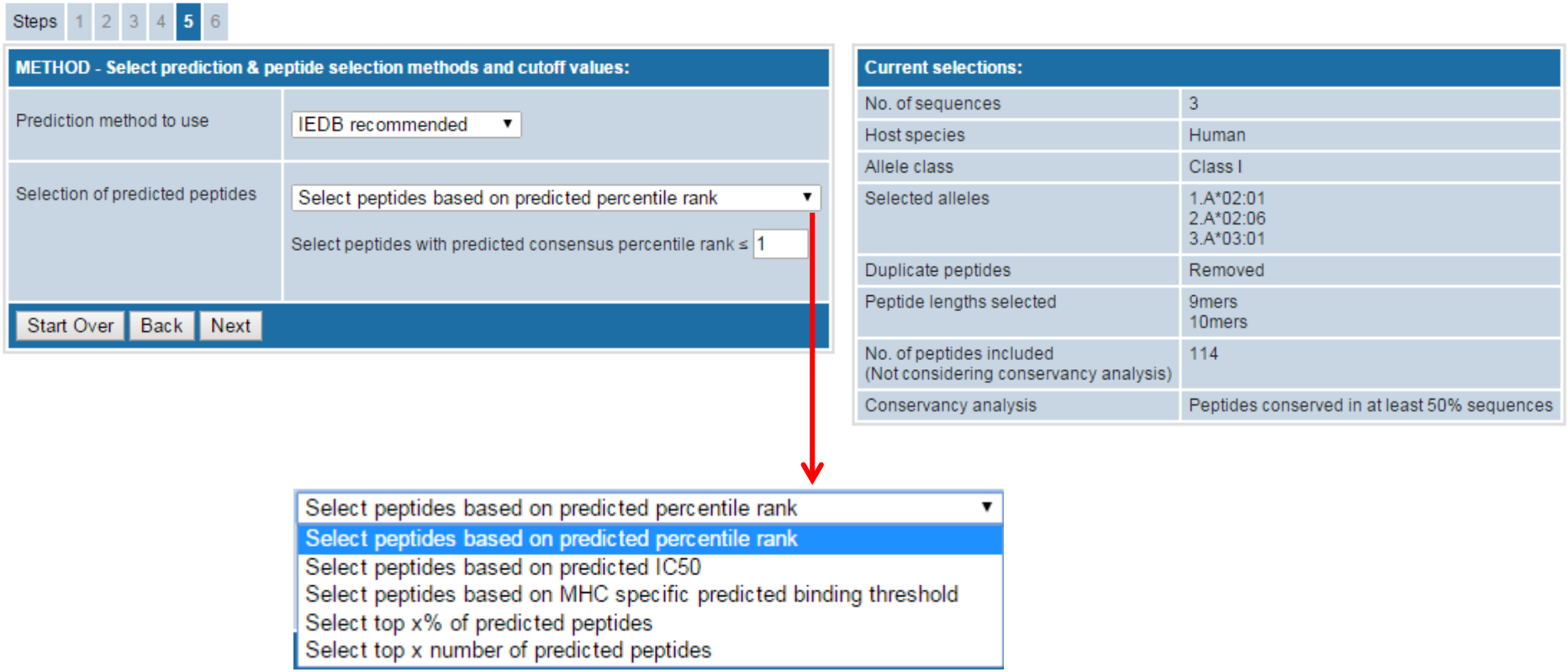
Use peptides conserved in 50% sequences ▼

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 5: Method - Class I



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 ▼
Selection of predicted peptides	Select peptides based on predicted IC50 ▼ Select peptides with predicted IC50 ≤ 500 nM

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
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

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 ▾
Selection of predicted peptides	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

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

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
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

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 ▼
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 (Final selection of predicted peptides done based on percentile rank)

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
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

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 ▼
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

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

Step 6: Review & Submit

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)	workshop
Email (optional - will notify when job is finished)	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: Web - Class I

Home | Help | Reference | Download | Contact

Prediction results - concise (Download table):

Seq #	Peptide start	Peptide end	Peptide	Percentile rank	Allele	Conservancy
1	5	14	IVLGLVLLSV	0.35	HLA-A*02:06	67%
1	10	19	VLLSVTVQGK	0.45	HLA-A*03:01	67%
1	5	14	IVLGLVLLSV	0.8	HLA-A*02:01	67%

Download results details:

Complete results	Prediction results of all peptides
Conservancy of peptides	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, NN and Combinatorial library methods.
1. TepiTool reference
2. Moutaftsi M, Peters B, Pasquetto V, Tscharke DC, Sidney J, Bui HH, Grey H, Sette A. 2006. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia

For complete list of references please click here: References

Results: Web - Class I

Input sequences:

Seq #	Seq title	Sequence
1	Seq_1	MKALIVLGLVLLSVTVQGKVFCELARTLKRLGMDGYRGISLANWMCLAKW
2	Seq_2	MLLALVCLLSCLANSDF
3	Seq_3	MKALIVLGLVLLSVTVQGKVFERCELAR

Other input parameters:

Input summary:	
No. of sequences	3
Host species	Human
Allele class	Class I
Alleles	A*02:01 A*02:06 A*03:01
Duplicate peptides	Removed
Peptide lengths selected	9mers 10mers
Peptide overlap	N/A
Conservancy analysis	Yes (Conservancy cutoff = 50% sequences)
Prediction method	IEDB recommended
Peptide selection criterion	Predicted percentile rank
Cutoff for peptide selection criterion	1
Job name	workshop
Email	spaul@lji.org

Results: Complete results

	allele	seq_num	start	end	length	peptide	method	percentile_rank	ann_ic50	ann_rank	smm_ic50	smm_rank	comblib_s	comblib_s	netmhcpa	netmhcpan_rank
2	HLA-A*02:01	2	1	9	9	MLLALVCLL	Consensus (ann/smm/comblib_sidney2008)	0.5	11	0.5	10.17	0.3	3.09E-05	1.8	-	-
3	HLA-A*02:01	1	40	48	9	SLANWMCLA	Consensus (ann/smm/comblib_sidney2008)	0.8	20	0.8	43.86	0.8	0.00017	9.1	-	-
4	HLA-A*03:01	1	41	49	9	LANWMCLAK	Consensus (ann/smm)	1.1	122	0.9	322.7	1.3	-	-	-	-
5	HLA-A*03:01	1	11	19	9	LLSVTVQGK	Consensus (ann/smm)	1.15	214	1.3	233.78	1	-	-	-	-
6	HLA-A*03:01	3	11	19	9	LLSVTVQGK	Consensus (ann/smm)	1.15	214	1.3	233.78	1	-	-	-	-
7	HLA-A*02:06	2	1	9	9	MLLALVCLL	Consensus (ann/smm)	1.35	24	1.6	34.96	1.1	-	-	-	-
8	HLA-A*02:01	1	6	14	9	VLGLVLLSV	Consensus (ann/smm/comblib_sidney2008)	1.4	42	1.4	40.28	0.7	4.27E-05	2.4	-	-
9	HLA-A*02:01	3	6	14	9	VLGLVLLSV	Consensus (ann/smm/comblib_sidney2008)	1.4	42	1.4	40.28	0.7	4.27E-05	2.4	-	-
10	HLA-A*02:06	1	16	24	9	VQGKVFCEL	Consensus (ann/smm)	1.45	24	1.6	37.98	1.3	-	-	-	-
11	HLA-A*02:01	1	8	16	9	GLVLLSVTV	Consensus (ann/smm/comblib_sidney2008)	2.2	89	2.2	89.97	1.5	0.0001	5.7	-	-
12	HLA-A*02:01	3	8	16	9	GLVLLSVTV	Consensus (ann/smm/comblib_sidney2008)	2.2	89	2.2	89.97	1.5	0.0001	5.7	-	-
13	HLA-A*02:01	2	4	12	9	ALVCLLSCL	Consensus (ann/smm/comblib_sidney2008)	2.4	118	2.4	101.42	1.6	0.000537	23	-	-
14	HLA-A*02:06	3	19	27	9	KVFERCELA	Consensus (ann/smm)	2.75	42	2.2	103.65	3.3	-	-	-	-
15	HLA-A*02:06	1	2	10	9	KALIVLGLV	Consensus (ann/smm)	2.9	66	3	86.81	2.8	-	-	-	-
16	HLA-A*02:06	3	2	10	9	KALIVLGLV	Consensus (ann/smm)	2.9	66	3	86.81	2.8	-	-	-	-
17	HLA-A*02:06	1	4	12	9	LIVLGLVLL	Consensus (ann/smm)	3.6	119	3.8	105.33	3.4	-	-	-	-
18	HLA-A*02:06	3	4	12	9	LIVLGLVLL	Consensus (ann/smm)	3.6	119	3.8	105.33	3.4	-	-	-	-
19	HLA-A*02:06	2	4	12	9	ALVCLLSCL	Consensus (ann/smm)	3.6	104	3.6	111.83	3.6	-	-	-	-
20	HLA-A*02:06	1	40	48	9	SLANWMCLA	Consensus (ann/smm)	3.9	49	2.4	183.89	5.4	-	-	-	-
21	HLA-A*02:06	1	8	16	9	GLVLLSVTV	Consensus (ann/smm)	4	219	5.3	81.01	2.7	-	-	-	-
22	HLA-A*02:06	3	8	16	9	GLVLLSVTV	Consensus (ann/smm)	4	219	5.3	81.01	2.7	-	-	-	-
23	HLA-A*02:06	1	6	14	9	VLGLVLLSV	Consensus (ann/smm)	4.55	318	6.1	92.8	3	-	-	-	-
24	HLA-A*02:06	3	6	14	9	VLGLVLLSV	Consensus (ann/smm)	4.55	318	6.1	92.8	3	-	-	-	-
25	HLA-A*02:01	1	3	11	9	ALIVLGLVL	Consensus (ann/smm/comblib_sidney2008)	4.7	766	4.7	257.1	3.3	0.000776	30	-	-
26	HLA-A*02:01	3	3	11	9	ALIVLGLVL	Consensus (ann/smm/comblib_sidney2008)	4.7	766	4.7	257.1	3.3	0.000776	30	-	-
27	HLA-A*02:01	1	4	12	9	LIVLGLVLL	Consensus (ann/smm/comblib_sidney2008)	5.1	911	5.1	367.38	4.1	0.00213	52	-	-
28	HLA-A*02:01	3	4	12	9	LIVLGLVLL	Consensus (ann/smm/comblib_sidney2008)	5.1	911	5.1	367.38	4.1	0.00213	52	-	-
29	HLA-A*02:01	3	19	27	9	KVFERCELA	Consensus (ann/smm/comblib_sidney2008)	5.1	436	4	562.48	5.1	0.000711	28	-	-
30	HLA-A*02:01	2	7	15	9	CLLSCLANS	Consensus (ann/smm/comblib_sidney2008)	5.9	1346	5.9	767.56	6.4	3.63E-05	2.1	-	-
31	HLA-A*03:01	2	1	9	9	MLLALVCLL	Consensus (ann/smm)	5.9	9050	6.5	2715.19	5.3	-	-	-	-
32	HLA-A*02:01	1	2	10	9	KALIVLGLV	Consensus (ann/smm/comblib_sidney2008)	6	1408	6	634.03	5.6	0.000425	20	-	-
33	HLA-A*02:01	3	2	10	9	KALIVLGLV	Consensus (ann/smm/comblib_sidney2008)	6	1408	6	634.03	5.6	0.000425	20	-	-
34	HLA-A*03:01	1	22	30	9	CELARTLKR	Consensus (ann/smm)	6.45	11796	8.4	2127.16	4.5	-	-	-	-
35	HLA-A*02:01	1	16	24	9	VQGKVFCEL	Consensus (ann/smm/comblib_sidney2008)	6.8	1947	6.6	859.23	6.8	0.01	75	-	-
36	HLA-A*03:01	3	15	23	9	TVQGKVFER	Consensus (ann/smm)	6.8	8195	6	4685.98	7.6	-	-	-	-
37	HLA-A*03:01	1	40	48	9	SLANWMCLA	Consensus (ann/smm)	7.15	7856	5.8	5751.75	8.5	-	-	-	-

Results: Peptide conservancy

	A	B	C	D	E	F	G	H
1	Peptides	Seq_num	Peptide_start	Peptide_end	Seq_1	Seq_2	Seq_3	Conservancy %
2	MKALIVLGL	1	1	9	1	0	1	67%
3	KALIVLGLV	1	2	10	1	0	1	67%
4	ALIVLGLVL	1	3	11	1	0	1	67%
5	LIVLGLVLL	1	4	12	1	0	1	67%
6	IVLGLVLLS	1	5	13	1	0	1	67%
7	VLGLVLLSV	1	6	14	1	0	1	67%
8	LGLVLLSVT	1	7	15	1	0	1	67%
9	GLVLLSVTV	1	8	16	1	0	1	67%
10	LVLLSVTVQ	1	9	17	1	0	1	67%
11	VLLSVTVQG	1	10	18	1	0	1	67%
12	LLSVTVQGK	1	11	19	1	0	1	67%
13	LSVTVQGKV	1	12	20	1	0	1	67%
14	SVTVQGKVF	1	13	21	1	0	1	67%
15	VTVQGKVFC	1	14	22	1	0	0	33%
16	TVQGKVFCE	1	15	23	1	0	0	33%
17	VQGKVFCEL	1	16	24	1	0	0	33%
18	QGKVFCELA	1	17	25	1	0	0	33%
19	GKVFCELAR	1	18	26	1	0	0	33%
20	KVFCELART	1	19	27	1	0	0	33%
21	VFCELARTL	1	20	28	1	0	0	33%
22	FCELARTLK	1	21	29	1	0	0	33%
23	CELARTLKR	1	22	30	1	0	0	33%
24	ELARTLKRL	1	23	31	1	0	0	33%
25	LARTLKRLG	1	24	32	1	0	0	33%
26	ARTLKRLGM	1	25	33	1	0	0	33%
27	RTLKRLGMD	1	26	34	1	0	0	33%
28	TLKRLGMDG	1	27	35	1	0	0	33%

Results: Email

Binding prediction results for job "workshop" Inbox x

TepiTool-results-noreply@tepitool.iedb.org 3:35 PM (1 minute ago)

to me

Binding prediction for your job "workshop" is finished. Below are the concise results based on your input parameters. Please go back to the browser running TepiTool for complete details.

Seq #	Peptide start	Peptide end	Peptide	Percentile rank	Allele	Conservancy
1	5	14	IVLGLVLLSV	0.35	HLA-A*02:06	67%
1	10	19	VLLSVTVQGK	0.45	HLA-A*03:01	67%
1	5	14	IVLGLVLLSV	0.8	HLA-A*02:01	67%

Download concise results table
Download complete results table
Download conservancy of peptides

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

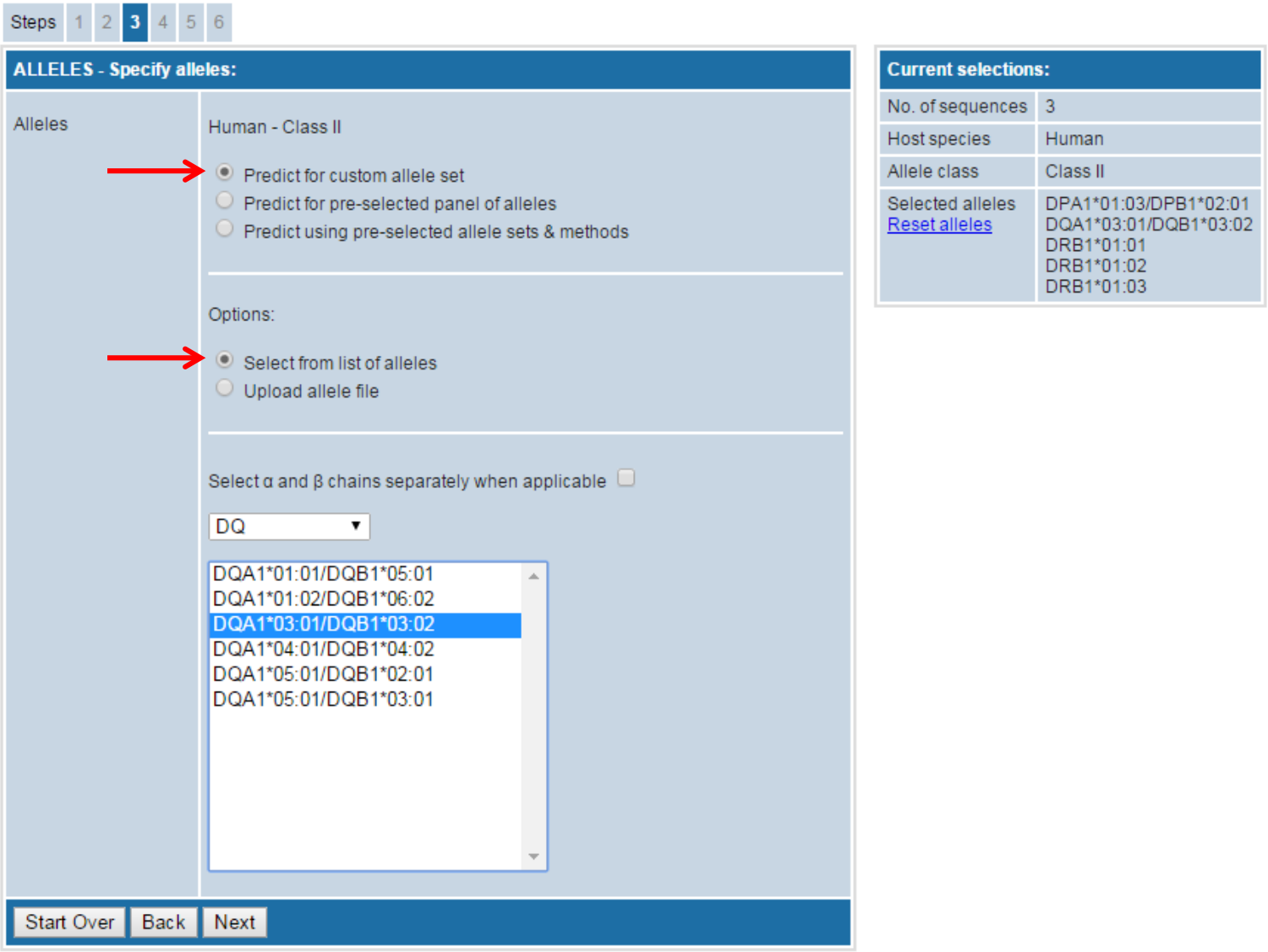
The MHC I binding predictions were done with IEDB analysis resource (TepiTool [1]) using Consensus method [2] which employs SMM, ANN and Combinatorial library methods.

1. Paul, S., S idney, 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. Moutaftsi M, Peters B, Pasquetto V, Tscharke DC, Sidney J, Bui HH, Grey H, Sette A. 2006. A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. *Nat Biotechnol.* 24: 817-819.

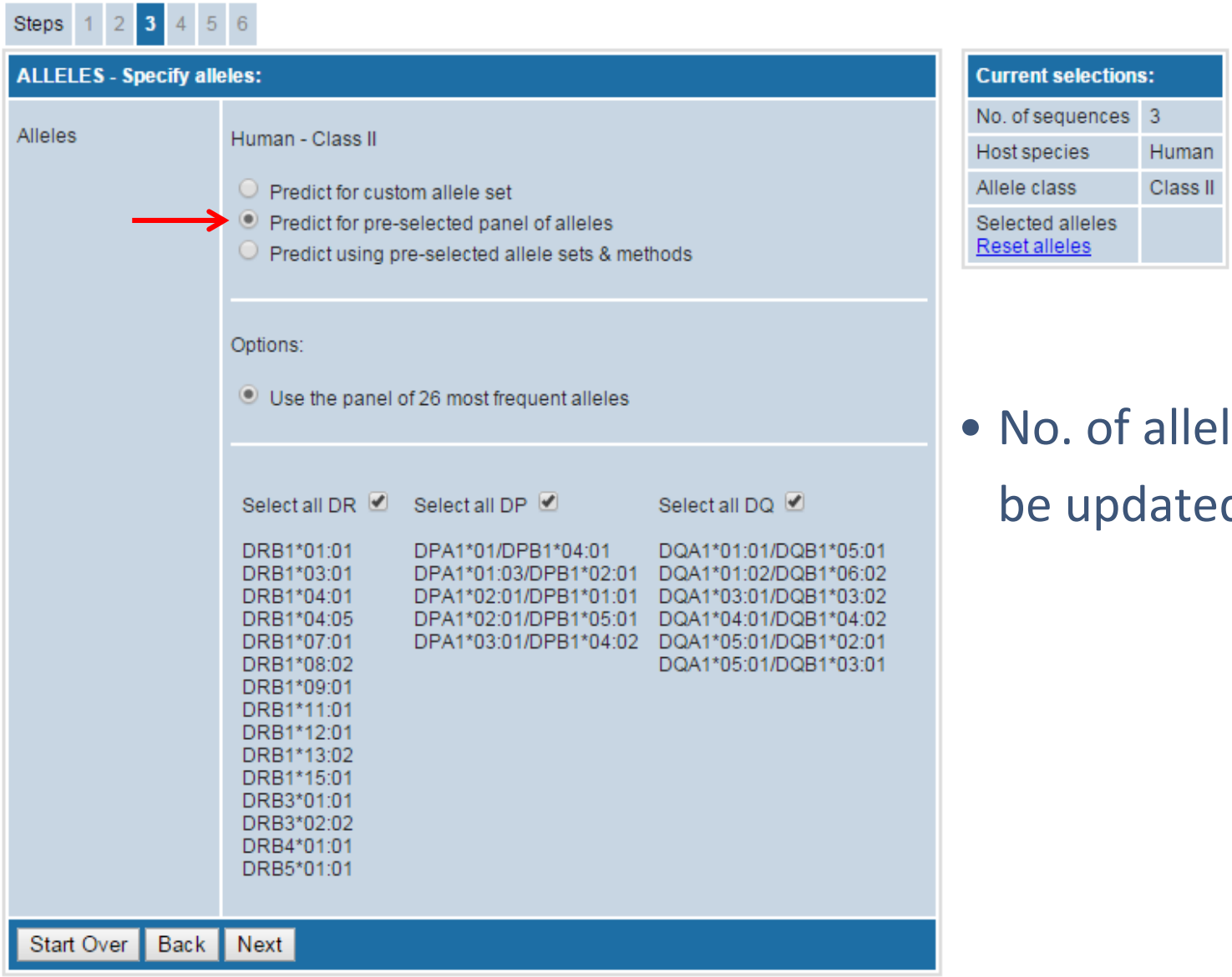
For complete list of references please click here: References

Click here to go to Tepitool home page

Step 3: Alleles - Class II

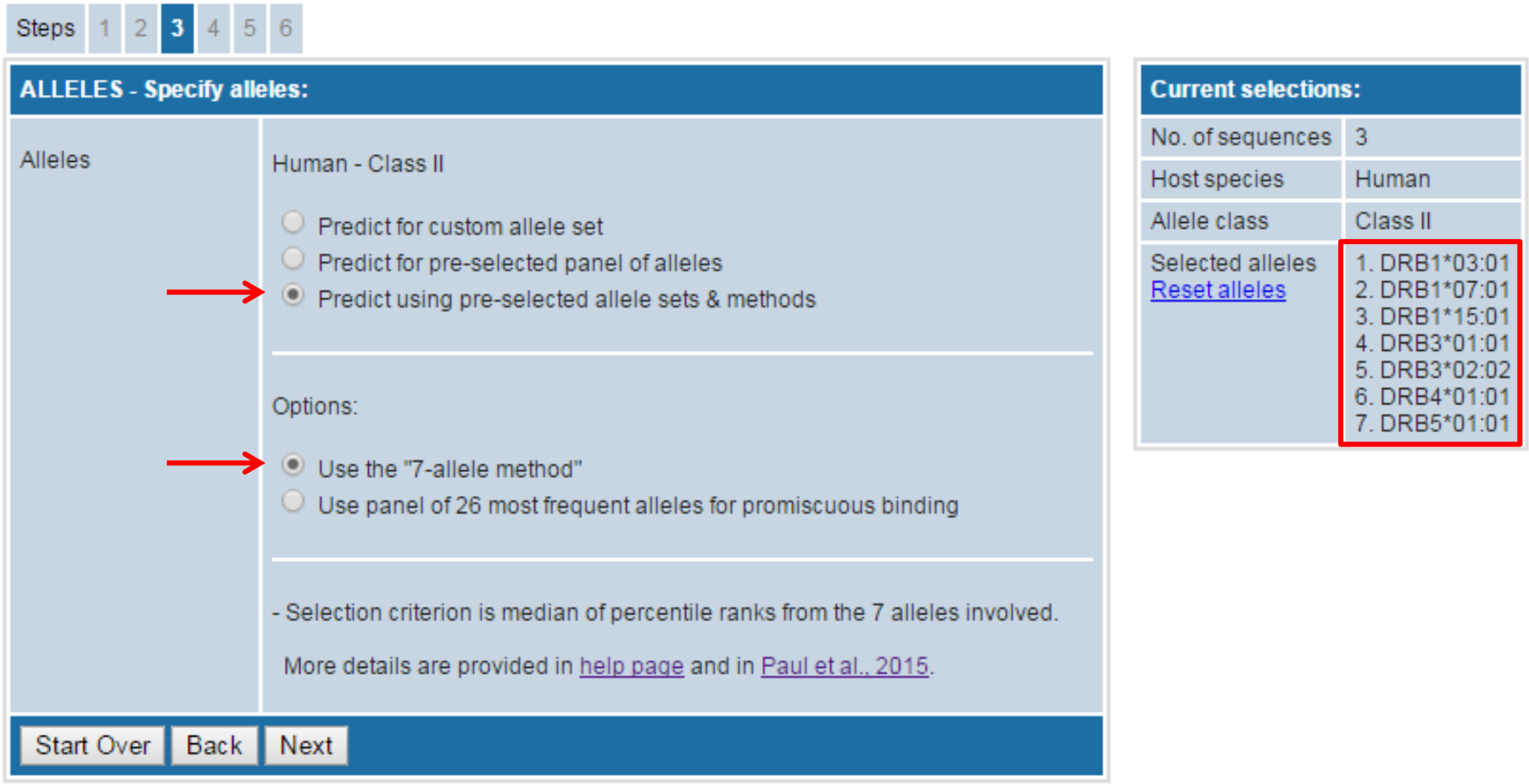


Step 3: Alleles - Class II

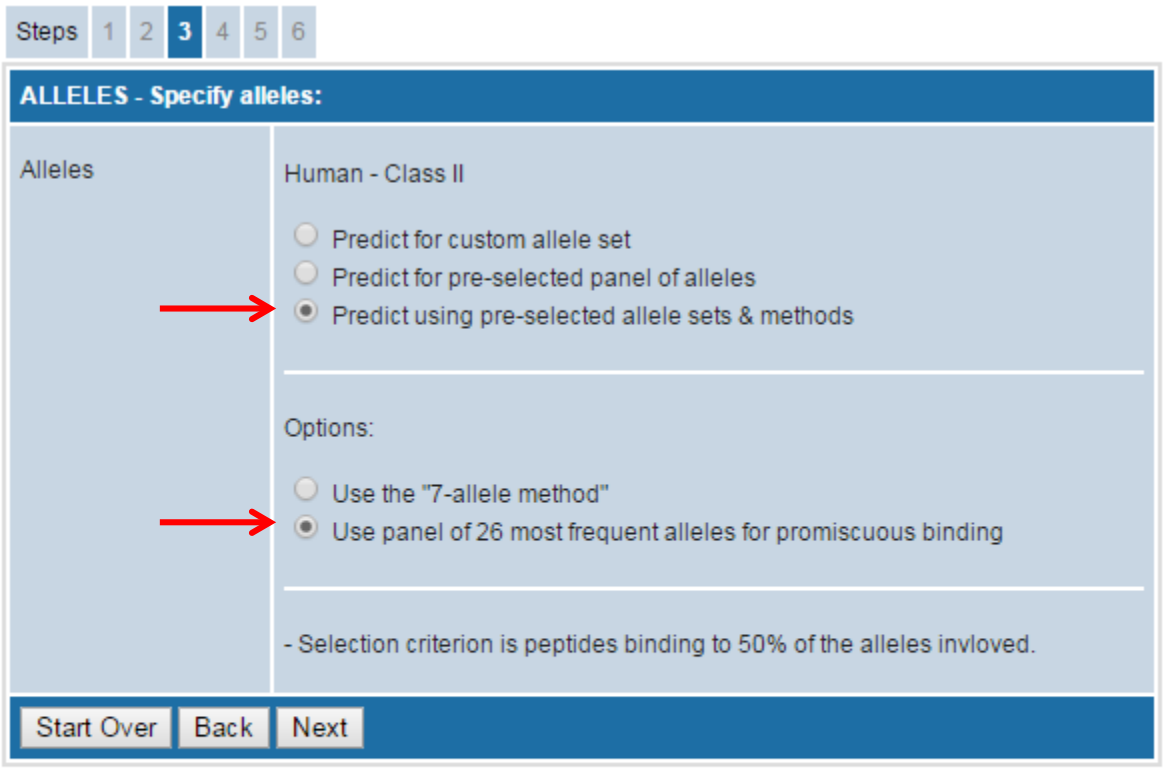


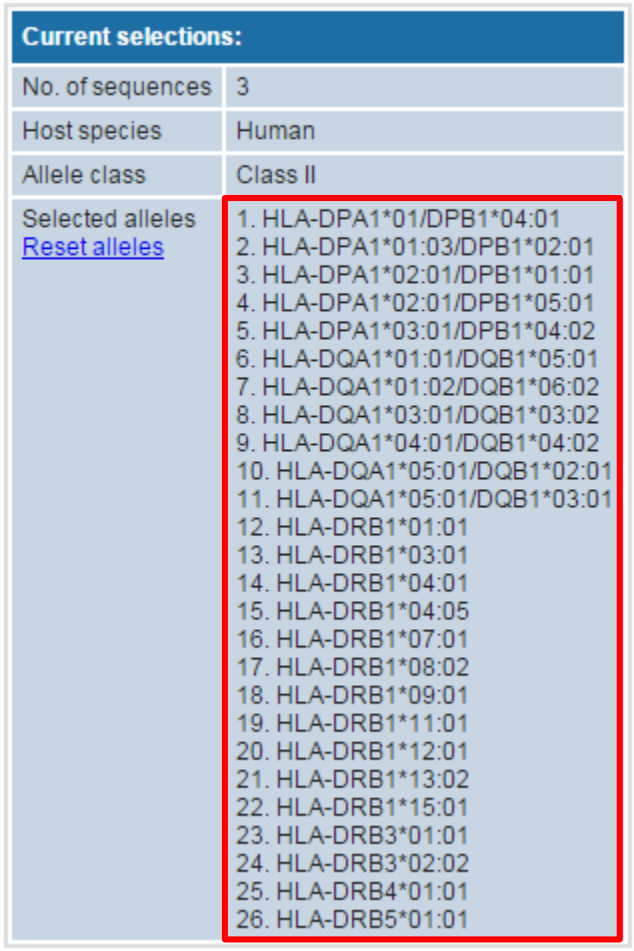
- No. of alleles will be updated to 27

Step 3: Alleles - Class II



Step 3: Alleles - Class II





- No. of alleles will be updated to 27

Step 4: Peptides - Class II

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 50% sequences ▾

Start Over | Back | Next

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 4: Peptides - Class II

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 8. Approximate no. of peptides to be considered for prediction = 10
Conservancy analysis (Uses only peptides conserved in specified % of sequences)	( ) No (•) Yes Use peptides conserved in 50% sequences ▾

Start Over | Back | Next

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 4: Peptides - Class II

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. Desired no. of overlapping residues for 15mers - No. of overlapping residues fixed at 10. Approximate no. of peptides to be considered for prediction = 14
Conservancy analysis (Uses only peptides conserved in specified % of sequences)	○ No ◉ Yes Use peptides conserved in 50% sequences ▾

Start Over | Back | Next

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 4: Peptides - Class II

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

Desired no. of overlapping residues for 15mers

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 50% sequences ▼

Start Over | Back | Next

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 4: Peptides - Class II (7-allele method & panel of 26 most frequent alleles)

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

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Alleles inolved	1. HLA-DRB1*03:01 2. HLA-DRB1*07:01 3. HLA-DRB1*15:01 4. HLA-DRB3*01:01 5. HLA-DRB3*02:02 6. HLA-DRB4*01:01 7. HLA-DRB5*01:01

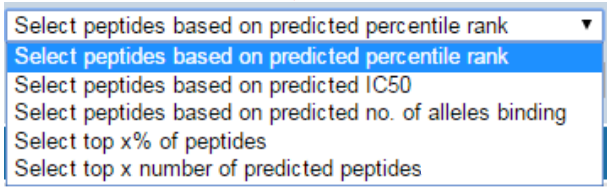
Step 5: Method - Class II

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 consenus percentile rank ≤ 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



Step 5: Method - Class II

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 no. of alleles binding ▾ Select peptides that bind to at least 50 % alleles (binding determined by IEDB consensus percentile rank ≤ 20.0)

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

Step 5: Method - Class II (7-allele method)

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 ≤ 20

Start Over | Back | Next

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Alleles involved	1. HLA-DRB1*03:01 2. HLA-DRB1*07:01 3. HLA-DRB1*15:01 4. HLA-DRB3*01:01 5. HLA-DRB3*02:02 6. HLA-DRB4*01:01 7. HLA-DRB5*01:01
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included	12

Step 5: Method - Class II (panel of 26 most frequent alleles)

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

Current selections:	
No. of sequences	3
Host species	Human
Allele class	Class II
Alleles involved	1. DPA1*01/DPB1*04:01 2. DPA1*01:03/DPB1*02:01 3. DPA1*02:01/DPB1*01:01 4. DPA1*02:01/DPB1*05:01 5. DPA1*03:01/DPB1*04:02 6. DQA1*01:01/DQB1*05:01 7. DQA1*01:02/DQB1*06:02 8. DQA1*03:01/DQB1*03:02 9. DQA1*04:01/DQB1*04:02 10. DQA1*05:01/DQB1*02:01 11. DQA1*05:01/DQB1*03:01 12. DRB1*01:01 13. DRB1*03:01 14. DRB1*04:01 15. DRB1*04:05 16. DRB1*07:01 17. DRB1*08:02 18. DRB1*09:01 19. DRB1*11:01 20. DRB1*12:01 21. DRB1*13:02 22. DRB1*15:01 23. DRB3*01:01 24. DRB3*02:02 25. DRB4*01:01 26. DRB5*01:01
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included	12

Results: Web - Class II

Home | Help | Reference | Download | Contact

Prediction results - concise (Download table):

Seq #	Peptide start	Peptide end	Peptide sequence	Consensus percentile rank	Allele	Conservancy
1	2	16	KALIVLGLVLLSVTV	2.74	HLA-DRB1*01:01	67.0%
1	1	15	MKALIVLGLVLLSVT	1.15	HLA-DRB1*01:02	67.0%
1	6	20	VLGLVLLSVTVQGKV	1.77	HLA-DRB1*01:02	67.0%

Download results details:

Non-redundant results	Prediction results with redundant peptides within each sequence removed - Includes positives and negatives
Complete results	Prediction results of all peptides
Conservancy of peptides	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,
1. TepiTool reference
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 Bio*
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 evaluati
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 m
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 an

For complete list of references please click here: References

Results: Non-redundant results (Class II)

	A	B	C	D	E	F	G	
1	allele	seq_num	start	end	peptide	method	percentile_rank	c
2	HLA-DPA1*01:03/DPB1*02:01	1	36	50	YRGISLANWMCLAKW	Consensus (comb.lib./smm/nn)	14.46	C
3	HLA-DPA1*01:03/DPB1*02:01	1	10	24	VLLSVTVQGKVFCEL	Consensus (comb.lib./smm/nn)	17.26	L
4	HLA-DPA1*01:03/DPB1*02:01	1	15	29	TVQGKVFCELARTLK	Consensus (comb.lib./smm/nn)	23.57	V
5	HLA-DPA1*01:03/DPB1*02:01	1	1	15	MKALIVLGLVLLSVT	Consensus (comb.lib./smm/nn)	24.97	I
6	HLA-DPA1*01:03/DPB1*02:01	1	20	34	VFCELARTLKRLGMD	Consensus (comb.lib./smm/nn)	30.44	V
7	HLA-DPA1*01:03/DPB1*02:01	1	31	45	LGMDGYRGISLANWM	Consensus (comb.lib./smm/nn)	31.43	L
8	HLA-DPA1*01:03/DPB1*02:01	1	26	40	RTLKRLGMDGYRGIS	Consensus (comb.lib./smm/nn)	53.98	L
9	HLA-DPA1*01:03/DPB1*02:01	2	1	15	MLLALVCLLSCLANS	Consensus (comb.lib./smm/nn)	22.65	M
10	HLA-DPA1*01:03/DPB1*02:01	3	13	27	SVTVQGKVFERCELA	Consensus (comb.lib./smm/nn)	11.07	K
11	HLA-DPA1*01:03/DPB1*02:01	3	1	15	MKALIVLGLVLLSVT	Consensus (comb.lib./smm/nn)	24.97	I
12	HLA-DPA1*01:03/DPB1*02:01	3	6	20	VLGLVLLSVTVQGKV	Consensus (comb.lib./smm/nn)	29.86	G
13	HLA-DQA1*03:01/DQB1*03:02	1	1	15	MKALIVLGLVLLSVT	Consensus (comb.lib./smm/nn)	15.75	A
14	HLA-DQA1*03:01/DQB1*03:02	1	6	20	VLGLVLLSVTVQGKV	Consensus (comb.lib./smm/nn)	21.69	G
15	HLA-DQA1*03:01/DQB1*03:02	1	33	47	MDGYRGISLANWMCL	Consensus (comb.lib./smm/nn)	27.59	G
16	HLA-DQA1*03:01/DQB1*03:02	1	11	25	LLSVTVQGKVFCELA	Consensus (comb.lib./smm/nn)	42.15	T
17	HLA-DQA1*03:01/DQB1*03:02	1	17	31	QGKVFCELARTLKRL	Consensus (comb.lib./smm/nn)	47.43	V
18	HLA-DQA1*03:01/DQB1*03:02	1	28	42	LKRLGMDGYRGISLA	Consensus (comb.lib./smm/nn)	69.96	D
19	HLA-DQA1*03:01/DQB1*03:02	1	22	36	CELARTLKRLGMDGY	Consensus (comb.lib./smm/nn)	81.47	F
20	HLA-DQA1*03:01/DQB1*03:02	2	2	16	LLALVCLLSCLANSD	Consensus (comb.lib./smm/nn)	28.42	L
21	HLA-DQA1*03:01/DQB1*03:02	3	1	15	MKALIVLGLVLLSVT	Consensus (comb.lib./smm/nn)	15.75	A
22	HLA-DQA1*03:01/DQB1*03:02	3	6	20	VLGLVLLSVTVQGKV	Consensus (comb.lib./smm/nn)	21.69	G
23	HLA-DQA1*03:01/DQB1*03:02	3	11	25	LLSVTVQGKVFERCE	Consensus (comb.lib./smm/nn)	67.65	G
24	HLA-DRB1*01:01	1	2	16	KALIVLGLVLLSVTV	Consensus (comb.lib./smm/nn)	2.74	A
25	HLA-DRB1*01:01	1	7	21	LGLVLLSVTVQGKVF	Consensus (comb.lib./smm/nn)	10.4	G
26	HLA-DRB1*01:01	1	17	31	QGKVFCELARTLKRL	Consensus (comb.lib./smm/nn)	19.65	E
27	HLA-DRB1*01:01	1	33	47	MDGYRGISLANWMCL	Consensus (comb.lib./smm/nn)	31.83	G
28	HLA-DRB1*01:01	1	28	42	LKRLGMDGYRGISLA	Consensus (comb.lib./smm/nn)	33.84	M
29	HLA-DRB1*01:01	1	22	36	CELARTLKRLGMDGY	Consensus (comb.lib./smm/nn)	42.05	E
30	HLA-DRB1*01:01	1	12	26	LSVTVQGKVFCELAR	Consensus (comb.lib./smm/nn)	56.52	S

Redundancy removal to solve the issue of overlapping peptides

- **Post-processing:**
  - Remove largely overlapping peptides after prediction

	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	528	542	HLEFWEGVFTGLTHI	2.57	FWEGVFTGL	6.9	19.59	FWEGVFTGL	302	0.58	FWEGVFTGL	4
4	HLA-DPA1*01/DPB1*0401	1	526	540	QDHLEFWEGVFTGLT	2.62	FWEGVFTGL	6.9	19.59	FWEGVFTGL	310	0.62	EFWEGVFTG	4
5	HLA-DPA1*01/DPB1*0401	1	529	543	LEFWEGVFTGLTHID	3.13	FWEGVFTGL	6.9	19.59	FWEGVFTGL	308	0.61	FWEGVFTGL	5
6	HLA-DPA1*01/DPB1*0401	1	525	539	CQDHLEFWEGVFTGL	3.26	FWEGVFTGL	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	40	54	AQTFLATCINGVCWT	4.08	QTFLATCIN	728.23	45.84	FLATCINGV	746	3.39	FLATCINGV	7
10	HLA-DPA1*01/DPB1*0401	1	263	277	SPITYSTYGKFLADG	4.08	TYSTYGKFL	2.38	15.24	ITYSTYGKF	828	4.08	ITYSTYGKF	5

Other versions

- Run using command line or script
  - Write scripts to run them easily
  - Integrate into your own pipeline

API version

API (Application Programming Interface)

- A programming interface where the client can send parameters to the server over internet and get the processed results back.

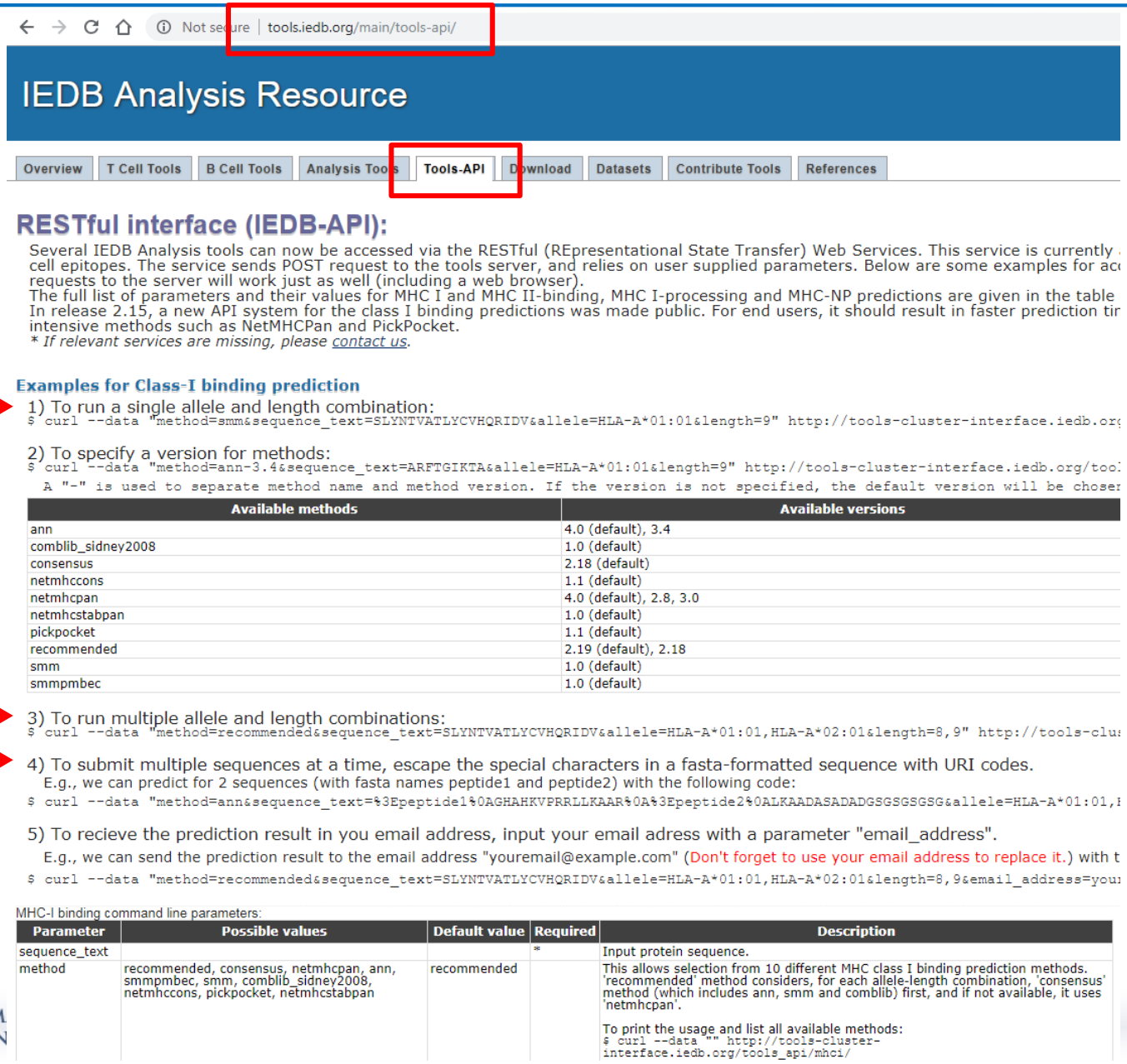
IEDB tools API

- Both class I & class II tools can be accessed via the IEDB RESTful Web Services. Other tools are also available.
- Sends request to the tools server at LJI and does prediction based on user supplied parameters.

Advantages:

- No need to install tools on your machine
- No need to use the web interface
- Freely available to all users
- Automatic update without re-installing
- Build custom scripts/tools or integrate into your pipeline
- Submit multiple sequences/alleles/peptide lengths in same command
- Submit large data set and receive results in email

IEDB tools API



Not secure | tools.iedb.org/main/tools-api/

IEDB Analysis Resource

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

RESTful interface (IEDB-API):

Several IEDB Analysis tools can now be accessed via the RESTful (REpresentational State Transfer) Web Services. This service is currently
cell epitopes. The service sends POST request to the tools server, and relies on user supplied parameters. Below are some examples for ac
requests to the server will work just as well (including a web browser).
The full list of parameters and their values for MHC I and MHC II-binding, MHC I-processing and MHC-NP predictions are given in the table
In release 2.15, a new API system for the class I binding predictions was made public. For end users, it should result in faster prediction tir
intensive methods such as NetMHCPan and PickPocket.
** If relevant services are missing, please contact us.*

Examples for Class-I binding prediction

1) To run a single allele and length combination:

```
$ curl --data "method=smm&sequence_text=SLYNTVATLYCVHQRIDV&allele=HLA-A*01:01&length=9" http://tools-cluster-interface.iedb.org
```

2) To specify a version for methods:

```
$ curl --data "method=ann-3.4&sequence_text=ARFTGIKTA&allele=HLA-A*01:01&length=9" http://tools-cluster-interface.iedb.org/tool
```

A "-" is used to separate method name and method version. If the version is not specified, the default version will be chose

Available methods	Available versions
ann	4.0 (default), 3.4
comblib_sidney2008	1.0 (default)
consensus	2.18 (default)
netmhccons	1.1 (default)
netmhcpan	4.0 (default), 2.8, 3.0
netmhcstabpan	1.0 (default)
pickpocket	1.1 (default)
recommended	2.19 (default), 2.18
smm	1.0 (default)
smmpmbec	1.0 (default)

3) To run multiple allele and length combinations:

```
$ curl --data "method=recommended&sequence_text=SLYNTVATLYCVHQRIDV&allele=HLA-A*01:01,HLA-A*02:01&length=8,9" http://tools-clus
```

4) To submit multiple sequences at a time, escape the special characters in a fasta-formatted sequence with URI codes.
E.g., we can predict for 2 sequences (with fasta names peptide1 and peptide2) with the following code:

```
$ curl --data "method=ann&sequence_text=%3Epeptide1%0AGHAHKVPRRLLKAAR%0A%3Epeptide2%0ALKAADASADADGSGSGSGSG&allele=HLA-A*01:01,
```

5) To recieve the prediction result in you email address, input your email adress with a parameter "email_address".
E.g., we can send the prediction result to the email address "youremail@example.com" (Don't forget to use your email address to replace it.) with t

```
$ curl --data "method=recommended&sequence_text=SLYNTVATLYCVHQRIDV&allele=HLA-A*01:01,HLA-A*02:01&length=8,9&email_address=you
```

MHC-I binding command line parameters:

Parameter	Possible values	Default value	Required	Description
sequence_text			*	Input protein sequence.
method	recommended, consensus, netmhcpan, ann, smmpmbec, smm, comblib_sidney2008, netmhccons, pickpocket, netmhcstabpan	recommended		This allows selection from 10 different MHC class I binding prediction methods. 'recommended' method considers, for each allele-length combination, 'consensus' method (which includes ann, smm and comblib) first, and if not available, it uses 'netmhcpan'. To print the usage and list all available methods: $ curl --data "" http://tools-cluster-interface.iedb.org/tools_api/mhci/

IEDB tools API sample command – class I

```
sinu@ubuntu:~/Desktop$ curl --data "method=recommended&sequence_text=SLYNTVATLYCVHQRIDV&allele=HLA-A*01:01&
length=9&email_address=spaul@lji.org" http://tools-cluster-interface.iedb.org/tools_api/mhci/

allele  seq_num start   end     length  peptide method  percentile_rank ann_ic50        ann_rank        smm
_ic50   smm_rank        comblib_sidney2008_score        comblib_sidney2008_rank netmhcpan_ic50  netmhcpan_r
ank
HLA-A*01:01     1       2       10      9       LYNTVATLY       Consensus (ann/smm)     1.1     9049.25 0.9
1286.92 1.3     -       -       -       -
HLA-A*01:01     1       4       12      9       NTVATLYCV       Consensus (ann/smm)     4.2     13556.93  2
.9      6061.08 5.5     -       -       -       -
HLA-A*01:01     1       8       16      9       TLYCVHQRI       Consensus (ann/smm)     15.0    16287.20  6
36774.77        24      -       -       -       -
HLA-A*01:01     1       3       11      9       YNTVATLYC       Consensus (ann/smm)     15.25   18322.54  9
.5      27896.52        21      -       -       -       -
HLA-A*01:01     1       5       13      9       TVATLYCVH       Consensus (ann/smm)     17.6    15675.17  5
.2      49607.80        30      -       -       -       -
HLA-A*01:01     1       1       9       9       SLYNTVATL       Consensus (ann/smm)     28.5    19635.42  1
7       85418.12        40      -       -       -       -
HLA-A*01:01     1       10      18      9       YCVHQRIDV       Consensus (ann/smm)     31.5    23625.86  3
1       56565.29        32      -       -       -       -
HLA-A*01:01     1       7       15      9       ATLYCVHQR       Consensus (ann/smm)     34.0    23237.48  2
9       83090.24        39      -       -       -       -
HLA-A*01:01     1       6       14      9       VATLYCVHQ       Consensus (ann/smm)     70.0    30975.41  7
2       282195.45       68      -       -       -       -
HLA-A*01:01     1       9       17      9       LYCVHQRID       Consensus (ann/smm)     81.0    32972.64  8
3       491529.97       79      -       -       -       -
sinu@ubuntu:~/Desktop$
```

IEDB tools API sample command – class II

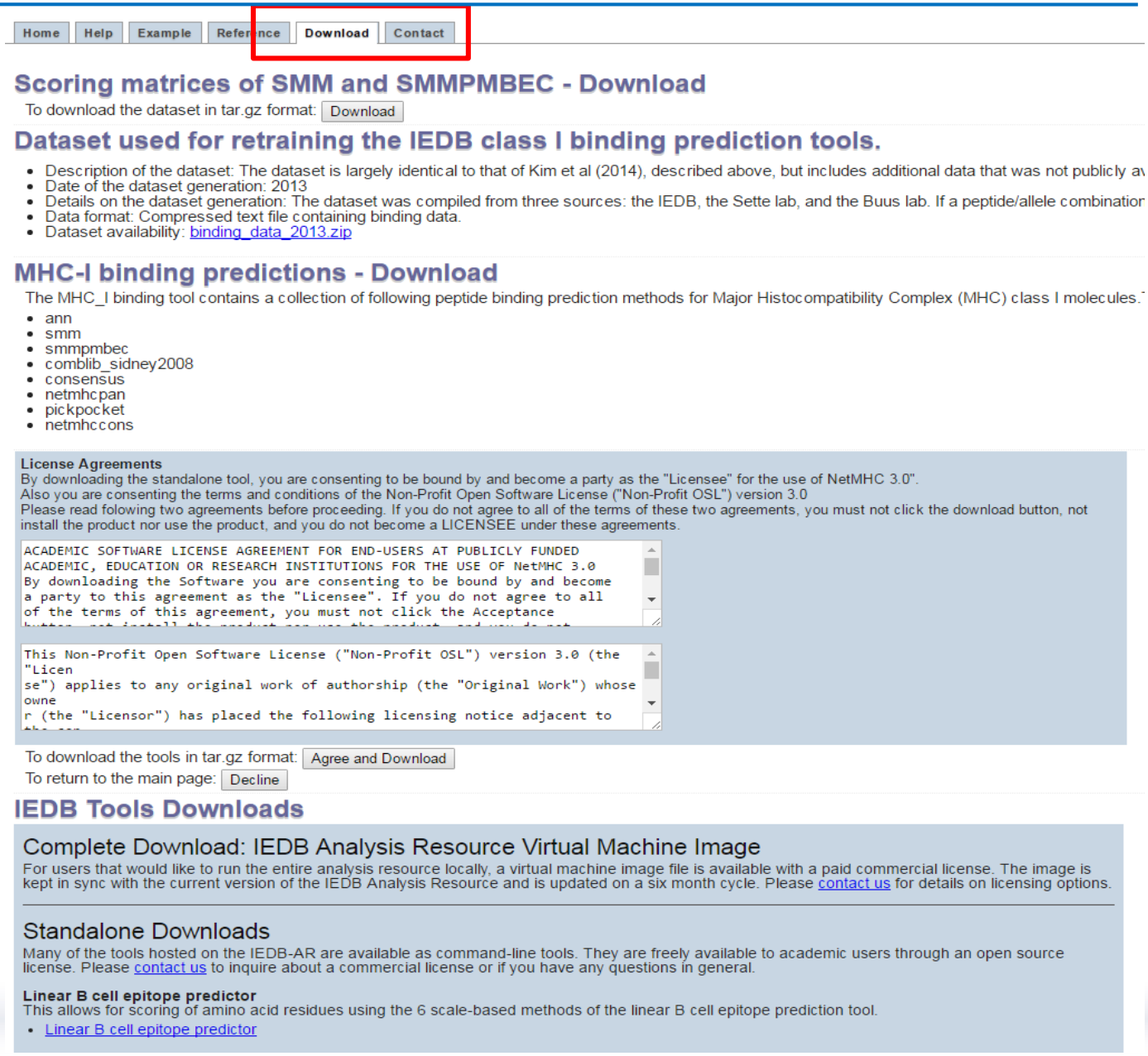
```
sinu@ubuntu:~/Desktop$ curl --data "method=recommended&sequence_text=%3Epeptide1%0AGHAHKVPRRLLKAARMSAPCLRA&
allele=HLA-DRB1*01:01&email address=spaul@lji.org" http://tools-cluster-interface.iedb.org/tools api/mhcii/
```

allele	seq_num	start	end	peptide	method	percentile_rank	comblib_core	comblib_score	comblib_rank	smm_align_core	smm_align_ic50	smm_align_rank	nn_align_core	nn_align_ic50	nn_align_rank	netmhciipan_core	netmhciipan_ic50	netmhciipan_rank	sturniolo_core	sturniolo_score	sturniolo_rank
HLA-DRB1*01:01	1	5	19	KVPRRLLKAARMSAP	Consensus (comb.lib./smm/nn)	3.49	RRLLKAARM	1.15	19.23	RRLLKAARM	19	3.49	RRLLKAARM	7.80	3.36	-	-	-	-	-	-
HLA-DRB1*01:01	1	4	18	HKVPRRLLKAARMSA	Consensus (comb.lib./smm/nn)	3.72	RRLLKAARM	1.15	19.23	RRLLKAARM	20	3.72	RRLLKAARM	7.30	2.91	-	-	-	-	-	-
HLA-DRB1*01:01	1	6	20	VPRRLLKAARMSAPC	Consensus (comb.lib./smm/nn)	6.24	RRLLKAARM	1.15	19.23	RRLLKAARM	20	3.72	RRLLKAARM	11.40	6.24	-	-	-	-	-	-
HLA-DRB1*01:01	1	3	17	AHKVPRRLLKAARMS	Consensus (comb.lib./smm/nn)	6.87	RRLLKAARM	1.15	19.23	RRLLKAARM	35	6.87	RRLLKAARM	8.80	4.22	-	-	-	-	-	-
HLA-DRB1*01:01	1	2	16	HAHKVPRRLLKAARM	Consensus (comb.lib./smm/nn)	7.08	HAHKVPRRL	0.19	12.42	VPRRLLKAA	36	7.08	RRLLKAARM	10.70	5.73	-	-	-	-	-	-
HLA-DRB1*01:01	1	8	22	RRLLKAARMSAPCLR	Consensus (comb.lib./smm/nn)	7.48	AARMSAPCL	0.61	16.87	RRLLKAARM	38	7.48	AARMSAPCL	10.30	5.42	-	-	-	-	-	-
HLA-DRB1*01:01	1	7	21	PRRLLKAARMSAPCL	Consensus (comb.lib./smm/nn)	7.68	AARMSAPCL	0.61	16.87	RRLLKAARM	39	7.68	RRLLKAARM	13.20	7.47	-	-	-	-	-	-
HLA-DRB1*01:01	1	9	23	RLLKAARMSAPCLRA	Consensus (comb.lib./smm/nn)	13.70	AARMSAPCL	0.61	16.87	AARMSAPCL	73	13.70	AARMSAPCL	10.80	5.80	-	-	-	-	-	-
HLA-DRB1*01:01	1	1	15	GHAHKVPRRLLKAAR	Consensus (comb.lib./smm/nn)	55.55	HAHKVPRRL	0.19	12.42	AHKVPRRLL	824	55.55	AHKVPRRLL	523.40	57.47	-	-	-	-	-	-

Standalone version

Standalone version

Can be downloaded from “Download” tab



Standalone version

- Available for class I, class II & some other tools also.
- Runs on Linux (Can use Vmware or Virtualbox to create virtual machine to run Linux on Windows machines).

Advantages:

- No internet needed.
- Implement tools on your own machine.
- Large amount of data (genome scale).
- Repetitive analysis.
- Free for non-profit & academia.
- Available for industry at a nominal license fee.

Standalone version sample command – class I

```
sinu@ubuntu:~/tools/mhc_i$ ./src/predict_binding.py IEDB_recommended HLA-A*02:01 9 test.fasta
```

allele	seq_num	start	end	length	peptide	method	percentile_rank	ann_ic50	ann_rank	smm_ic50	smm_rank	comblib_sidney2008_score	comblib_sidney2008_rank	netmhcpan_ic50	netmhcpan_rank
HLA-A*02:01	1	11	19	9	SGATWVDLV	Consensus (ann/smm/comblib_sidney2008)	8.7	8066.17	2.9	1390.30472906	8.7	0.000502342589522	22	-	-
HLA-A*02:01	1	6	14	9	FLEGVSGAT	Consensus (ann/smm/comblib_sidney2008)	101	988.18	2.2	1742.24810171	10	0.000322106879128	17	-	-
HLA-A*02:01	1	2	10	9	SNRDFLEGV	Consensus (ann/smm/comblib_sidney2008)	221	6517.03	22	9956.57569908	23	0.000153038253218	8.2	-	-
HLA-A*02:01	1	12	20	9	GATWVDLVL	Consensus (ann/smm/comblib_sidney2008)	237	192.69	2.7	9486.58713289	23	0.00774818532776	80	-	-
HLA-A*02:01	1	5	13	9	DFLEGVSGA	Consensus (ann/smm/comblib_sidney2008)	272	9823.04	49	15852.9467196	27	0.000586273143353	25	-	-
HLA-A*02:01	1	9	17	9	GVSGATWVD	Consensus (ann/smm/comblib_sidney2008)	333	3325.2	57	27996.9034762	33	0.000708108813694	28	-	-
HLA-A*02:01	1	14	22	9	TWVDLVLEG	Consensus (ann/smm/comblib_sidney2008)	342	5247.9	37	28914.121391	34	0.00080463700174	30	-	-

```
sinu@ubuntu:~/tools/mhc_i$ ./src/predict_binding.py IEDB_recommended HLA-A*02:01 9 test.fasta > result.txt
```

Standalone version sample command – class II

```
sinu@ubuntu:~/tools/mhc_ii$ python mhc_II_binding.py IEDB_recommended HLA-DRB1*03:01 test.fasta

allele  seq_num start   end     peptide method  consensus_percentile_rank       comblib_core    comblib_scc
re      comblib_rank    smm_align_core  smm_align_ic50  smm_align_rank  nn_align_core   nn_align_ic50   nn_
align_rank      netmhciipan_core        netmhciipan_ic50        netmhciipan_rank        sturniolo_core  stu
rniolo_score    sturniolo_rank
HLA-DRB1*03:01  1       1       15      MSNRDFLEGVSGATW Consensus(SMM,NN,Sturniolo)     27.74   -       - -
FLEGVSGAT       9343.0  19.11   FLEGVSGAT       15398.6 72.13   -       -       -       FLEGVSGAT       -1.
2       27.74
HLA-DRB1*03:01  1       2       16      SNRDFLEGVSGATWV Consensus(SMM,NN,Sturniolo)     27.74   -       - -
FLEGVSGAT       8986.0  18.32   FLEGVSGAT       13753.3 69.06   -       -       -       FLEGVSGAT       -1.
2       27.74
HLA-DRB1*03:01  1       3       17      NRDFLEGVSGATWVD Consensus(SMM,NN,Sturniolo)     27.74   -       - -
FLEGVSGAT       9085.0  18.56   FLEGVSGAT       12966.4 67.52   -       -       -       FLEGVSGAT       -1.
2       27.74
HLA-DRB1*03:01  1       4       18      RDFLEGVSGATWVDL Consensus(SMM,NN,Sturniolo)     27.74   -       - -
FLEGVSGAT       8523.0  17.28   FLEGVSGAT       12159.0 65.84   -       -       -       FLEGVSGAT       -1.
2       27.74
HLA-DRB1*03:01  1       5       19      DFLEGVSGATWVDLV Consensus(SMM,NN,Sturniolo)     37.04   -       - -
FLEGVSGAT       18841.0 37.04   VSGATWVDL       12588.1 66.77   -       -       -       FLEGVSGAT       -1.
2       27.74
HLA-DRB1*03:01  1       6       20      FLEGVSGATWVDLVL Consensus(SMM,NN,Sturniolo)     36.26   -       - -
FLEGVSGAT       18363.0 36.26   VSGATWVDL       9927.8  60.7    -       -       -       FLEGVSGAT       -1.
2       27.74
```

- Detailed instructions available in README file

Versions of IEDB Analysis Resource tools

Web

- http://tools.iedb.org
- Client uses browsers to submit data
- Predictions run on IEDB tools server
- Can be run on Windows/Mac/Linux
- Internet is needed
- May not be suitable for very very large data sets
- Automatically updated

Standalone

- http://tools.iedb.org/main/download
- Uses command line interface
- Downloaded from IEDB website
- Installed and run on local machine
- Runs on Linux only (can use virtual machines to run Linux on other OS)
- Internet not needed once installed
- Better for very large data sets
- Need to update for every release
- Free to academia/non-profit; License fee for industry

API

- http://tools.iedb.org/main/tools-api
- Uses command line interface
- Predictions run on IEDB server
- Clients send parameters to IEDB server using commands or scripts
- Internet is needed
- Can be used to make custom scripts for use with large data sets
- Automatically updated
- Free to all

Summary

- Comparison of class I & II tools
- How the tool works
- How to use the tool – web version
- Recommendations – method, thresholds
- Prediction of promiscuous binders
- “7-allele” method
- TepiTool
- Other versions
  - API/RESTful interface
  - Standalone

La Jolla Institute FOR ALLERGY AND IMMUNOLOGY | Life Without Disease.

Thank you