IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

MHC Binding Predictions

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Presented by:
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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

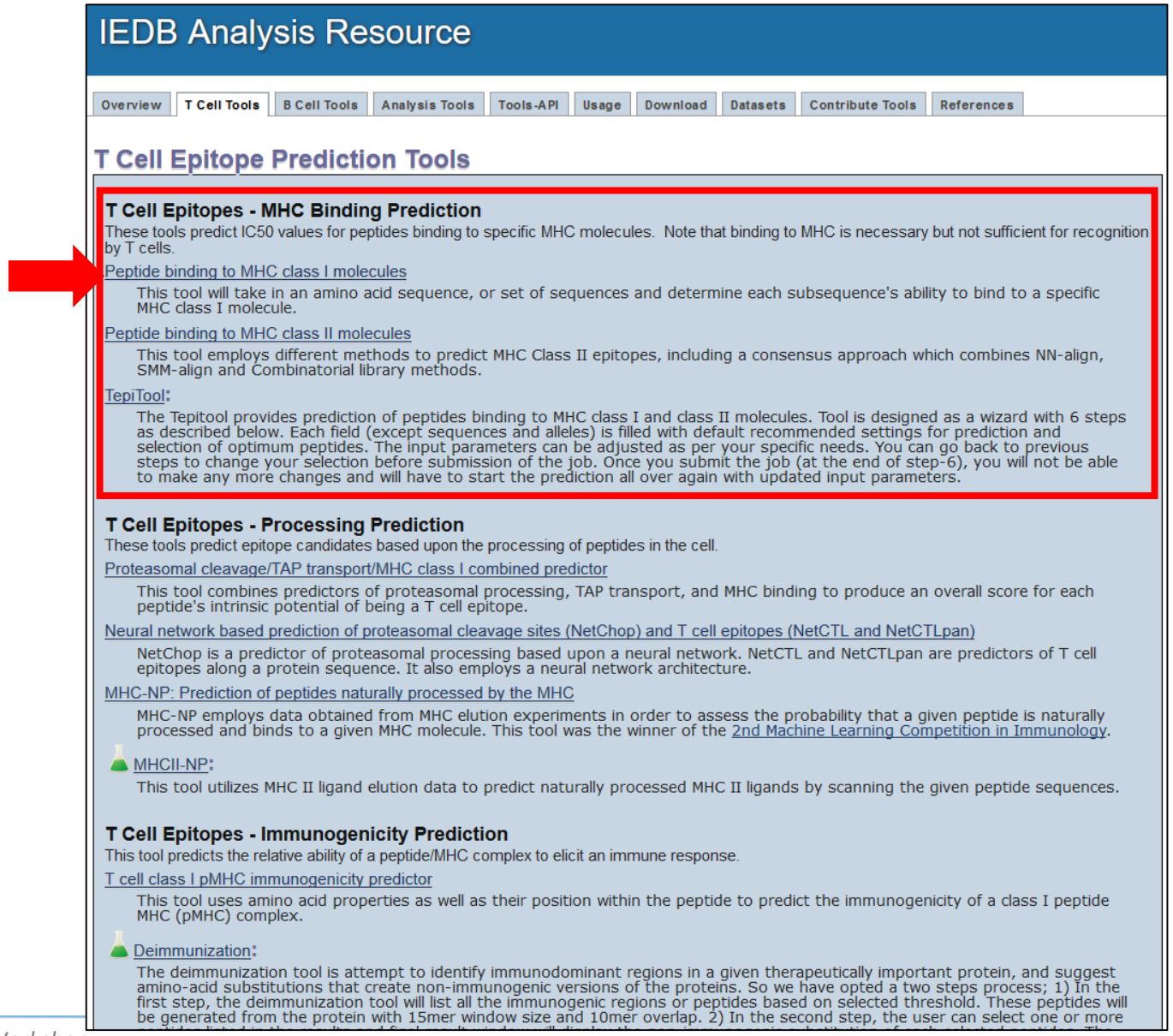
Peptide	IC_{50} (nM)
ASFCGSPY	51.4
LTDFGLSK	739.3
FTSFFYRY	1285.0
KSVFNSLY	1466.0
RDWAHNSL	1804.6
FSSCPVAY	1939.4
RNWAHSSL	2201.7
LSCAASGF	2830.1
LASIDLKY	3464.0

+

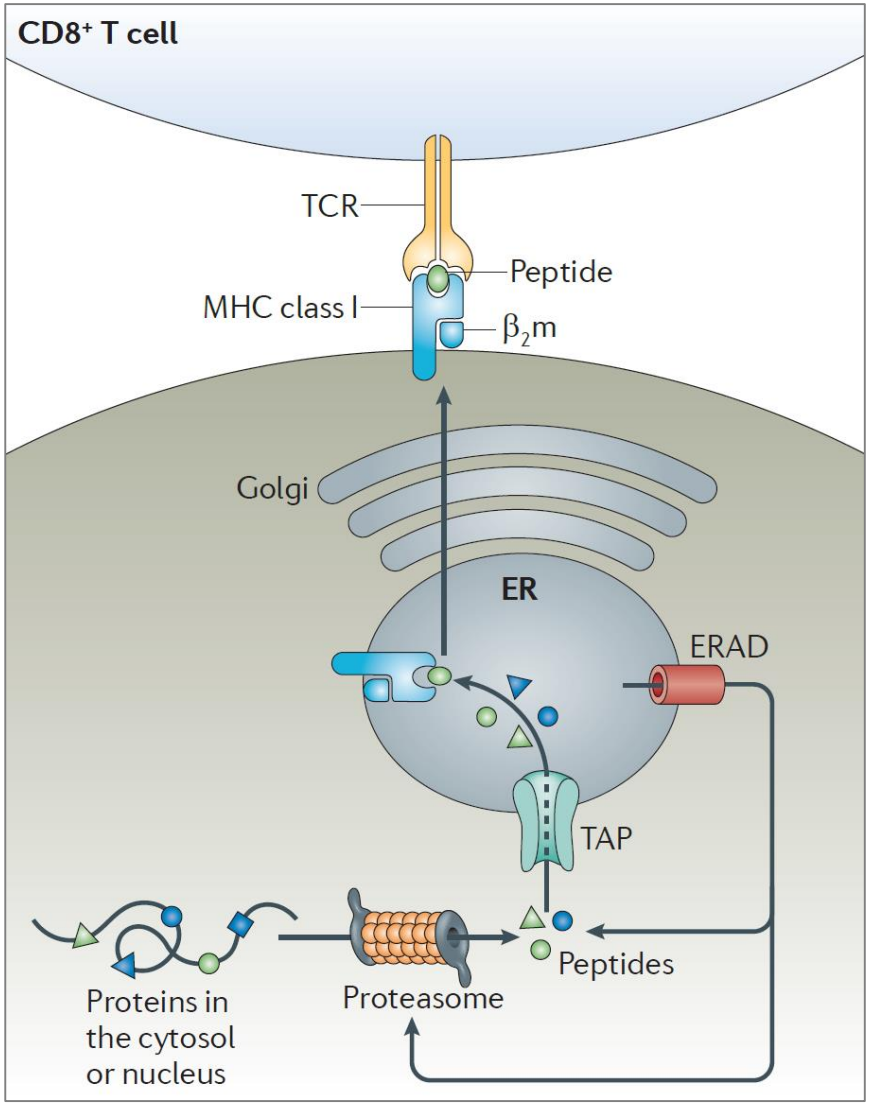
Machine learning algorithms

MHC I Binding Prediction

tools.iedb.org/main/tcell/



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”

Class I MHC molecule

- Expressed by almost all nucleated cells
- Presents antigen to **CD8+ T cells** (Cytotoxic T cells)
- One MHC encoded polymorphic chain (α) (2nd chain – β2-microglobulin)
- The binding groove is **closed** at both ends and can accommodate peptides of **8-15 AA**
- Only **α chain** impacts binding

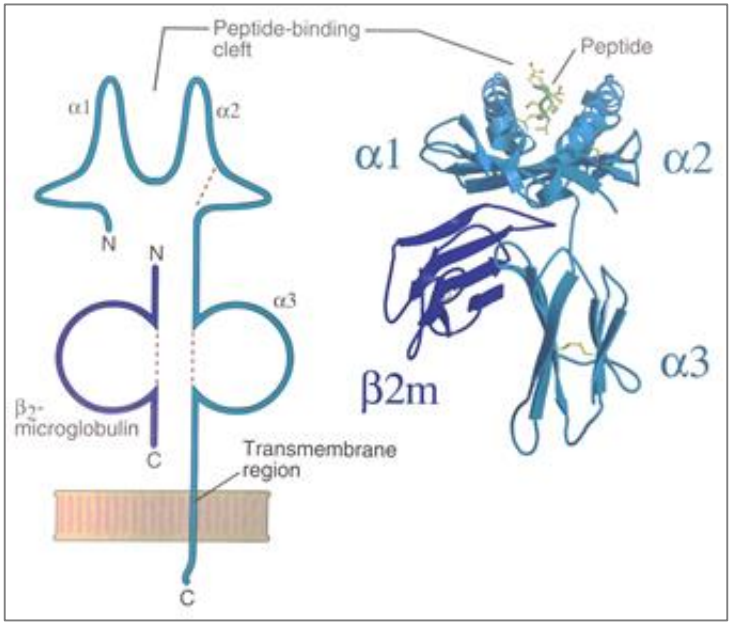
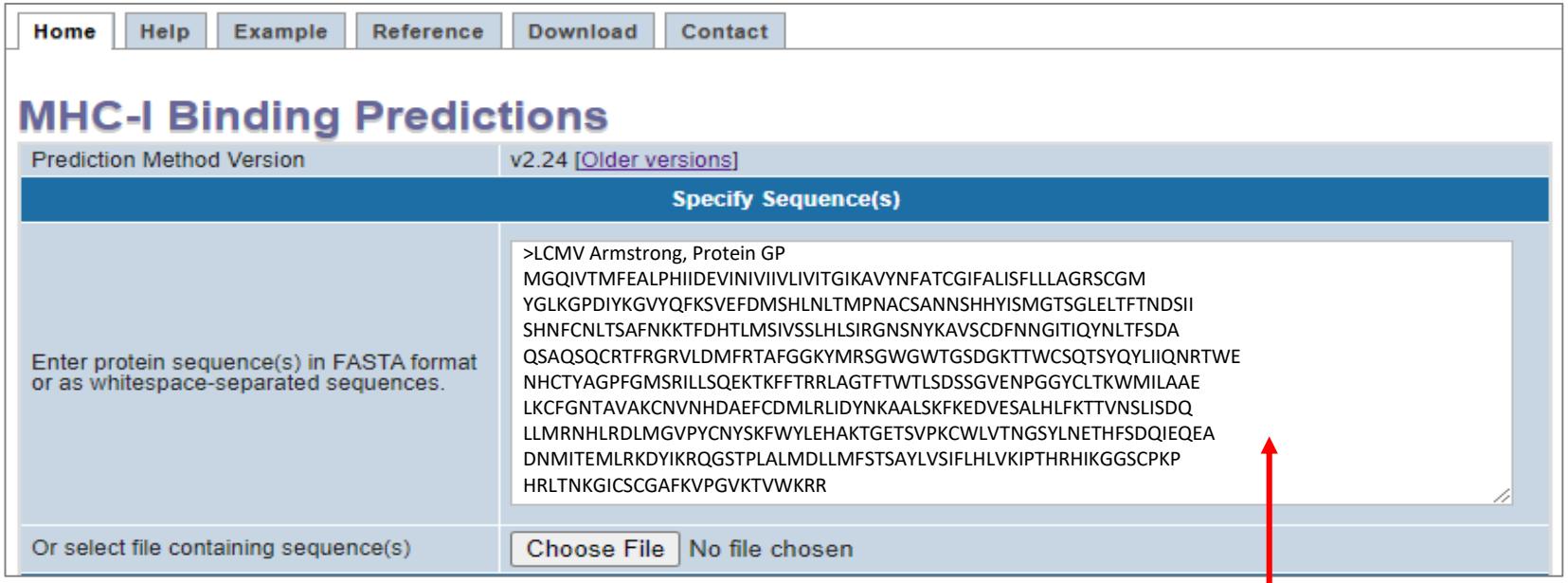


Figure Source
Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman

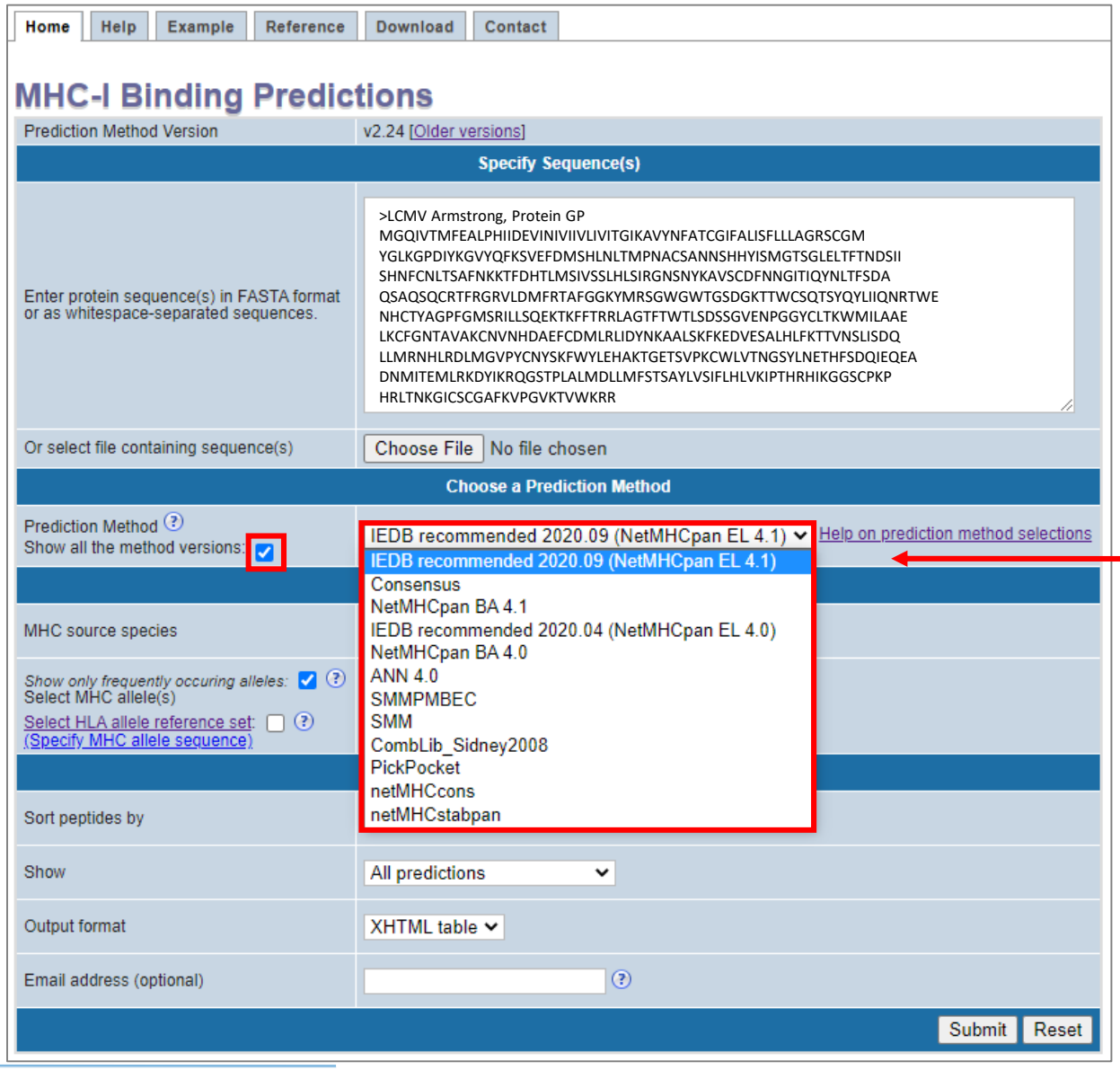
MHC-I binding prediction - example

tools.iedb.org/mhci/



Epitope sequence
(copy or upload)

MHC-I binding prediction - example



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Prediction method

MHC class I binding prediction methods available

Method	Reference	Performance Reported
NetMHCpan EL - 4.1	Reynisson et al., 2020	0.978 AUC (evaluated on EL data)
NetMHCpan EL - 4.0	Paul et al., 2020	0.977 AUC (average)
NetMHCpan BA - 4.1	Reynisson et al., 2020	0.893 AUC (evaluated on BA data)
NetMHCpan BA - 4.0	Paul et al., 2020	0.975 AUC (average)
Consensus	Moutaftsi et al., 2006	
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)

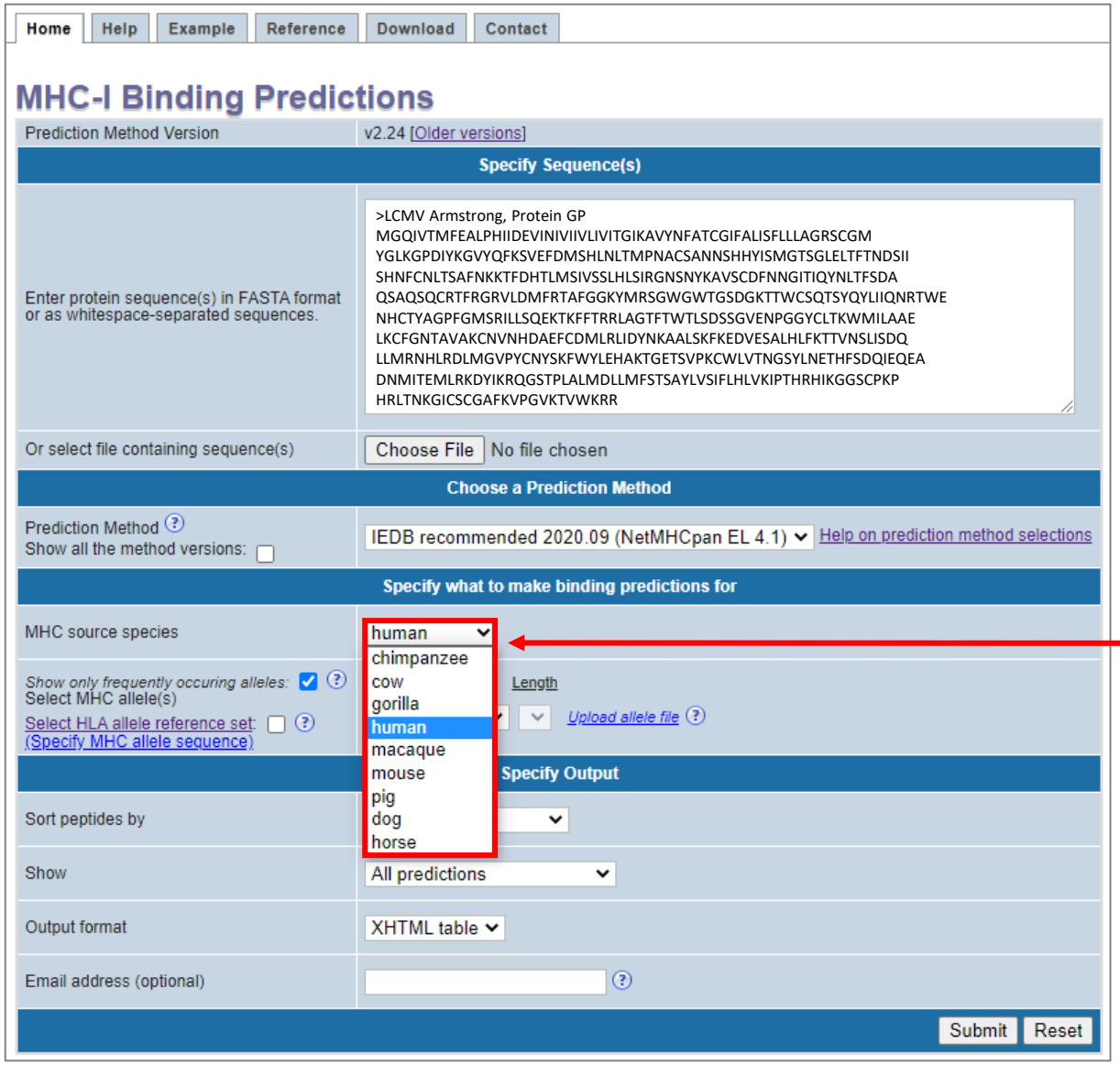
Guidelines: Choosing the prediction method

- Suggested method = “IEDB recommended”
  - Employs NetMHCpan EL 4.1 across all alleles
  - Provides binding affinity & percentile rank for each method separately as well

- Recommendation will change with the new benchmark studies

IEDB Tools Version	Recommended Method
2020.09 (current)	NetMHCPan 4.1 EL
2020.04	NetMHCPan 4.0 EL
2.22 and earlier	Consensus, if available; otherwise, NetMHCpan

MHC-I binding prediction – example



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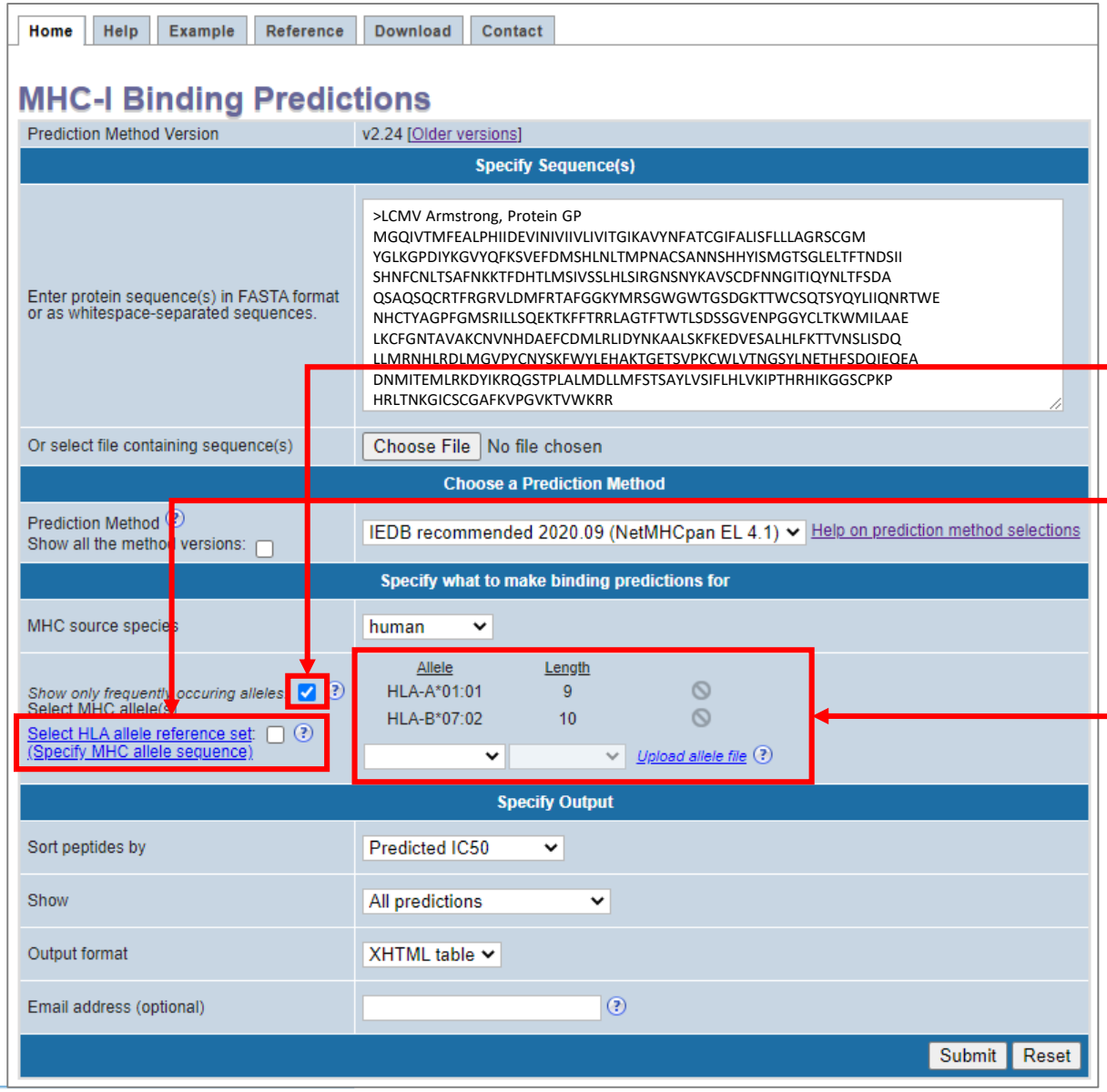
Choose species

MHC-I binding prediction – example



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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

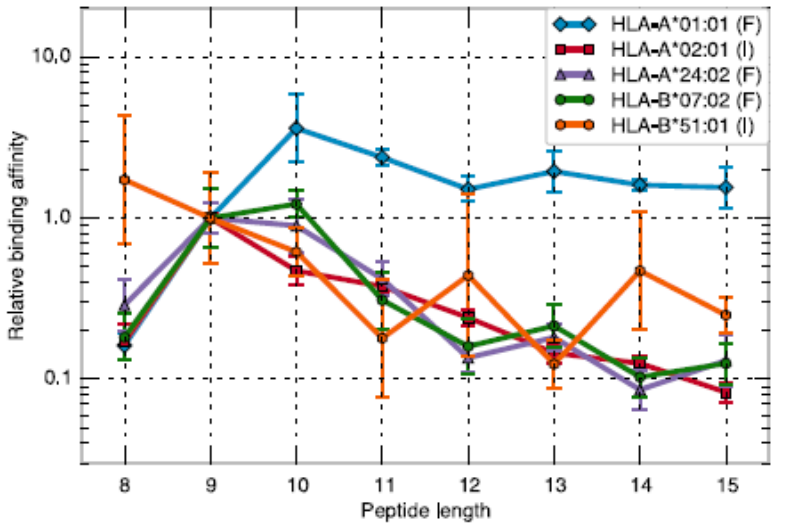

FIGURE 1. Peptide binding-length preference for five common HLA alleles. The length preference for each HLA was determined by measuring

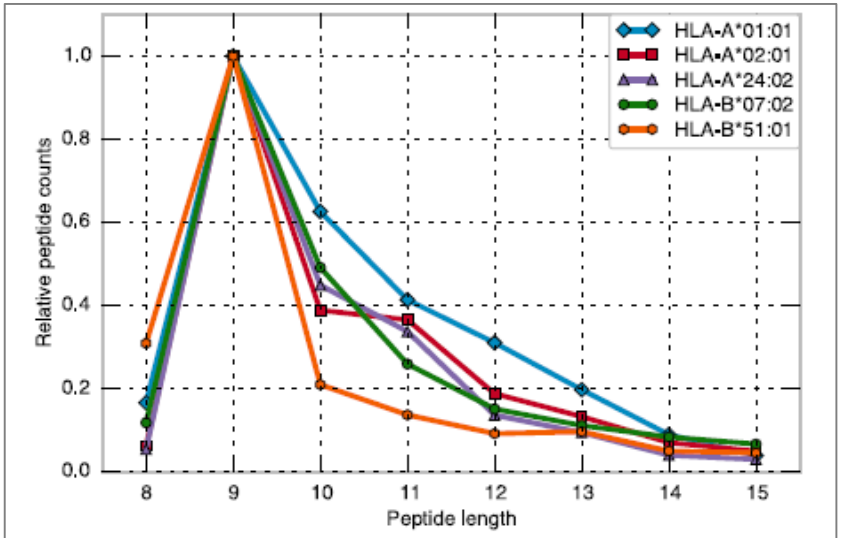

FIGURE 2. Length profiles of naturally presented peptides for five HLA molecules. Large datasets of HLA-I ligands were determined by the elu-

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[1], McMurtrey CP[2], Sidney J[3], Bardet W[2], Osborn SC[2], Kaever T[3], Sette A[3], Hildebrand WH[2], Nielsen M[4], Peters B[5].

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

NetMHCpan prediction methods allow **FASTA sequence input**

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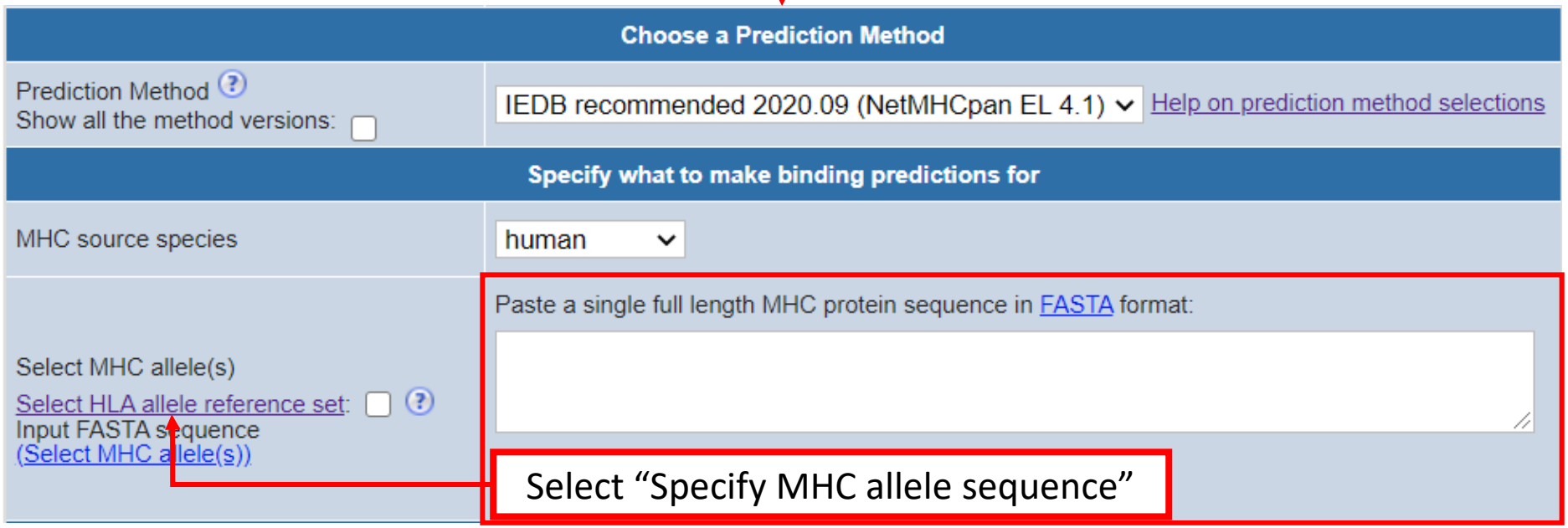

MHC-I binding prediction – example

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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

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MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	LCMV Armstrong, Protein GP	MGQIVTMFEALPHIIDEVINIVIIVLIVITGIKAVYNFATCGIFALISFL LLAGRSCGMYGLKGPDIYKGVYQFKSVEFDMSHLNLTMPNACSANNSHHY ISMGTSGLELTFTNDSIISHNFCNLTSAFNKKTFDHTLMSIVSSLHLSIR GNSNYKAVSCDFNNGITIQYNLTFSDAQSAQSQCRTFRGRVLDMFRTAFG GKYMRSGWGWTGSDGKTTWCSQTSYQYLIIQNRTWENHCTYAGPFGMSRI LLSQEKTKFFTRRLAGTFTWTLSDSSGVENPGGYCLTKWMILAAELKCFG NTAVAKCNVNHDAEFCDMLRLIDYNKAALSKFKEDVESALHLFKTTVNSL ISDQLLMRNHLRDLMGVPYCNYSKFWYLEHAKTGETSVPKCWLVTNGSYL NETHFSDQIEQEADNMITEMLRKDYIKRQGSTPLALMDLLMFSTSAYLVS IFLHLVKIPTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR

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	GPFGMSRILL	Consensus (ann/smm)	1.35
HLA-A*01:01	1	191	199	9	VLDMFRTAF	Consensus (ann/smm)	1.6

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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

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Individual scores for different methods

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

Download result

Citations

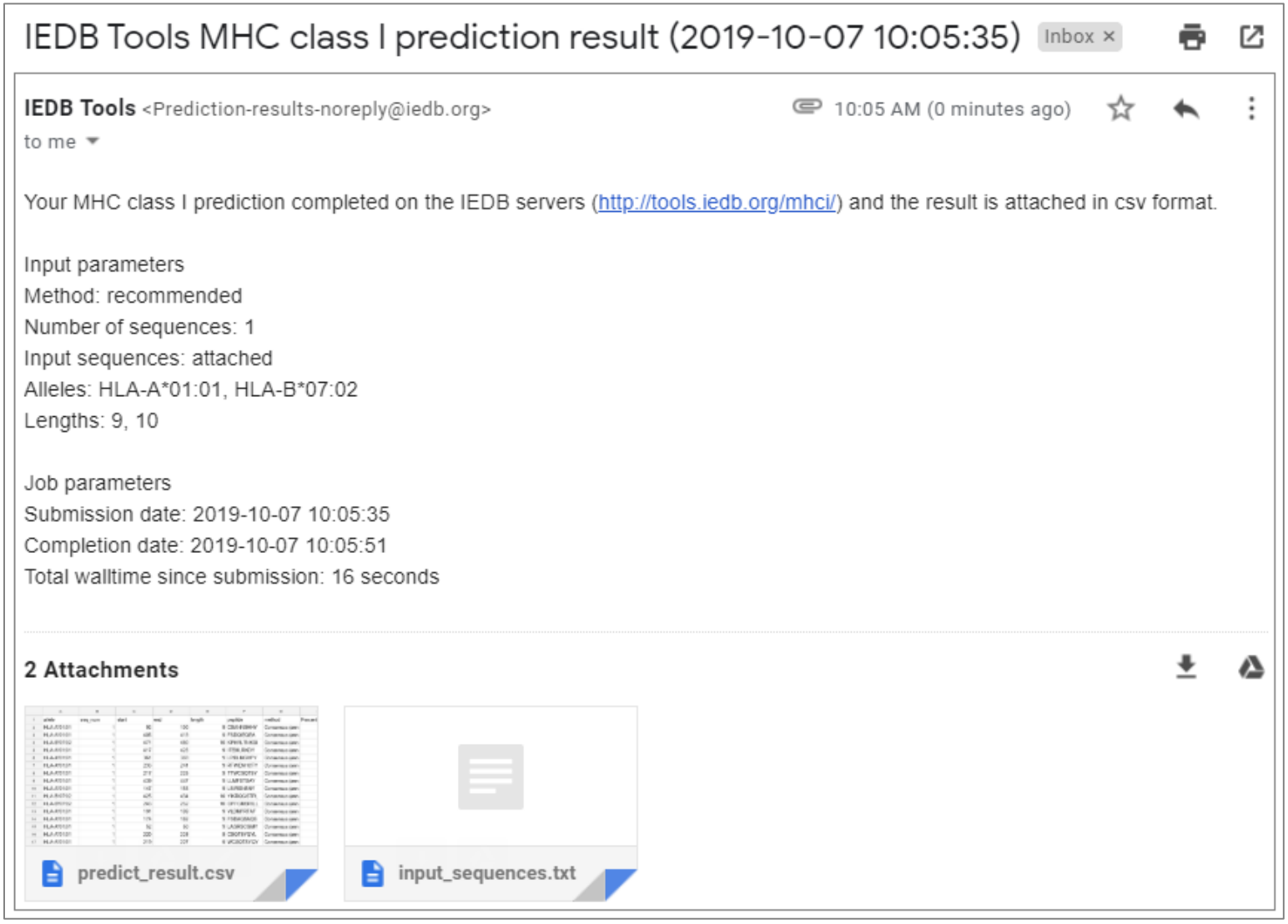
Check to expand the result:

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	FSDAQSAQS	Consensus (ann/smm)	1.75	2437.12	1.7	1934.42	1.8

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	LRDLMGVPY	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	GPFGMSRILL	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	LMGVPYCNY	Consensus (ann/smm)	2.75	5142.58	2.7	3009.89	2.8
27	HLA-A*01:01	1	104	112	9	GTSGLELTF	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



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

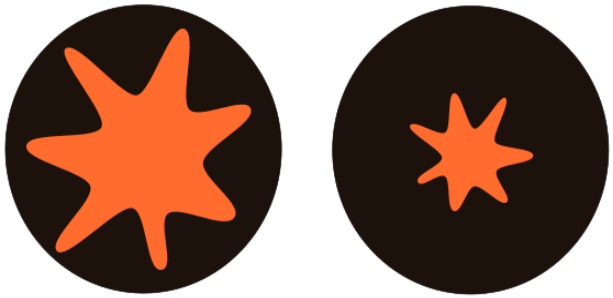
- Select based on **allele specific binding affinity** threshold

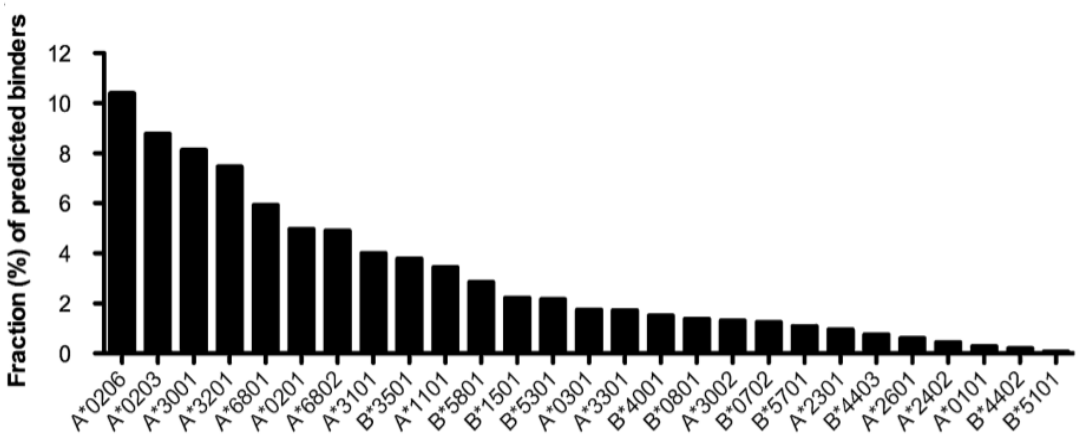
Different peptide-binding repertoires

- 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[#1], Weiskopf D[#1], Angelo MA[1], Sidney J[1], Peters B[1], Sette A[1].

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) fo
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 <= 1% for each (MHC allele, length) combination to cover most of the immune responses.[1, 2] 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.[3] 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.[4] 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 IC50 <500nM)
- Current studies suggest that allele specific thresholds can be derived

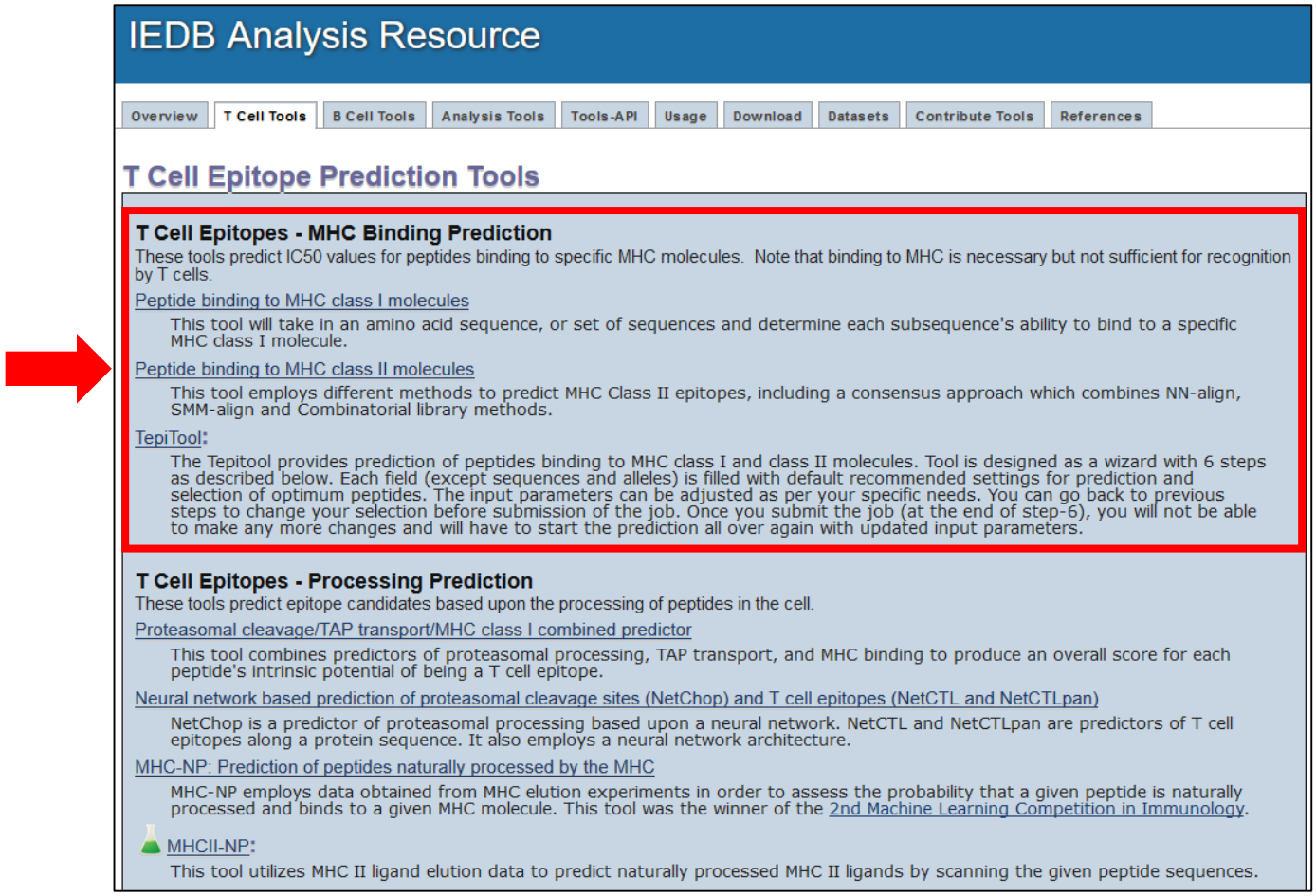
Alternate approaches for selecting binders

- Change threshold values depending on your need
  - e.g. in case you have too few or too many predicted binders.

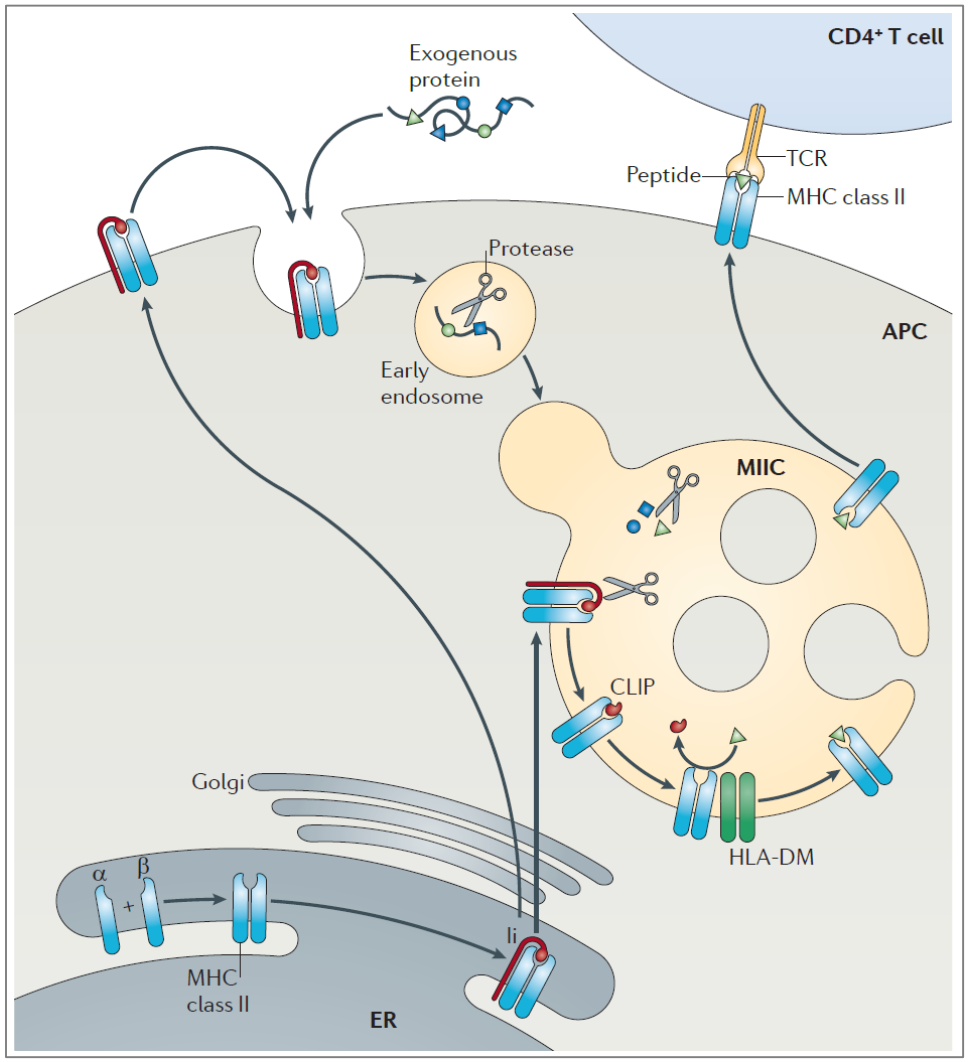
- Set a desired percentage within your peptide set (irrespective of IEDB percentile rank) in case you want to study a fixed number of best possible peptides.

Class II MHC Binding Prediction

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



Exogenous antigen processing pathway (class II)



- Antigens 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[1], Jongsma ML, Paul P, Bakke O.

PMID: 22076556 DOI: 10.1038/nri3084

Class II MHC molecule

- Only expressed by professional 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 peptides to **CD4+ T cells**

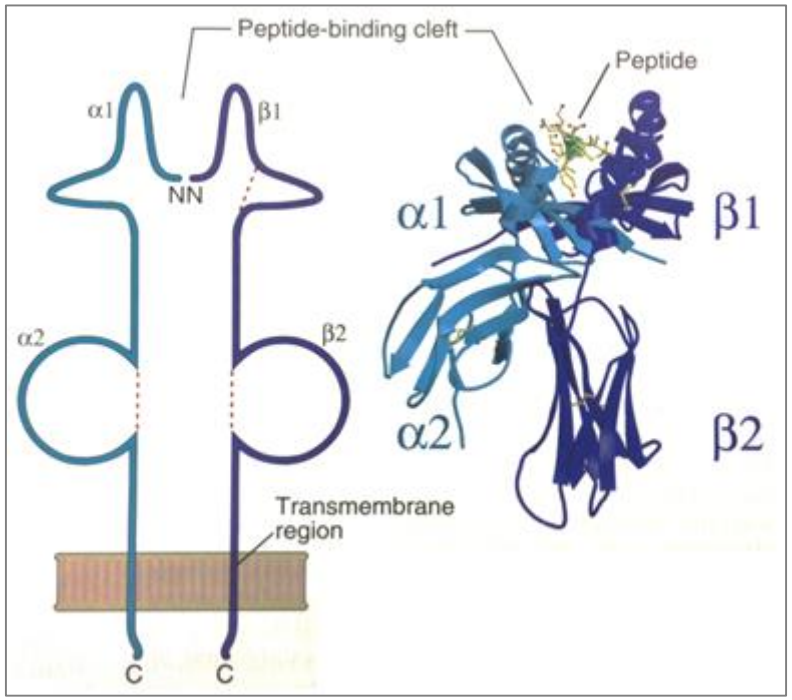


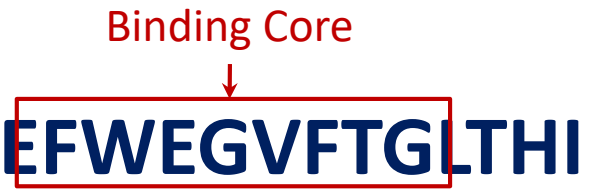
Figure Source
Cellular & Molecular Immunology, 5th Ed by Abbas and Lichtman

HLA Nomenclature

- Class I:
  - Only α chain is variable
    - HLA-B*07:02 (“B” is locus)
    - β2-microglobulin
- 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”

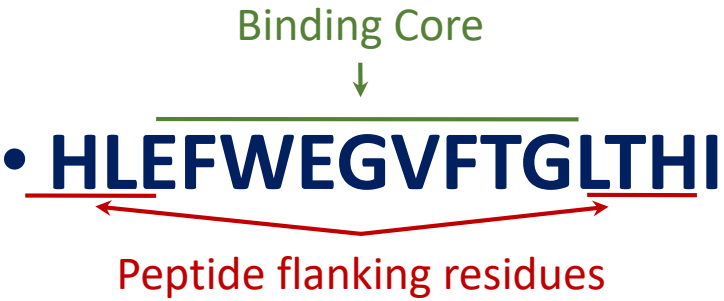
- 9 AA core within the peptide that interacts with the binding groove of MHC molecule



- 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 (to a lesser degree) influence binding.

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[1]	NetMHCIIpan-3.2	0.781[2]
SMM	0.894[3]	SMM-align	0.763[4]

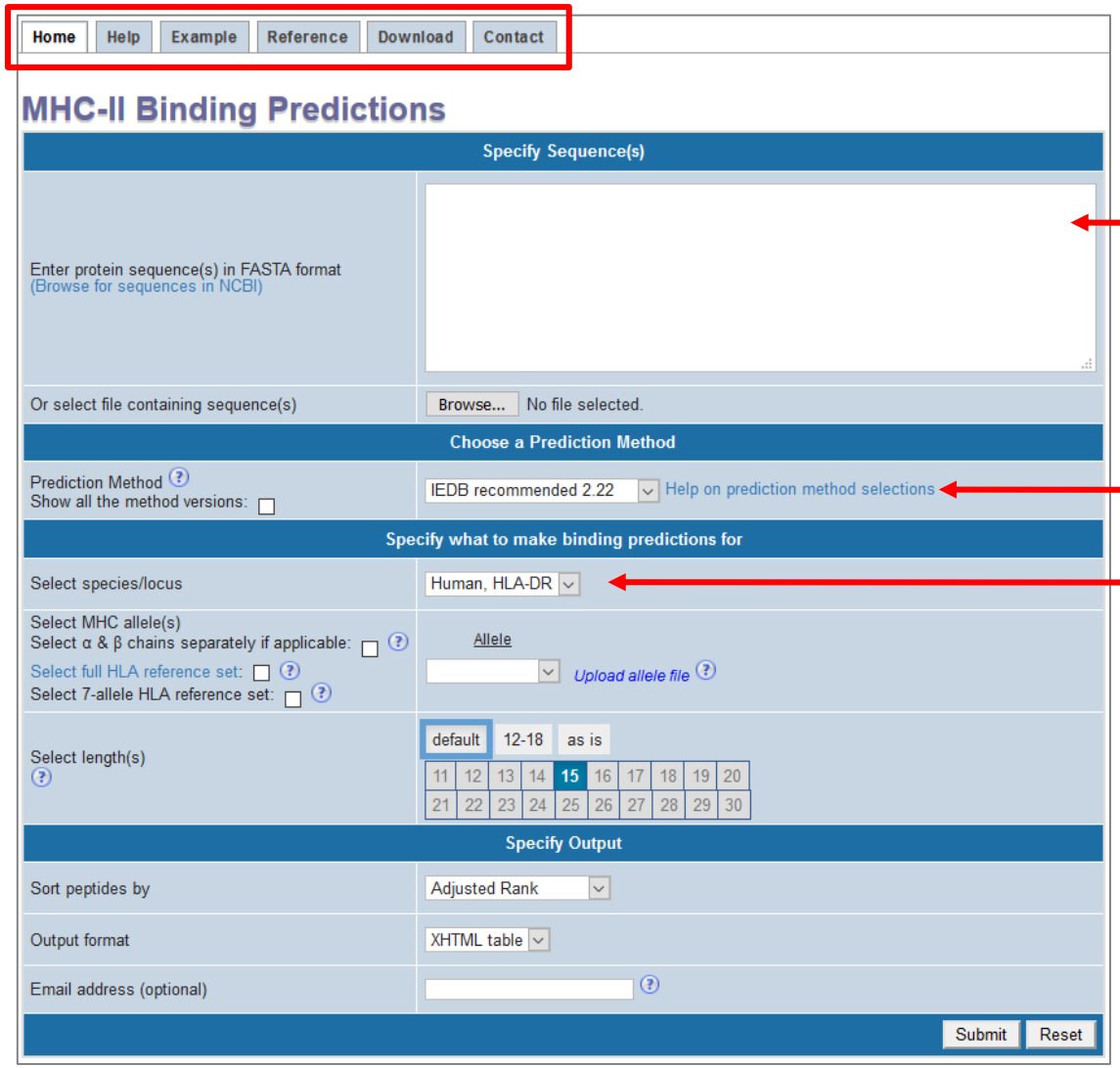
** The AUCs reported here are from different studies and obtained from different data sets*

- Less stringent 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/

Insert protein sequence(s)

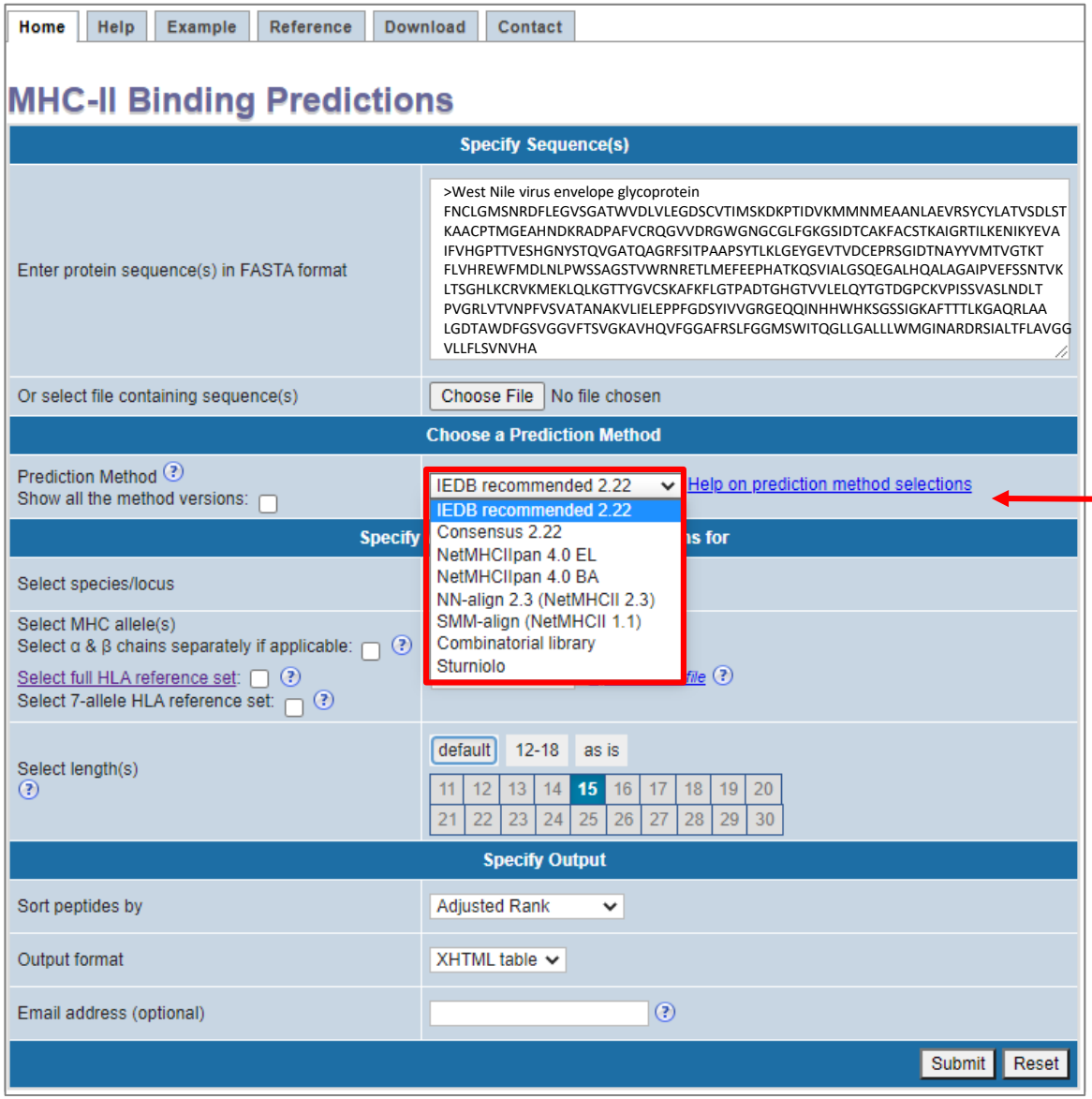
Select prediction method

Select species

Select allele(s) & length

Output options

MHC-II binding prediction - example



tools.iedb.org/mhcii/

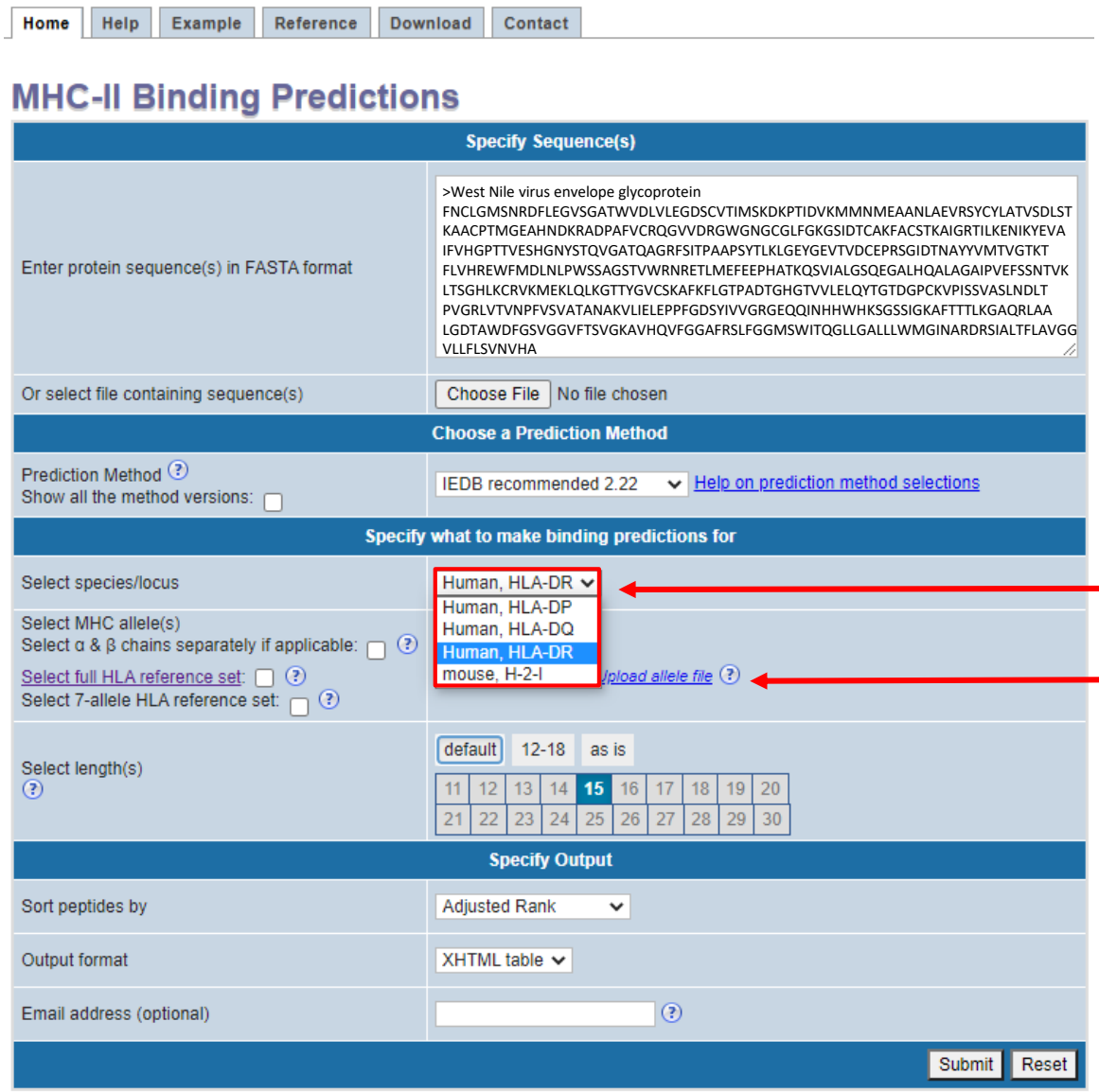
Prediction method

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/

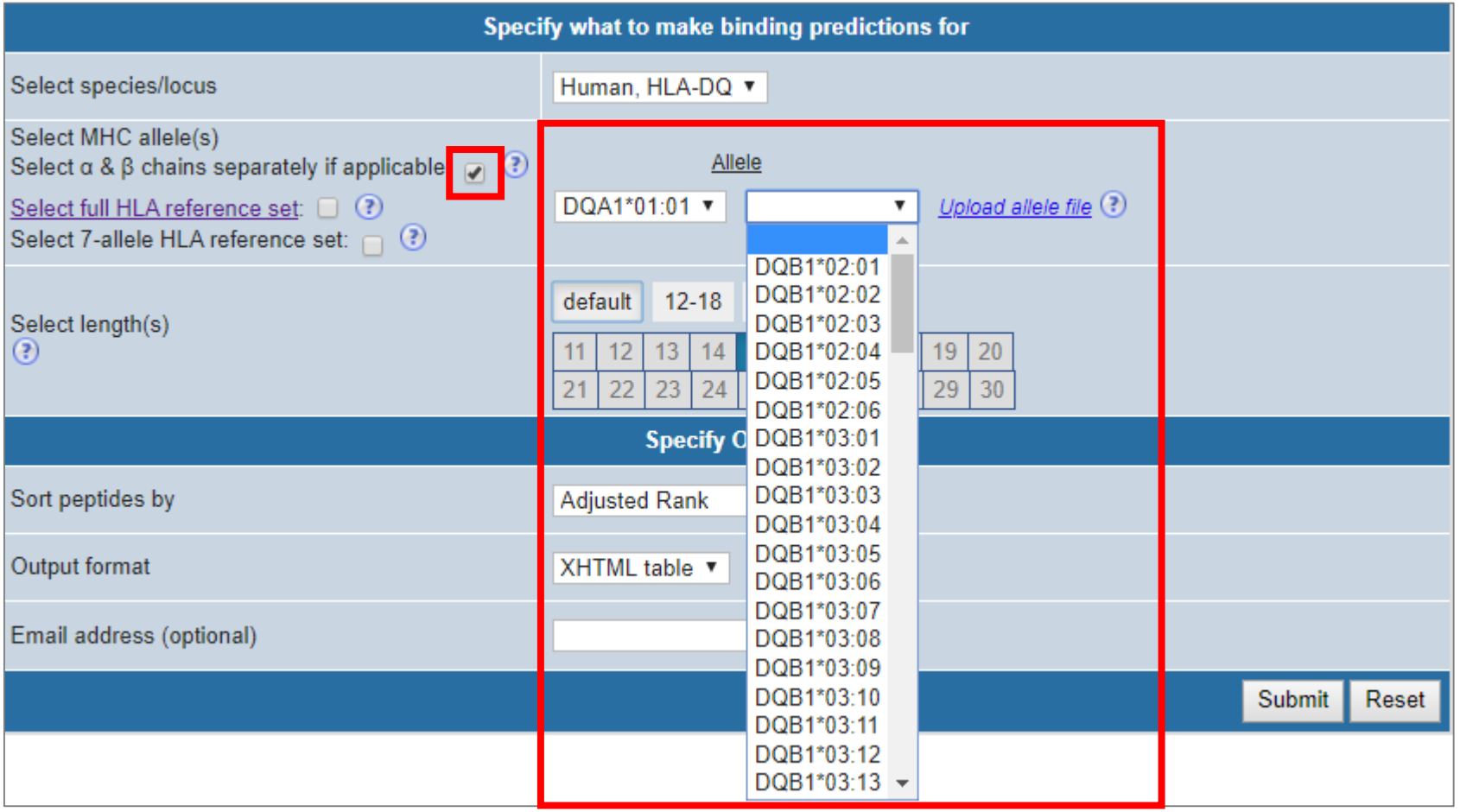
Choose species & locus

Select or upload alleles

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

Allele selection - α and β chains separately

tools.iedb.org/mhcii/

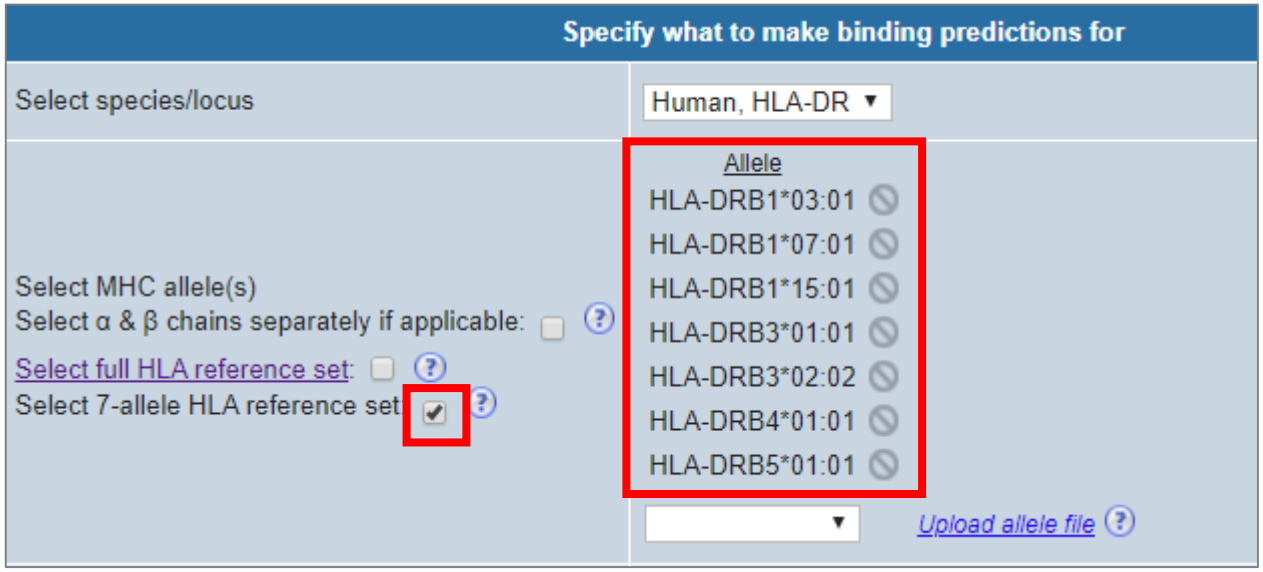


Allele selection – 27 allele reference set

tools.iedb.org/mhcii/

Allele selection – 7 allele set

tools.iedb.org/mhcii/



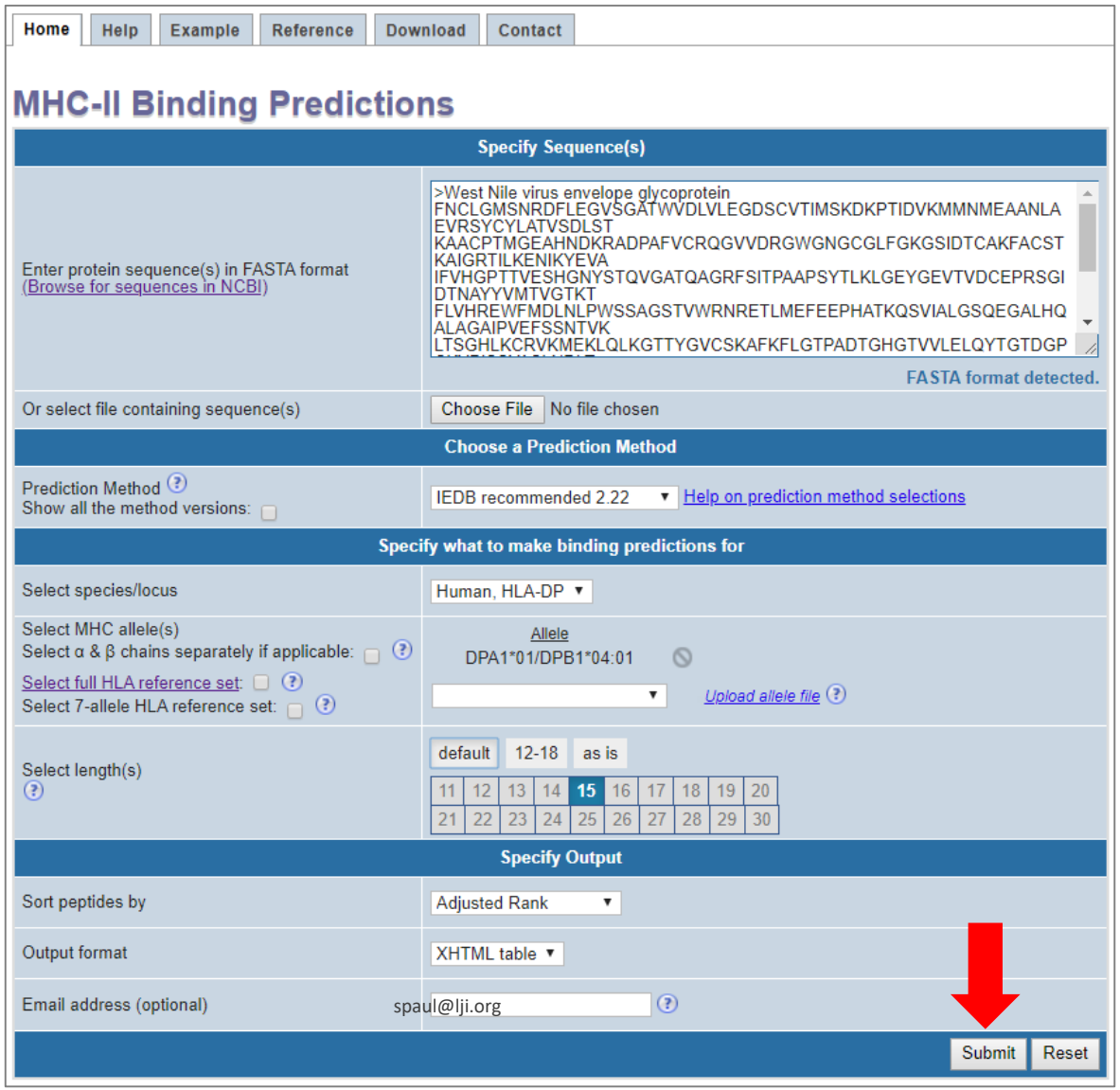
J Immunol Methods. 2015 Jul;422:28-34. doi: 10.1016/j.jim.2015.03.022. Epub 2015 Apr 7.

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

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

PMID: 25862607 PMCID: PMC4458426 DOI: 10.1016/j.jim.2015.03.022

MHC-II binding prediction – example



tools.iedb.org/mhcii/

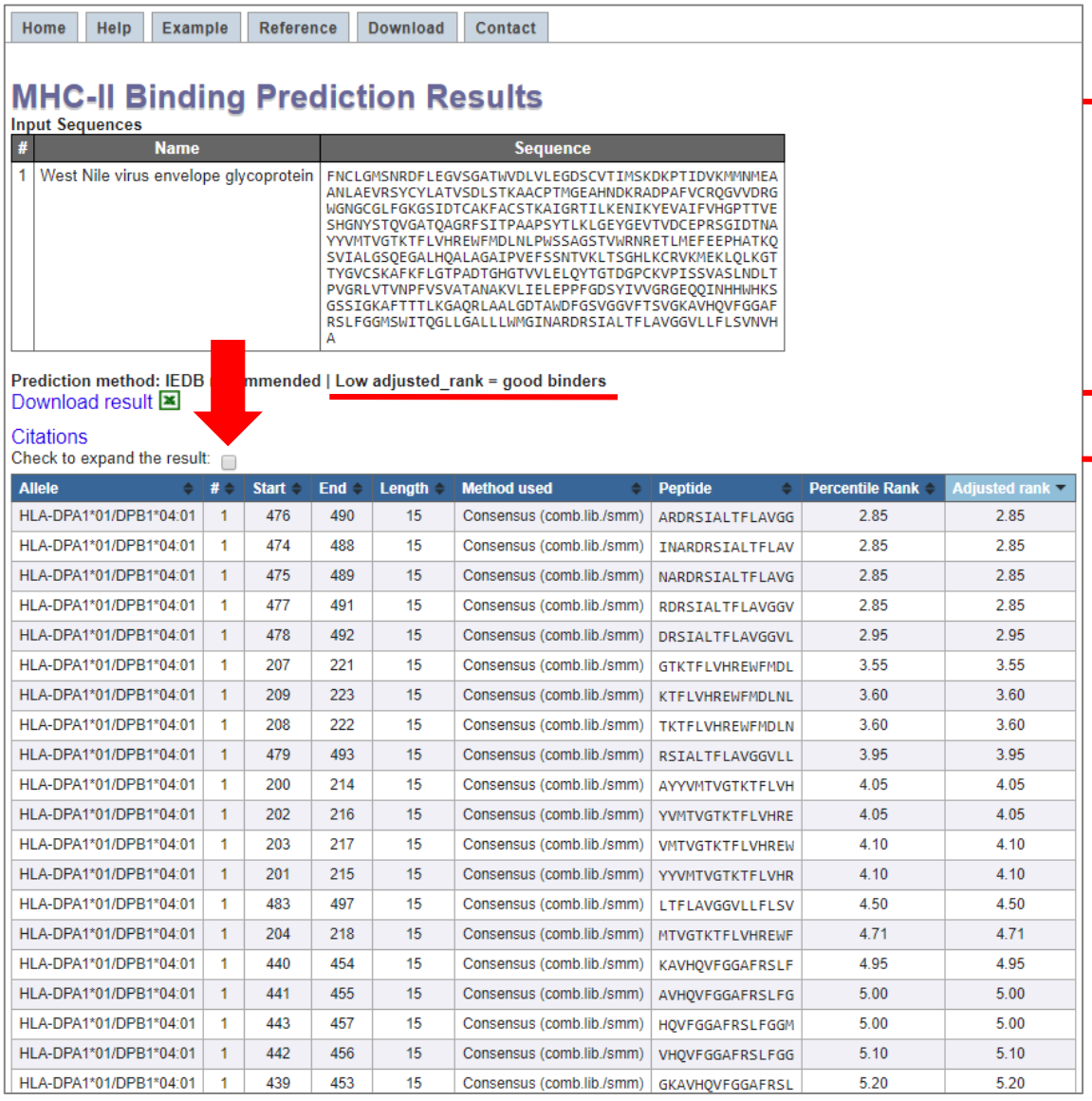
Length selection

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/

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/

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)	GTKTFLVHREWFMDL	3.55	3.55	KTFLVHREW	0.03	3.90	3.90	FLVHREWFM	230.00	3.20	3.20
HLA-DPA1*01/DPB1*04:01	1	209	223	15	Consensus (comb.lib./smm)	KTFLVHREWFMDLNL	3.60	3.60	KTFLVHREW	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)	TKTFLVHREWFMDLN	3.60	3.60	KTFLVHREW	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)	AYYVMTVGTKTFLVH	4.05	4.05	TVGTKTFLV	0.01	0.01	0.01	TVGTKTFLV	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	TVGTKTFLV	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	TVGTKTFLV	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)	YYVMTVGTKTFLVHR	4.10	4.10	TVGTKTFLV	0.01	0.01	0.01	VGTKTFLVH	585.00	8.20	8.20
HLA-	1	483	497	15	Consensus	LTFLAVGGVLLFLSV	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 ≤ **10.0** (Percentile rank on linear scale (0-100), lower value = better binder)
  - MHC binding affinity IC50 ≤ **1000nM**
- Select all peptides with IEDB percentile rank ≤ 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	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 15-mers with all possible 9-mer 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

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**

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[1], 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
VIQMYTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSG
GPLLCPAGHAVGLFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPAVPQSFQVAHLHAPTGSGK
STKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGVDPNIRTGVRTITTGSPITYSTYGKFLADGGCS
GGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVSHPNIEEVALSTTGEIPFY
GKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVSTDALMTGFTG
DFDSVIDCNTCVTQTVDFSLDPTFTIETTTLPQDAVSRTQRRGRTGRGKPGIYRFVAPGERPSGMFDSSV
LCECYDAGCAWYELTPAETTVRLRAYMNTPGLPVCQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENFPYL
VAYQATVCARAQAPPPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEVTLTHPITKYIMTCMSADLEVV
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: Human, HLA-DR

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
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./smm/nn)	GYKVLVLNPSVAATL	0.14	0.14
HLA-DRB1*01:01	1	221	235	15	Consensus (comb.lib./smm/nn)	QGYKVLVLNPSVAAT	0.14	0.14
HLA-DRB1*01:01	1	220	234	15	Consensus (comb.lib./smm/nn)	AQGYKVLVLNPSVAA	0.39	0.39
HLA-DRB1*01:01	1	223	237	15	Consensus (comb.lib./smm/nn)	YKVLVLNPSVAATLG	0.39	0.39
HLA-DRB1*01:01	1	224	238	15	Consensus (comb.lib./smm/nn)	KVLVLNPSVAATLGF	1.30	1.30
HLA-DRB1*01:01	1	219	233	15	Consensus (comb.lib./smm/nn)	AAQGYKVLVLNPSVA	1.80	1.80
HLA-DRB1*01:01	1	378	392	15	Consensus (comb.lib./smm/nn)	AAKLVALGINAVAYY	3.60	3.60
HLA-DRB1*01:01	1	379	393	15	Consensus (comb.lib./smm/nn)	AKLVALGINAVAYYR	3.60	3.60
HLA-DRB1*01:01	1	375	389	15	Consensus (comb.lib./smm/nn)	DELAAKLVALGINAV	3.60	3.60
HLA-DRB1*01:01	1	376	390	15	Consensus (comb.lib./smm/nn)	ELAAKLVALGINAVA	3.60	3.60
HLA-DRB1*01:01	1	377	391	15	Consensus (comb.lib./smm/nn)	LAAKLVALGINAVAY	3.60	3.60
HLA-DRB1*01:01	1	225	239	15	Consensus (comb.lib./smm/nn)	VLVLNPSVAATLGFG	3.90	3.90
HLA-DRB1*01:01	1	386	400	15	Consensus (comb.lib./smm/nn)	INAVAYYRGLDVSVI	6.00	6.00
HLA-DRB1*01:01	1	512	526	15	Consensus (comb.lib./smm/nn)	RLRAYMNTPGLPVCQ	6.00	6.00
HLA-DRB1*01:01	1	388	402	15	Consensus (comb.lib./smm/nn)	AVAYYRGLDVSVIPT	6.30	6.30
HLA-DRB1*01:01	1	389	403	15	Consensus (comb.lib./smm/nn)	VAYYRGLDVSVIPTS	6.30	6.30
HLA-DRB1*01:01	1	511	525	15	Consensus (comb.lib./smm/nn)	VRLRAYMNTPGLPVC	6.40	6.40
HLA-DRB1*01:01	1	555	569	15	Consensus (comb.lib./smm/nn)	ENFPYLVAYQATVCA	6.50	6.50
HLA-DRB1*01:01	1	557	571	15	Consensus (comb.lib./smm/nn)	FPYLVAYQATVCARA	6.50	6.50
HLA-DRB1*01:01	1	554	568	15	Consensus (comb.lib./smm/nn)	GENFPYLVAYQATVC	6.50	6.50
HLA-DRB1*01:01	1	387	401	15	Consensus (comb.lib./smm/nn)	NAVAYYRGLDVSVIP	6.50	6.50
HLA-DRB1*01:01	1	556	570	15	Consensus (comb.lib./smm/nn)	NFPYLVAYQATVCAR	6.50	6.50
HLA-DRB1*01:01	1	553	567	15	Consensus (comb.lib./smm/nn)	SGENFPYLVAYQATV	6.50	6.50
HLA-DRB1*01:01	1	380	394	15	Consensus (comb.lib./smm/nn)	KLVALGINAVAYYRG	7.30	7.30
HLA-DRB1*01:01	1	513	527	15	Consensus (comb.lib./smm/nn)	LRAYMNTPGLPVCQD	7.50	7.50
HLA-DRB1*01:01	1	558	572	15	Consensus (comb.lib./smm/nn)	PYLVAYQATVCARAQ	7.60	7.60
HLA-DRB1*01:01	1	559	573	15	Consensus (comb.lib./smm/nn)	YLVAYQATVCARAQA	7.60	7.60
HLA-DRB1*01:01	1	372	386	15	Consensus (comb.lib./smm/nn)	KKCDELAAKLVALGI	8.00	8.00
HLA-DRB1*01:01	1	514	528	15	Consensus (comb.lib./smm/nn)	RAYMNTPGLPVCQDH	8.00	8.00
HLA-DRB1*01:01	1	373	387	15	Consensus (comb.lib./smm/nn)	KCDELAAKLVALGIN	8.20	8.20
HLA-DPA1*03:01/DPB1*04:02	1	164	178	15	Consensus (comb.lib./smm)	AKAVDFIPVENLETT	8.30	8.30
HLA-DRB1*01:01	1	226	240	15	Consensus (comb.lib./smm/nn)	LVLNPSVAATLGFGA	8.60	8.60
HLA-DPA1*03:01/DPB1*04:02	1	37	51	15	Consensus (comb.lib./smm)	STATQTFLATCINGV	8.80	8.80
HLA-DPA1*03:01/DPB1*04:02	1	163	177	15	Consensus (comb.lib./smm)	VAKAVDFIPVENLET	8.85	8.85

Panel of 27 class II alleles to allow for global coverage

Locus	Molecule	Phenotype frequency		Locus	Molecule	Phenotype frequency
DRB1	DRB1*01:01	5.4		DQA1/DQB1	DQA1*05:01/DQB1*02:01	11.3
	DRB1*03:01	13.7			DQA1*05:01/DQB1*03:01	35.1
	DRB1*04:01	4.6			DQA1*03:01/DQB1*03:02	19.0
	DRB1*04:05	6.2			DQA1*04:01/DQB1*04:02	12.8
	DRB1*07:01	13.5			DQA1*01:01/DQB1*05:01	14.6
	DRB1*08:02	4.9			DQA1*01:02/DQB1*06:02	14.6
	DRB1*09:01	6.2			Combined	81.6
	DRB1*11:01	11.8		DPA1/DPB1	DPA1*02:01/DPB1*01:01	16.0
	DRB1*12:01	3.9			DPA1*01:03/DPB1*02:01	17.5
	DRB1*13:02	7.7			DPA1*01/DPB1*04:01	36.2
	DRB1*15:01	12.2			DPA1*03:01/DPB1*04:02	41.6
	Combined	71.1			DPA1*02:01/DPB1*05:01	21.7
DRB3/4/5	DRB3*01:01	26.1				
	DRB3*02:02	34.3				
	DRB4*01:01	41.8				
	DRB5*01:01	16.0				

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.

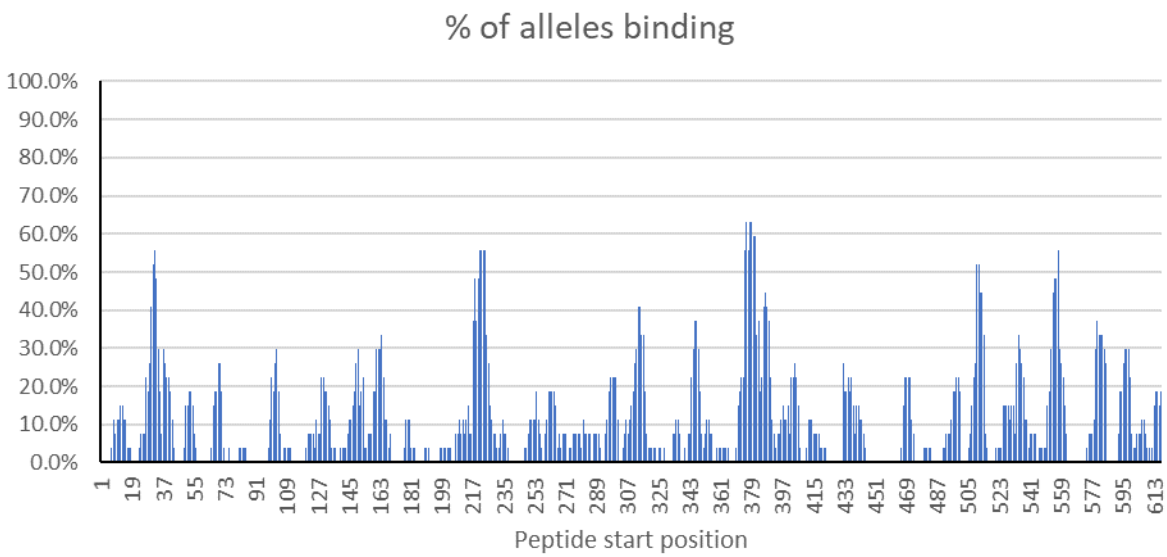
Greenbaum J[1], Sidney J, Chung J, Brander C, Peters B, Sette A.

PMID: 21305276 PMCID: PMC3626422 DOI: 10.1007/s00251-011-0513-0

Promiscuous binders

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

star	end	peptide	Count	%
31	45	GEVQIVSTATQTFLA	14	51.9%
32	46	EVQIVSTATQTFLAT	15	55.6%
221	235	QGYKVLVLNPSVAAT	15	55.6%
222	236	GYKVLVLNPSVAATL	15	55.6%
223	237	YKVLVLNPSVAATLG	15	55.6%
224	238	KVLVLNPSVAATLGF	15	55.6%
375	389	DELAAKLVALGINAV	15	55.6%
376	390	ELAAKLVALGINAVA	17	63.0%
377	391	LAAKLVALGINAVAY	15	55.6%
378	392	AAKLVALGINAVAYY	17	63.0%
379	393	AKLVALGINAVAYYR	17	63.0%
380	394	KLVALGINAVAYYRG	16	59.3%
381	395	LVALGINAVAYYRGL	16	59.3%
510	524	TVRLRAYMNTPGLPV	14	51.9%
511	525	VRLRAYMNTPGLPVC	14	51.9%
557	571	FPYLVAYQATVCARA	15	55.6%



“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 16-mer 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

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

PMID: 25862607 PMCID: PMC4458426 DOI: 10.1016/j.jim.2015.03.022

“7-allele” method

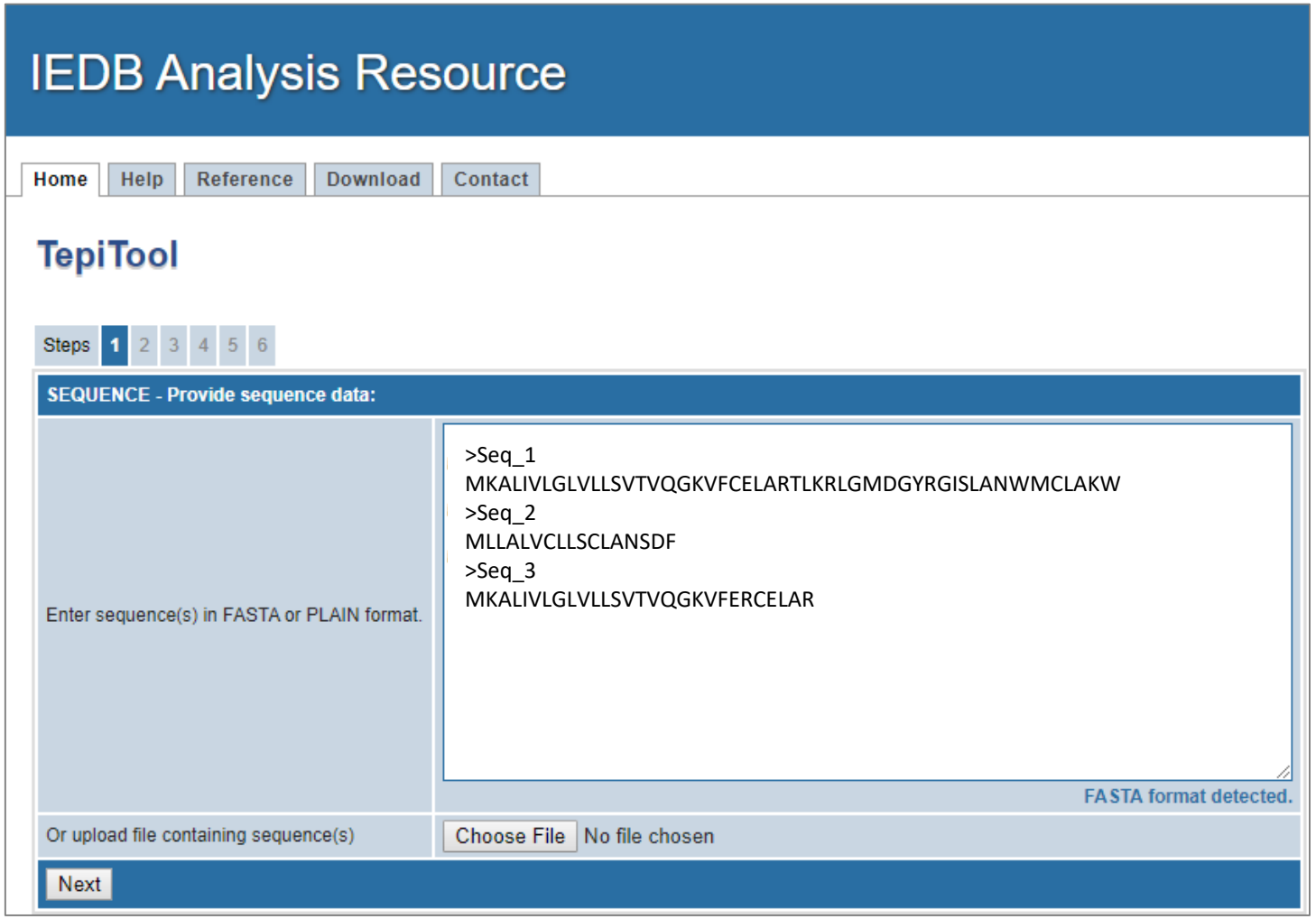
- Generate 15-mers overlapping by 10 AA residues
- Do binding prediction for the **7 selected alleles**
- Estimate the **median consensus percentile rank** (of the 7 alleles)
- Select all peptides with median consensus percentile rank **≤ 20.0**
- This set of peptides can capture ≈ 50% of the response
- These 7 alleles can be selected as a set
- This is implemented in **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)
- Input parameters can be adjusted as desired
- New methods incorporated

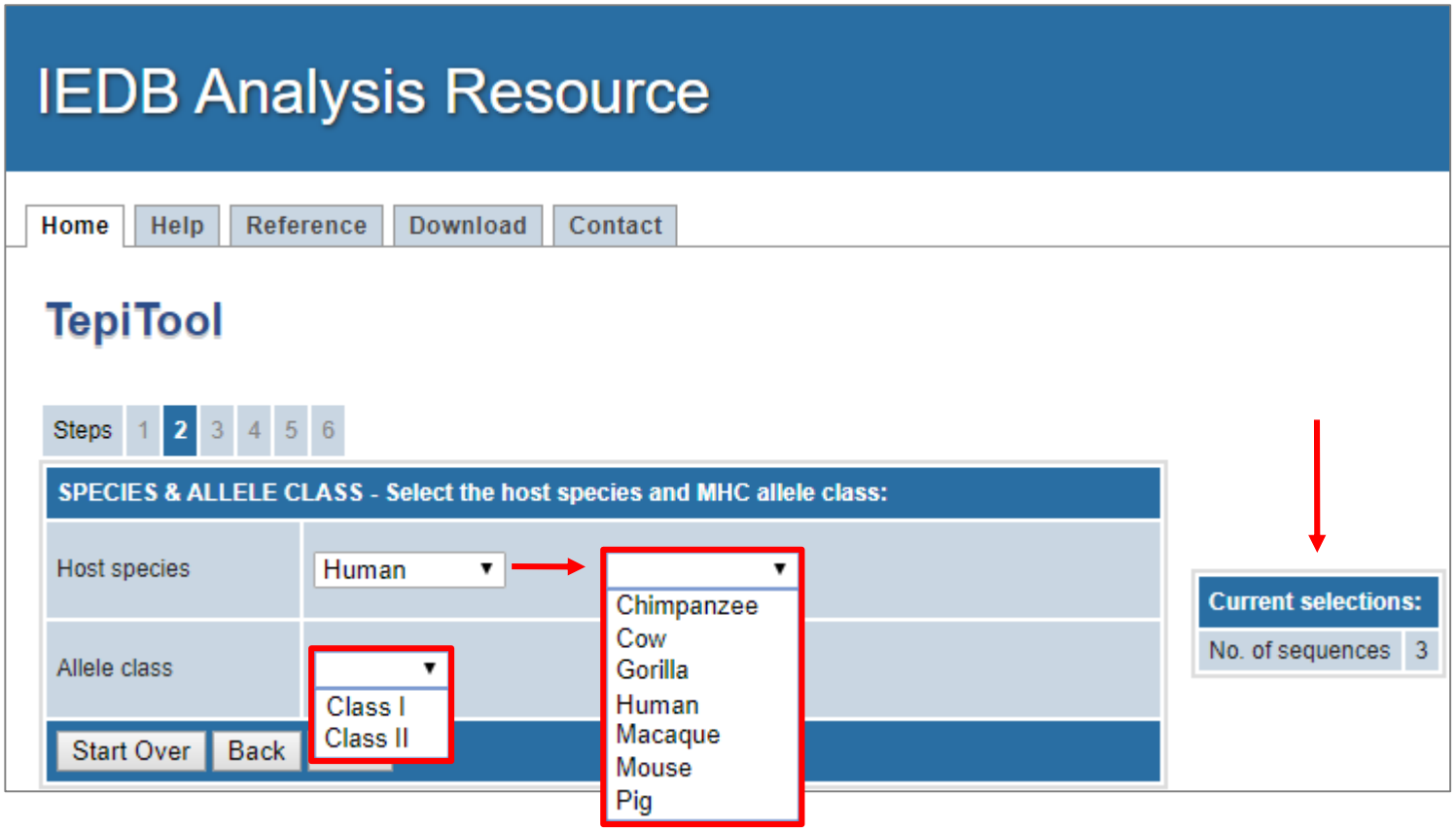
Step 1: Sequence data

tools.iedb.org/tepitool/



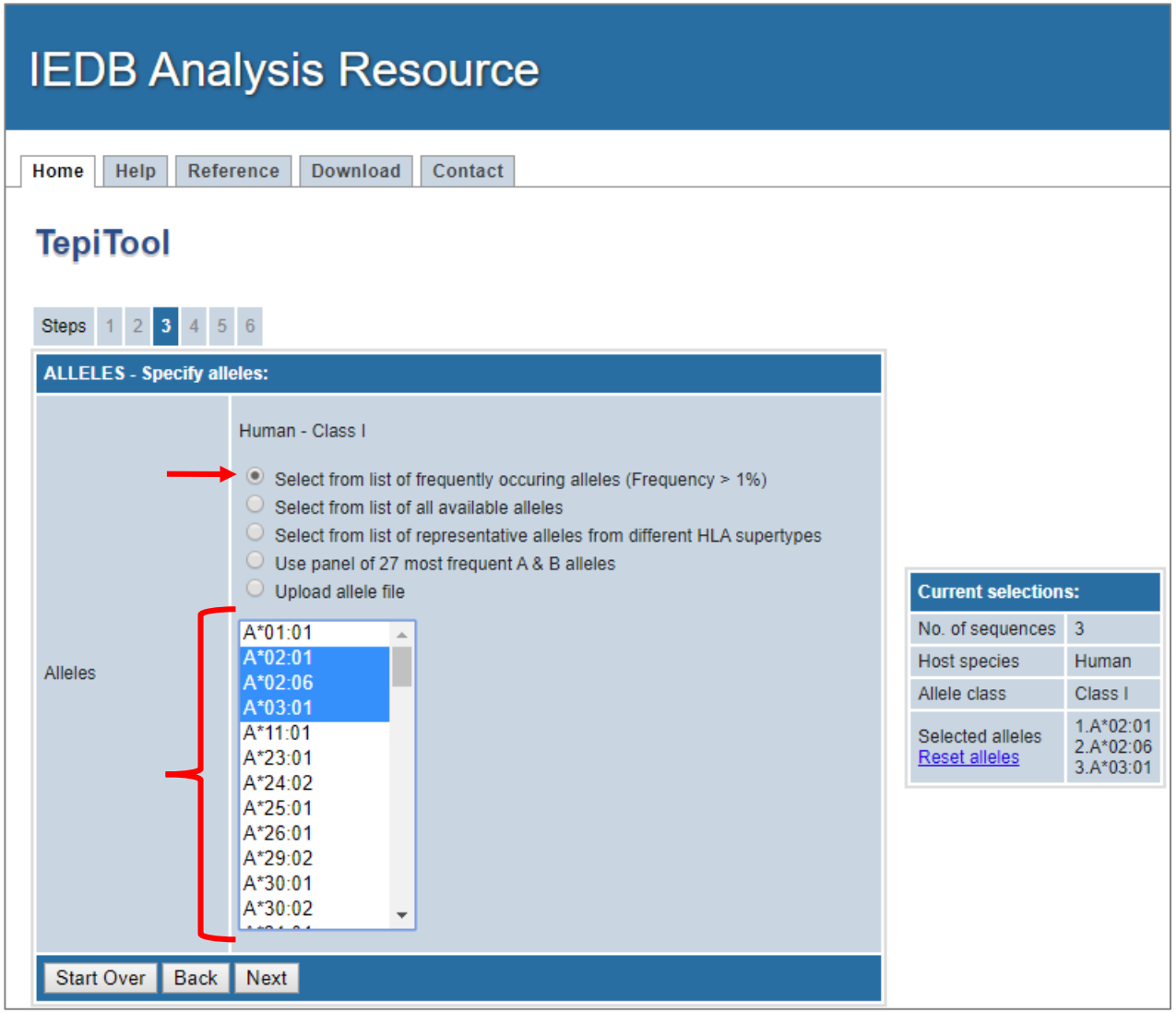
Step 2: Species & allele class

tools.iedb.org/tepitool/



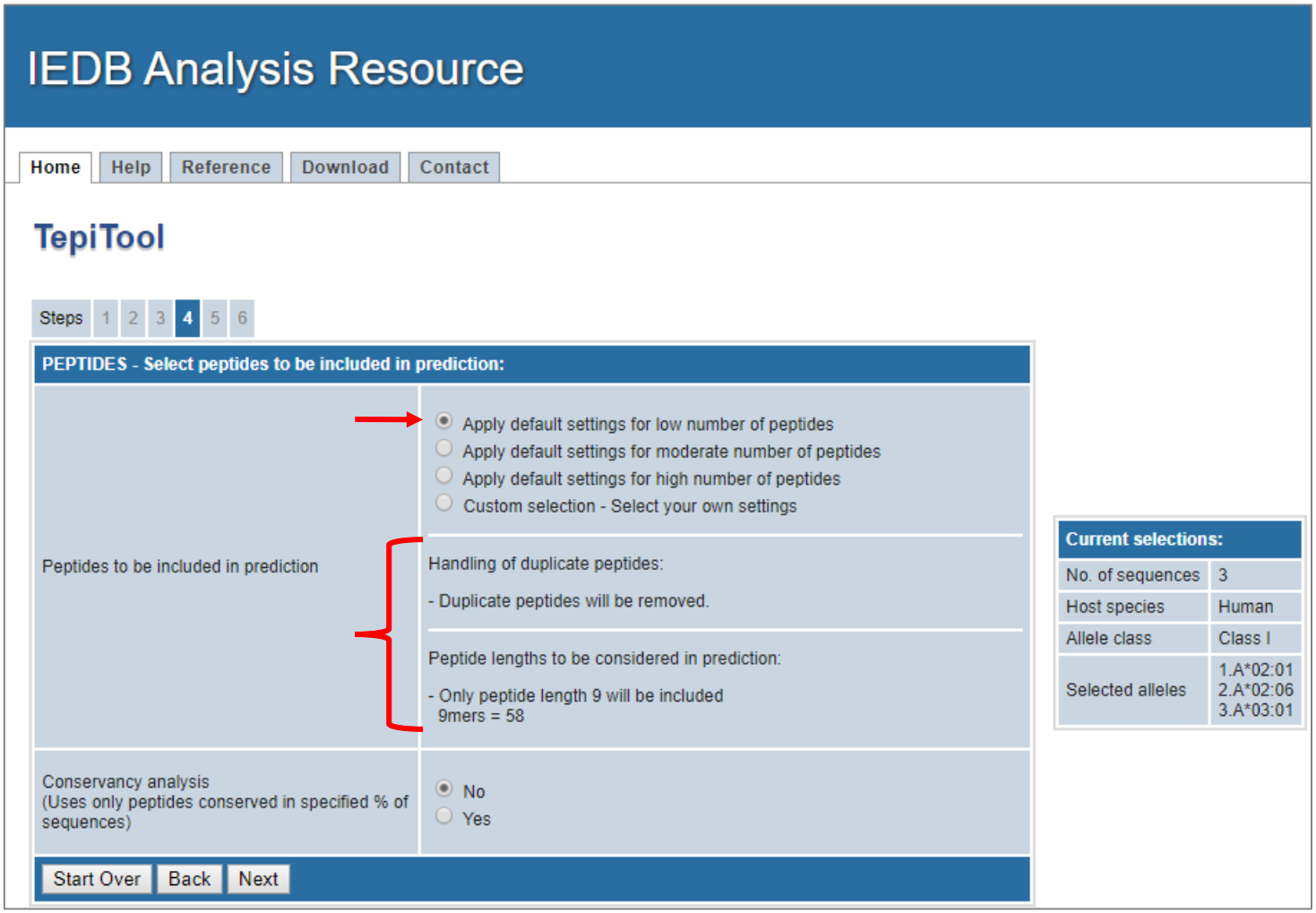
Step 3: Alleles - Class I

tools.iedb.org/tepitool/



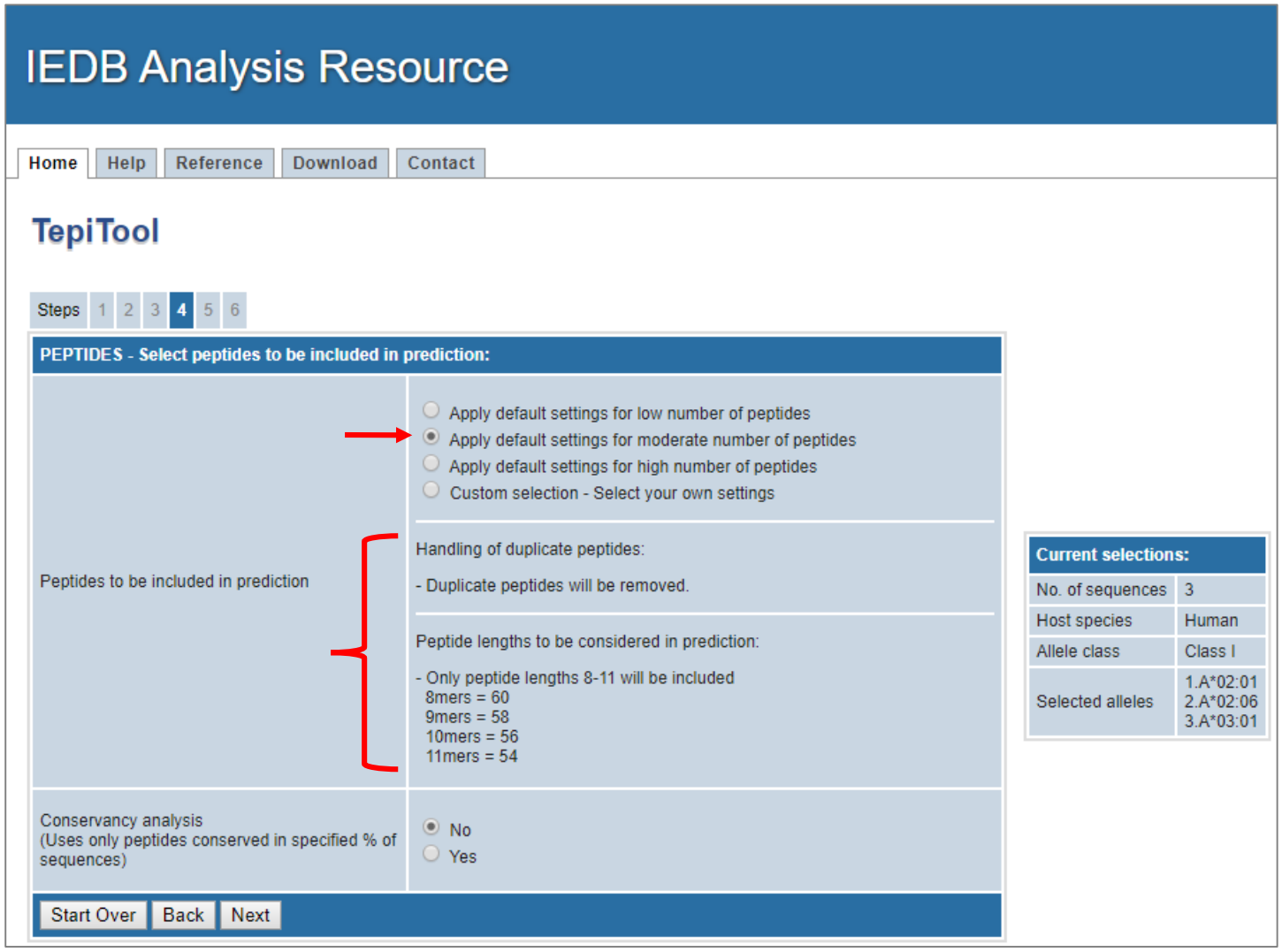
Step 4: Peptides - Class I

tools.iedb.org/tepitool/



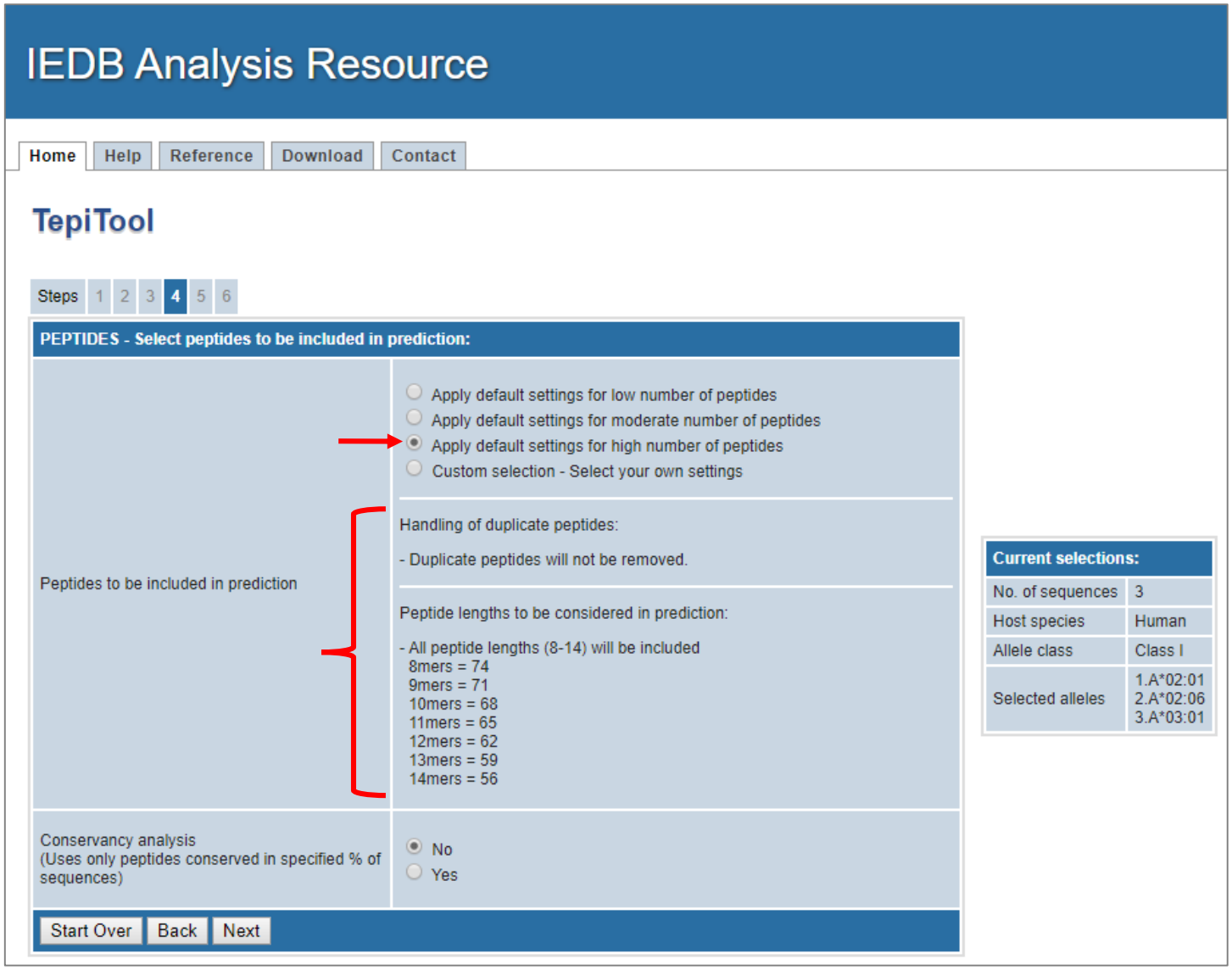
Step 4: Peptides - Class I

tools.iedb.org/tepitool/



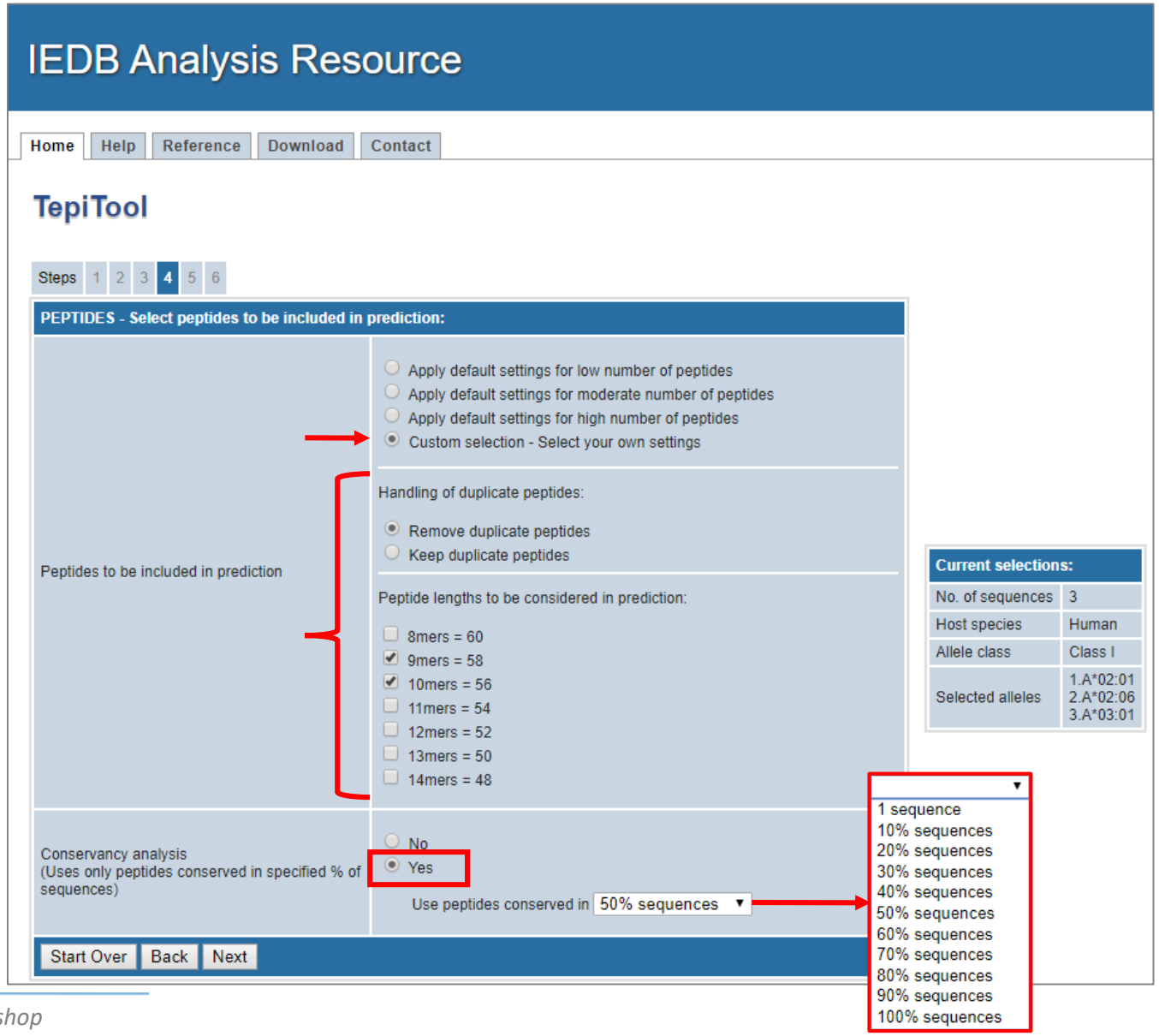
Step 4: Peptides - Class I

tools.iedb.org/tepitool/



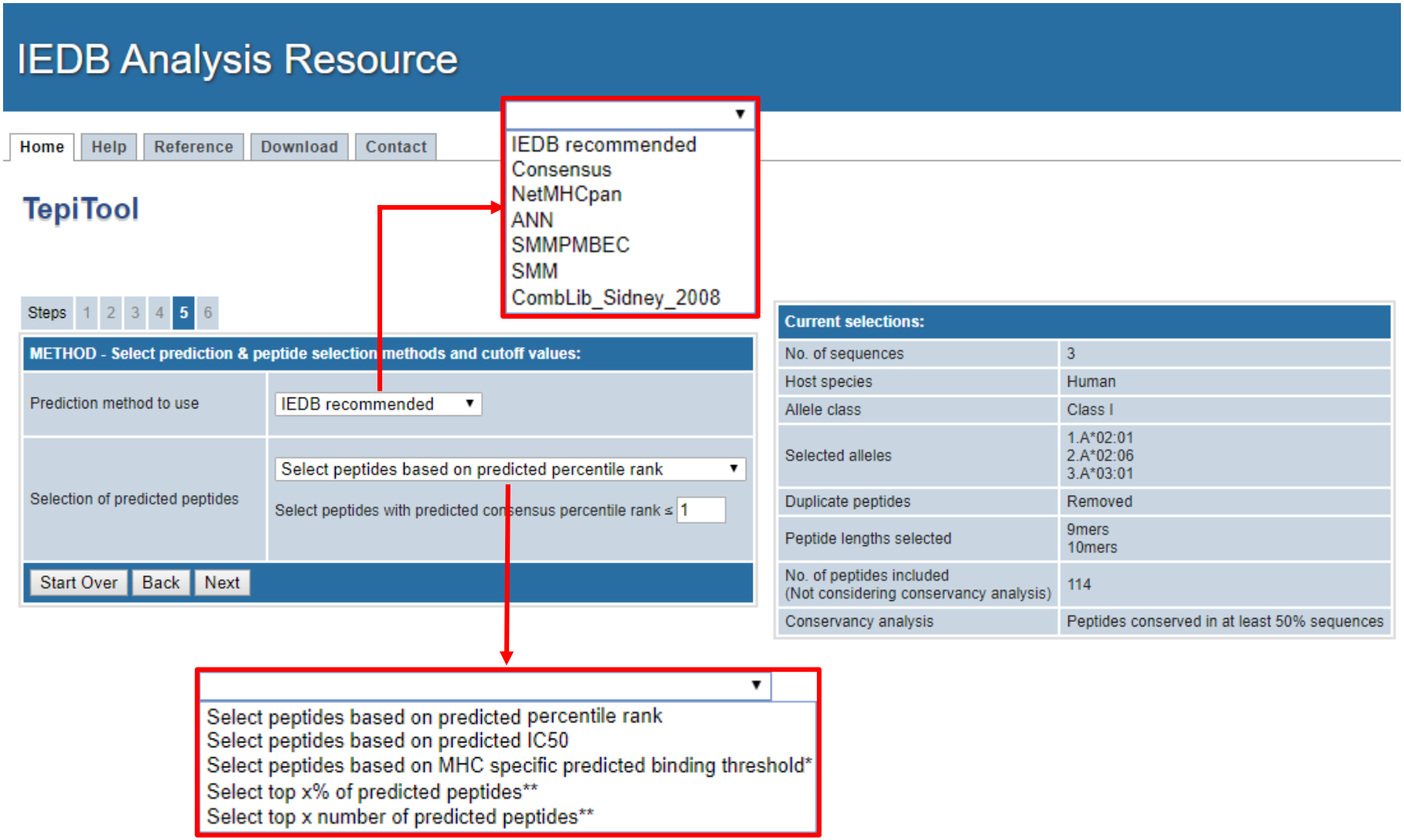
Step 4: Peptides - Class I

tools.iedb.org/tepitool/



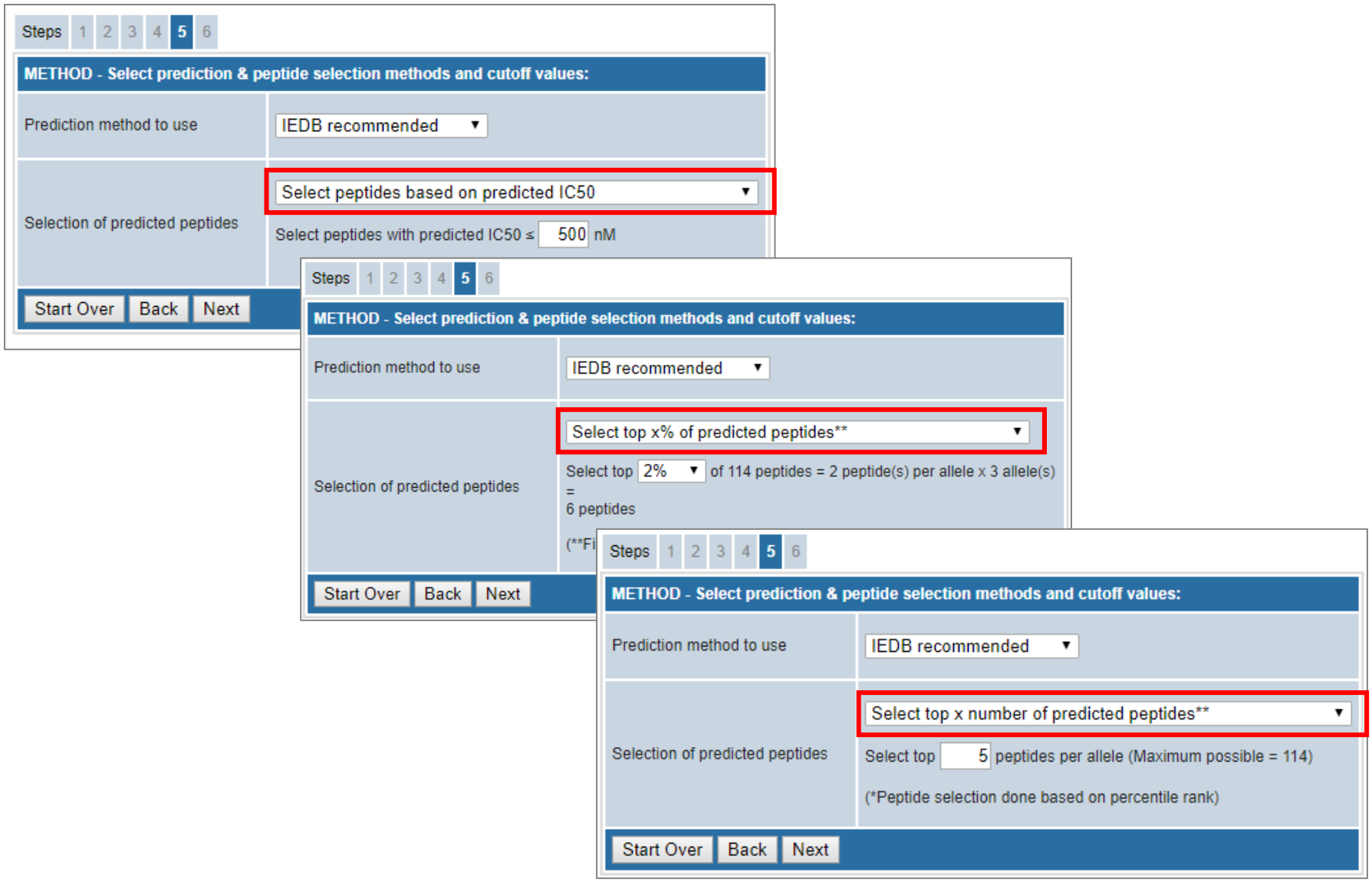
Step 5: Method - Class I

tools.iedb.org/tepitool/



Step 5: Method - Class I

tools.iedb.org/tepitool/

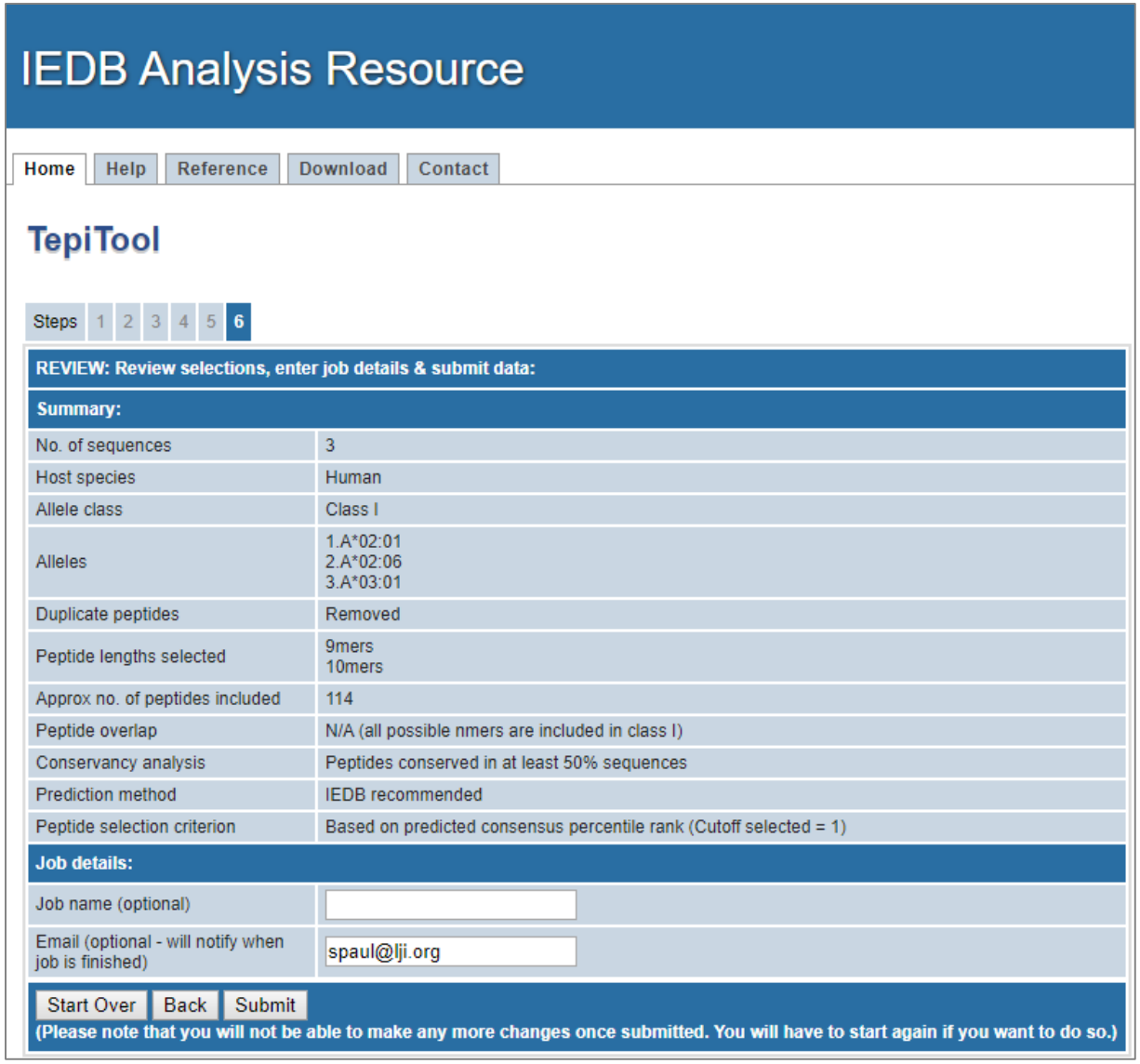


Step 5: Method - Class I

tools.iedb.org/tepitool/

Step 5: Method - Class I

tools.iedb.org/tepitool/



Results: Class I

tools.iedb.org/tepitool/

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TepiTool

Prediction results - concise (Download table):

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

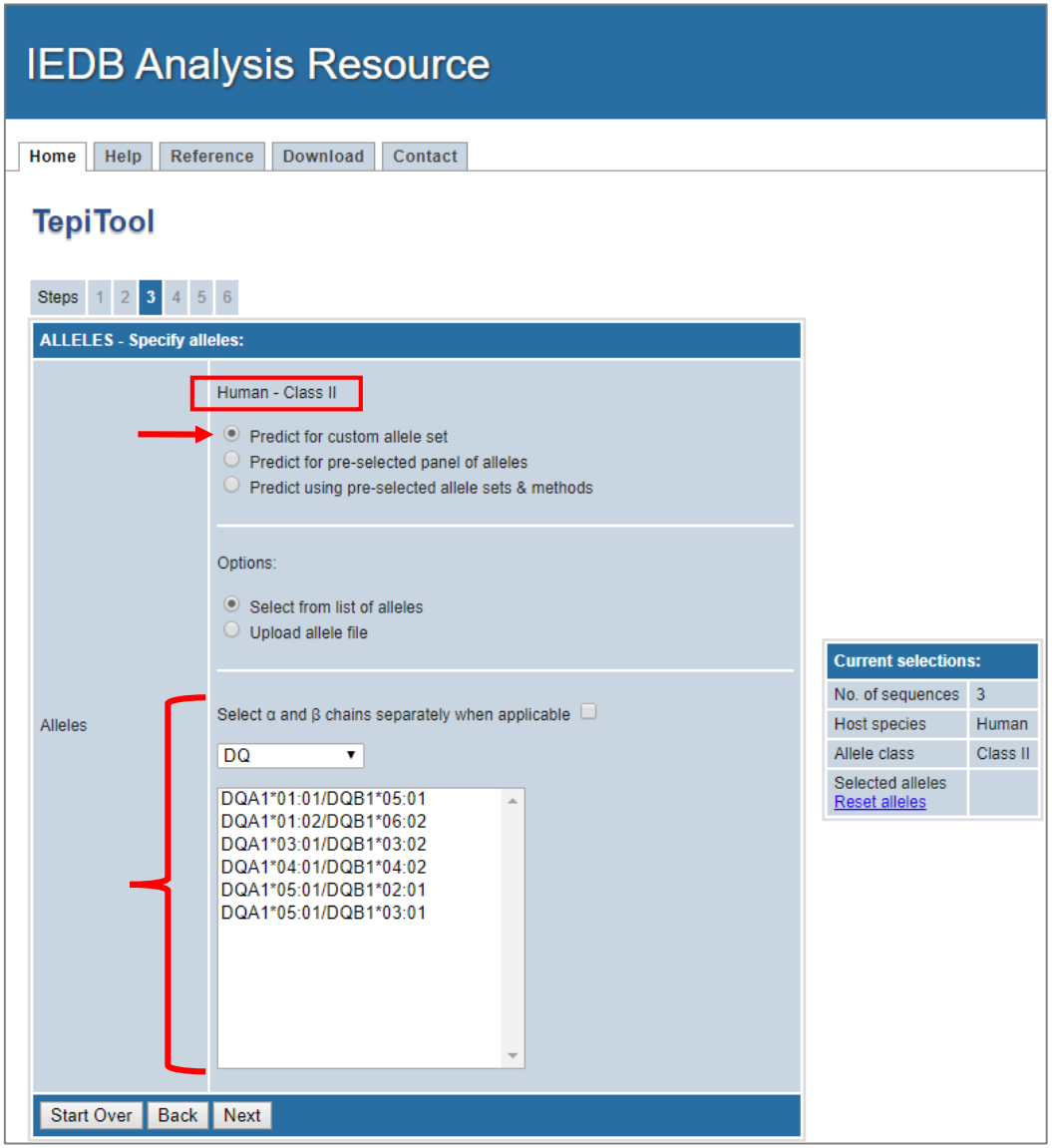
The MHC I binding predictions were done with IEDB analysis resource (TepiTool [1]) using Consensus method [2] which employs SMM, ANN and Combinatorial library methods.
1. Paul, S., Sidney, J., Sette, A., and Peters, B. 2016. TepiTool: A pipeline for computational prediction of T cell epitope candidates.*Curr. 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?

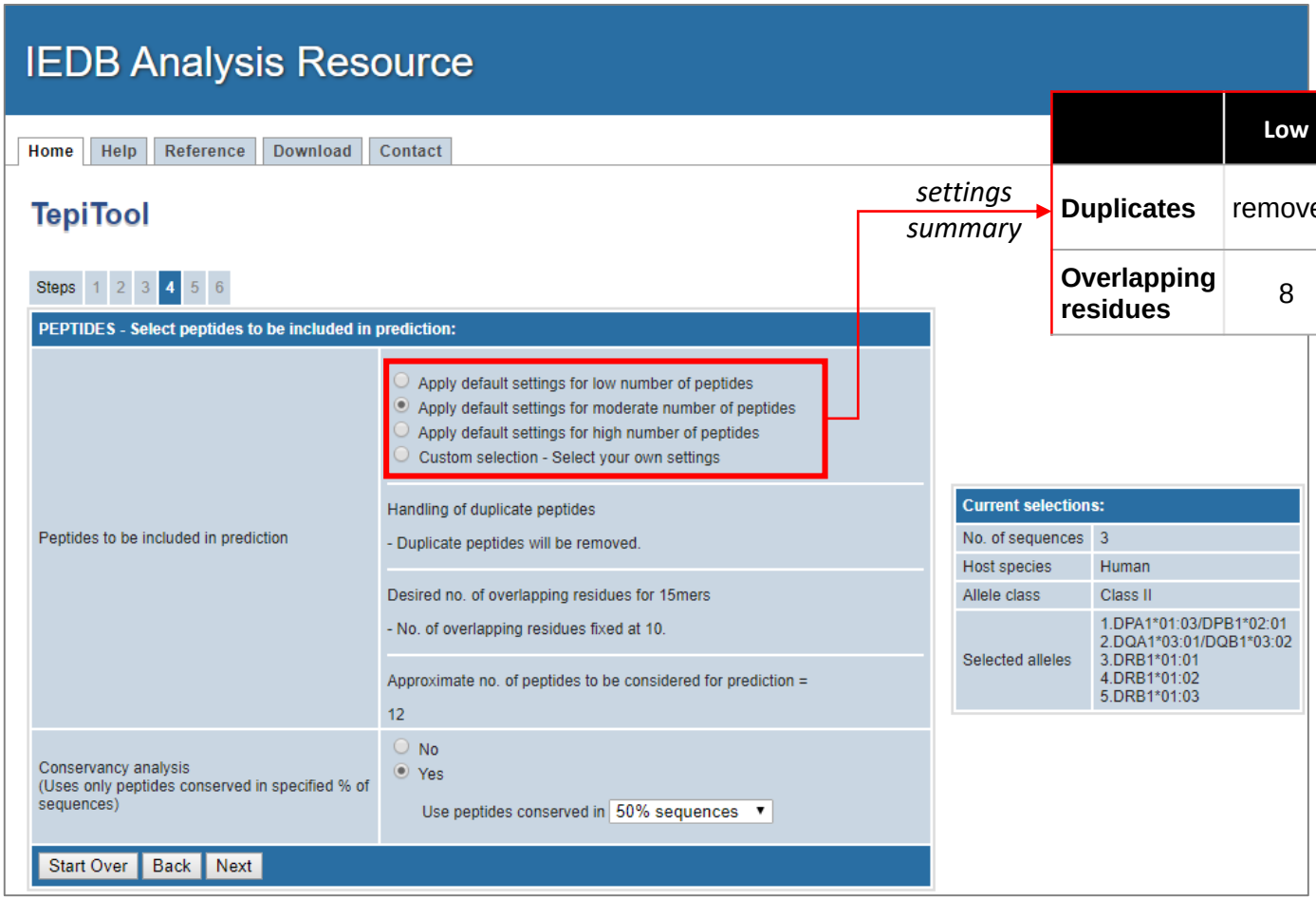
Step 3: Alleles - Class II

tools.iedb.org/tepitool/



Step 4: Peptides - Class II

tools.iedb.org/tepitool/

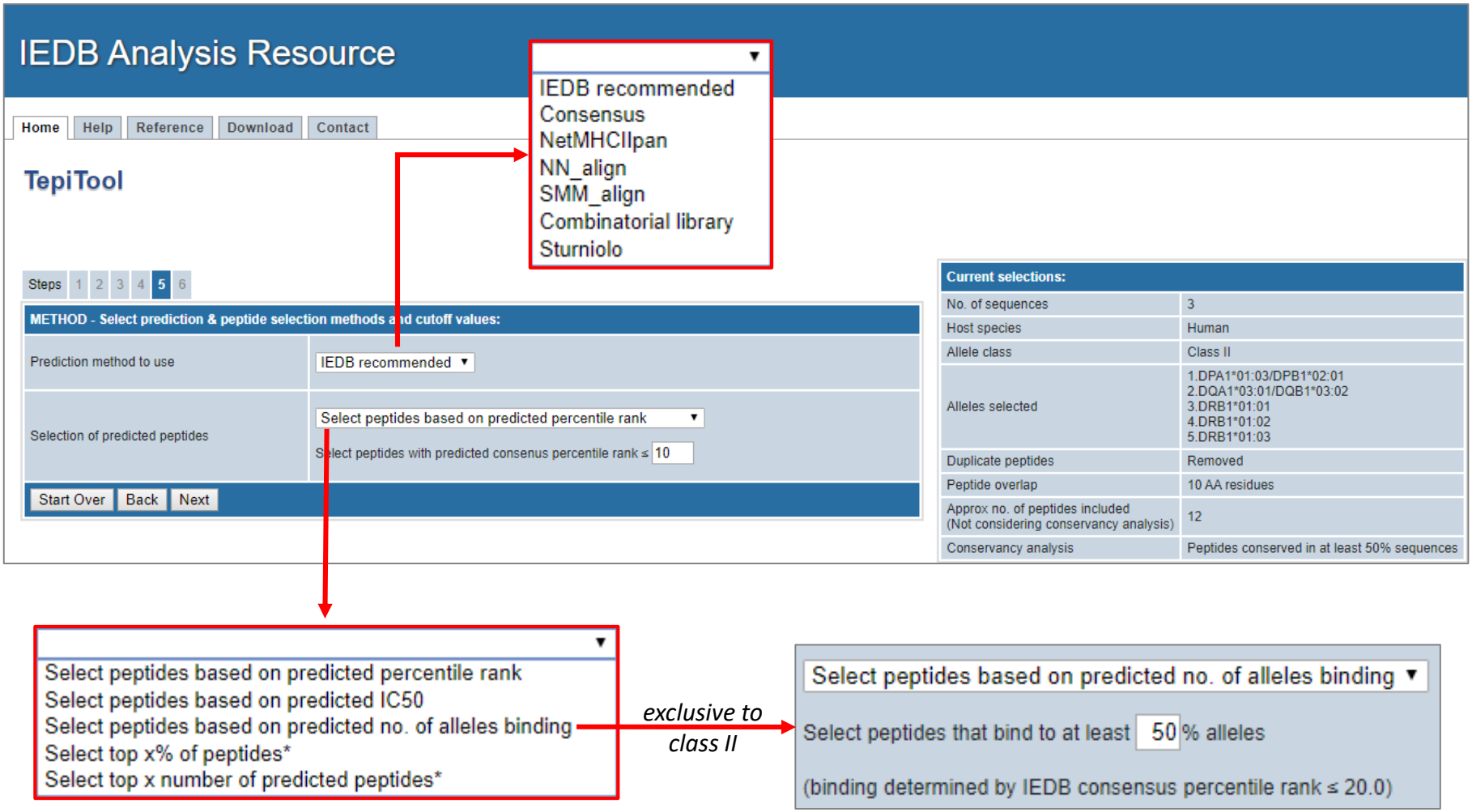


settings summary

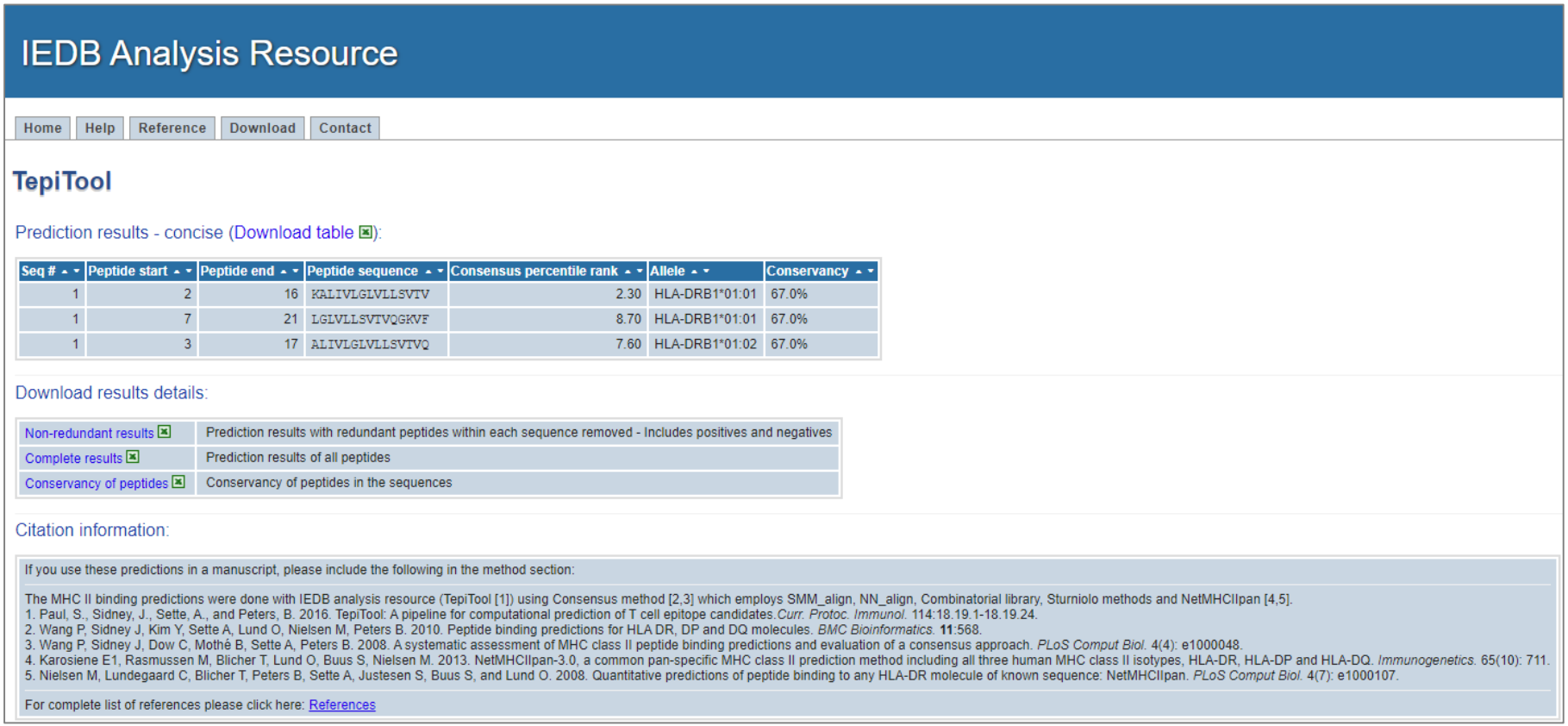
	Low	Moderate	High	Custom
Duplicates	removed	removed	not removed	user selects
Overlapping residues	8	10	10	user selects

Step 5: Method - Class II

tools.iedb.org/tepitool/



Results – Class II



IEDB Analysis Resource

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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	LGLVLLSVTVQGKVF	8.70	HLA-DRB1*01:01	67.0%
1	3	17	ALIVLGLVLLSVTVQ	7.60	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, NN_align, Combinatorial library, Sturniolo methods and NetMHCIIpan [4,5].

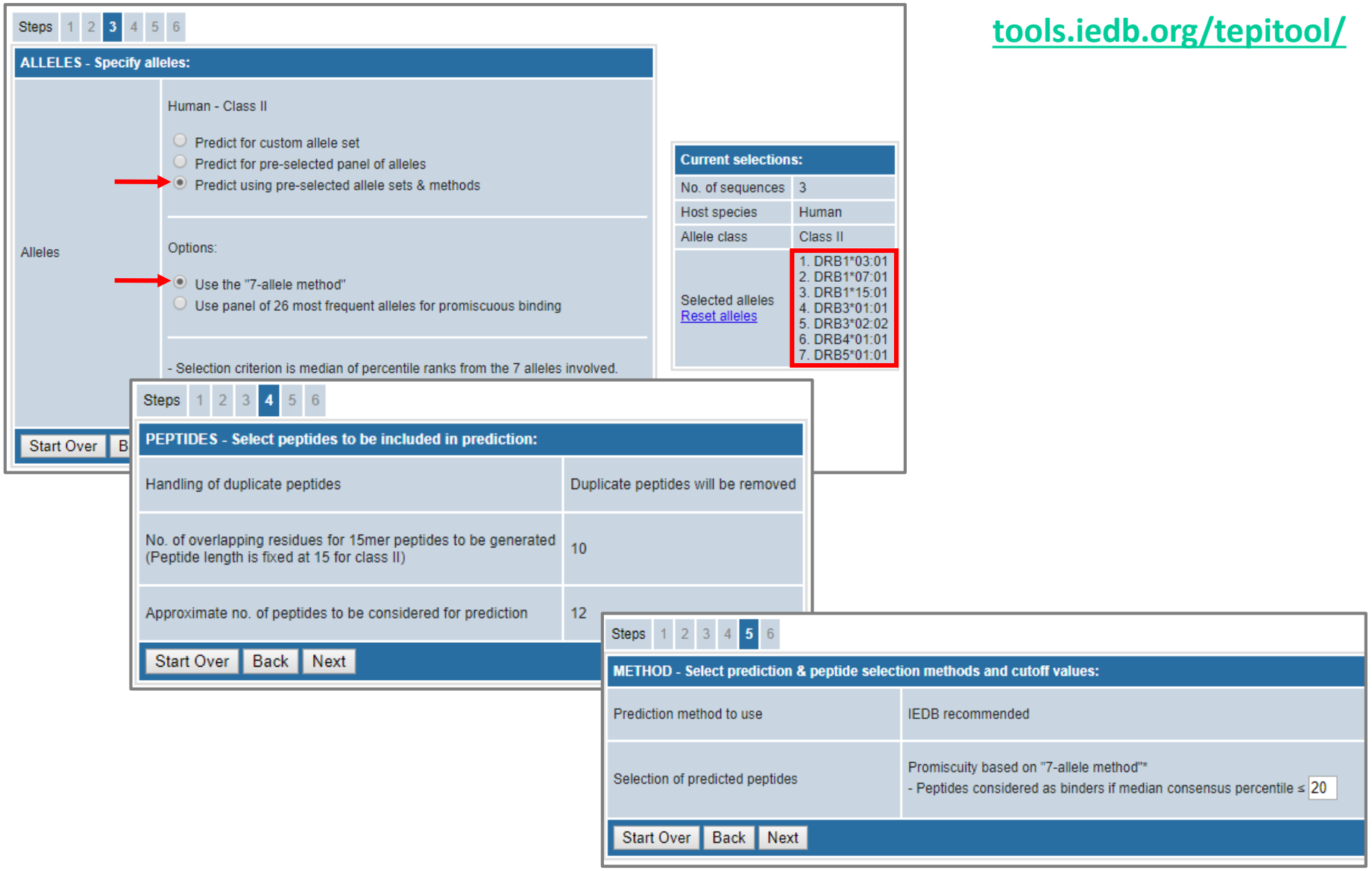
1. Paul, S., Sidney, J., Sette, A., and Peters, B. 2016. TepiTool: A pipeline for computational prediction of T cell epitope candidates.*Curr. Protoc. Immunol.* 114:18.19.1-18.19.24.
2. Wang P, Sidney J, Kim Y, Sette A, Lund O, Nielsen M, Peters B. 2010. Peptide binding predictions for HLA DR, DP and 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?

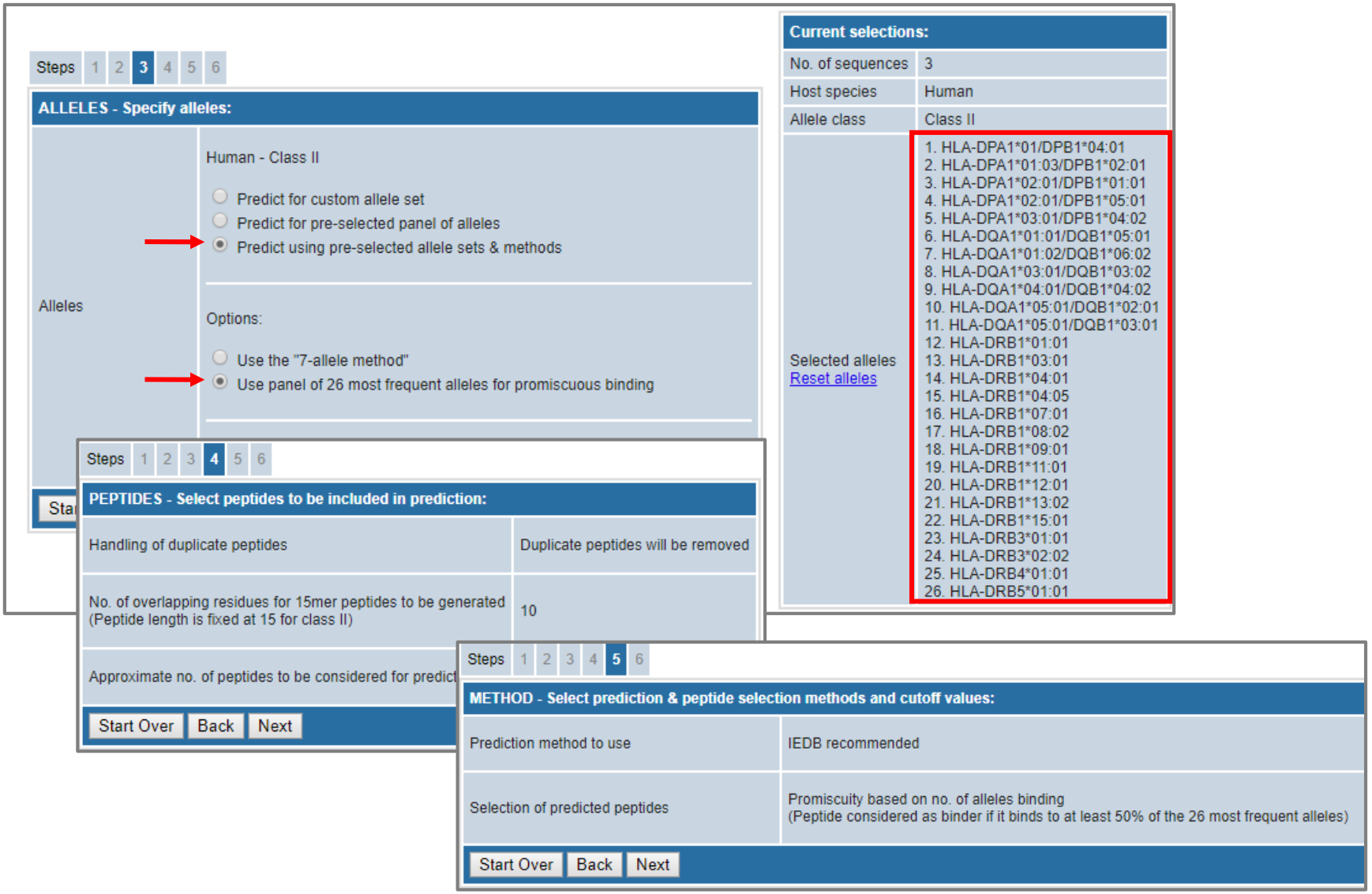
Step 3-5: Class II -7 allele method

tools.iedb.org/tepitool/



Step 3-5: Class II – promiscuous

tools.iedb.org/tepitool/



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-valiation 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^b & H-2K^b
- 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	https://www-bimas.cit.nih	Parker et al., 1994	http://www.jimmunol.c	Included
2	PREDEP	1995	http://margalit.huji.ac.il/T	Altuvia et al., 1995	https://www.sciencedi	Included
3	SYFPEITHI	1997	http://www.syfpeithi.de/b	Rammensee et al., 19	https://www.springer.c	Included
4	Rankpep	2002	http://imed.med.ucm.es/T	Reche et al., 2002	https://www.sciencedi	Included
5	ProPred1	2003	http://crdd.osdd.net/ragha	Singh and Raghava, 2	https://academic.oup.c	Included
6	SMM	2005	http://tools.iedb.org/mhci/	Peters and Sette, 2005	https://bmcbioinforma	Included
7	ARB	2005	http://tools.iedb.org/mhci/	Bui et al., 2005	https://link.springer.co	Included
8	IEDB Consensus	2006	http://tools.iedb.org/mhci/	Moutaftsi et al., 2006	https://www.nature.co	Included
9	SMMPMBEC	2009	http://tools.iedb.org/mhci/	Kim et al., 2009	https://bmcbioinforma	Included
10	PAComplex	2011	http://pacomplex.life.nctu	Liu et al., 2011	https://academic.oup.c	Included
11	NetMHCpan-3.0	2016	http://www.cbs.dtu.dk/ser	Nielsen and Andreatta	https://genomemedici	Included
12	NetMHC-4.0	2016	http://www.cbs.dtu.dk/ser	Andreatta and Nielser	https://academic.oup.c	Included
13	NetMHCpan-4.0***	2017	http://www.cbs.dtu.dk/ser	Jurtz et al., 2014	http://www.jimmunol.c	Included
14	MHCflurry***	2018	https://openvax.github.io/	O'Donnell et al., 2018	https://www.sciencedi	Included
15	MHCLovac	Not published yet	https://github.com/stefs3(	Stojanovic, S.	-	Included
16	CTLPred	2004	http://crdd.osdd.net/ragha	Bhasin and Raghava,	https://www.sciencedi	Excluded
17	EpiJen	2006	http://www.ddg-pharmfac	Doytchinova et al., 20	https://bmcbioinforma	Excluded (Trained mod
18	nHLAPred (ANNPred, ComPred)	2006	http://crdd.osdd.net/ragha	Bhasin and Raghava,	https://www.ias.ac.in/	Excluded
19	SVMHC	2006	http://abi.inf.uni-tuebinge	Donnes and Kohlbach	https://academic.oup.c	Excluded (Method not
20	SVRMHC	2006	http://c1.accurascience.co	Wan et al., 2006	https://bmcbioinforma	Excluded (Not working)
21	KISS	2007	http://cbio.ensmp.fr/kiss	Jacob and Vert, 2008	https://academic.oup.c	Excluded (Not working)
22	PickPocket	2009	http://www.cbs.dtu.dk/ser	Zhang et al., 2009	https://academic.oup.c	Excluded (Author sugge
23	Multipred2	2011	http://cvc.dfci.harvard.edu	Zhang et al., 2011	https://www.sciencedi	Excluded (Trained mod
24	NetMHCcons	2011	http://www.cbs.dtu.dk/ser	Karosiene et al., 2011	https://link.springer.co	Excluded (Author sugge
25	KernelRLSpanI	2014	https://github.com/guoxin	Shen et al., 2014	https://www.sciencedi	Excluded (Trained mod
26	NIELuter	2015	http://immunet.cn/nie/cgi-	Tang et al., 2015	https://www.sciencedi	Excluded (Trained mod
27	HONN	2015	Method not available	Kuksa et al., 2015	https://academic.oup.c	Excluded (Trained mod
28	ESMACS	2015	Method not available	Wan et al., 2015	https://pubs.acs.org/d	Excluded (Too resource
29	sNebula	2016	Method not available	Luo et al., 2016	https://www.nature.co	Excluded (Trained mod
30	HLaffy	2016	http://proline.biochem.iisc	Mukherjee et al., 2016	https://academic.oup.c	Excluded (Trained mod
31	ConvMHC	2017	http://jumong.kaist.ac.kr:8	Han and Kim, 2017	https://bmcbioinforma	Excluded (Trained mod
32	PSSMHCpan	2017	https://github.com/BGI201	Liu et al., 2017	https://academic.oup.c	Excluded (Trained mod
33	MixMHCpred	2017	https://github.com/Gfeller	Bassani-Sternberg et a	https://journals.plos.or	Excluded (Trained mod
34	HLA-CNN (HLA-bind)	2017	https://github.com/uci-cbc	Vang and Xie, 2017	https://academic.oup.c	Excluded (Trained mod
35	EDGE	2018	Model provided as part of	Bulik-Sullivan et al.	https://www.nature.co	Excluded (Not working)
36	MAM	2018	http://mhc.deepomics.org/	Xiao et al., 2018	https://bmcbioinforma	Excluded (Trained mod
37	DeepSeqPan	2019	https://github.com/pcpLiu	Liu et al., 2019	https://www.nature.co	Excluded (Trained mod
38	ForestMHC	2019	https://github.com/kmboe	Boehm et al., 2019	https://link.springer.co	Excluded (Trained mod
39	DeepMHC	Not published yet	http://mleg.cse.sc.edu/de	Hu and Liu	https://www.biorxiv.or	Excluded (Trained mod
40	AI-MHC	Not published yet	https://baras.pathology.jh	Sidhom et al.	https://www.biorxiv.or	Excluded (Trained mod
41	MHCSeqNet	Not published yet	https://github.com/cmbcu	Phloyphisut et al.	https://www.biorxiv.or	Excluded (Trained mod
42	ACME	Not published yet	https://github.com/HYsxe/	Hu et al.	https://www.biorxiv.or	Excluded (Trained mod
43	Deep-Learning-MHCI	Not published yet	https://github.com/altayg/	Altay, G.	https://www.biorxiv.or	Excluded (Trained mod
44	MHCnuggets	Not published yet	https://karchinlab.org/app	Bhattacharya et al.	https://www.biorxiv.or	Excluded (Author sugge

RESEARCH ARTICLE

Benchmarking predictions of MHC class I restricted T cell epitopes in a comprehensively studied model system

Sinu Paul[1], Nathan P. Croft[2,3], Anthony W. Purcell[2,3], David C. Tscharke[4], Alessandro Sette[1,5], Morten Nielsen[6,7], Bjoern Peters[1,5]*

Dataset

- Comprehensive epitope dataset from Vaccinia virus

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

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

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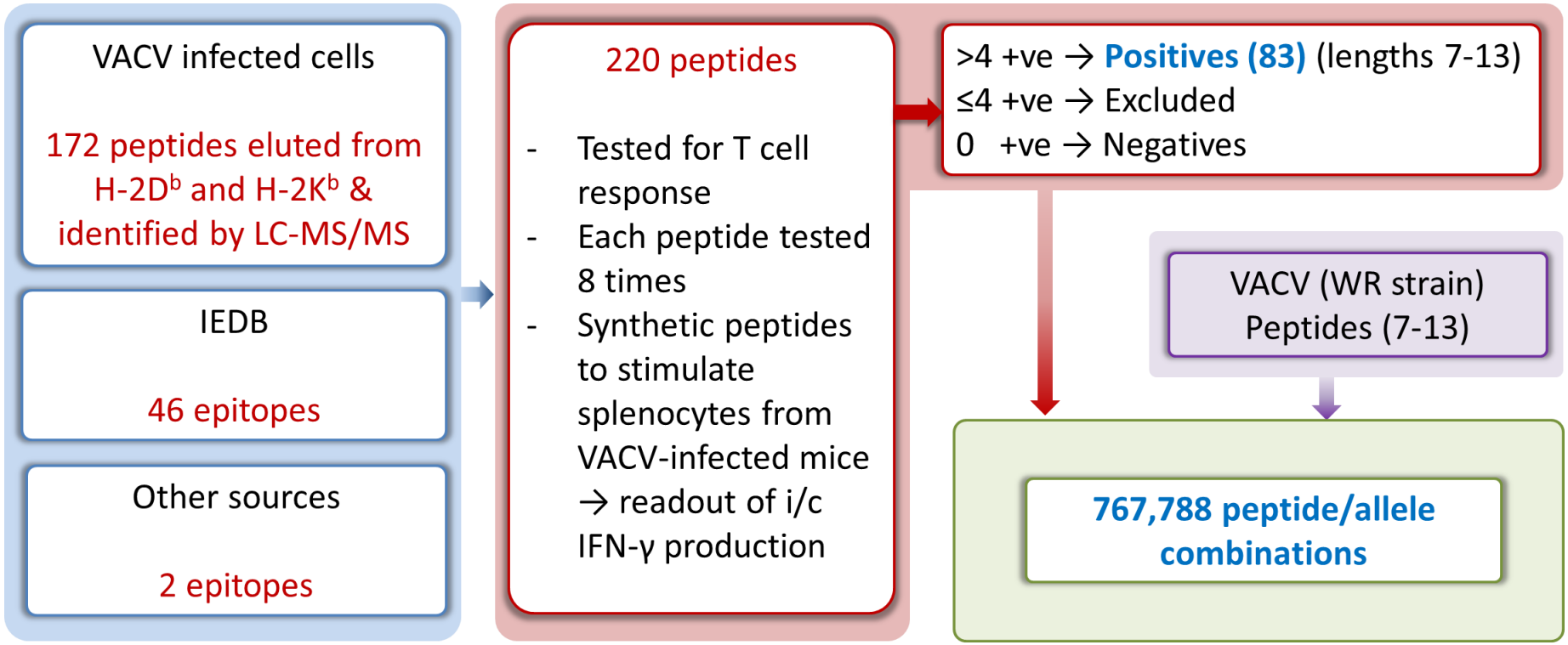
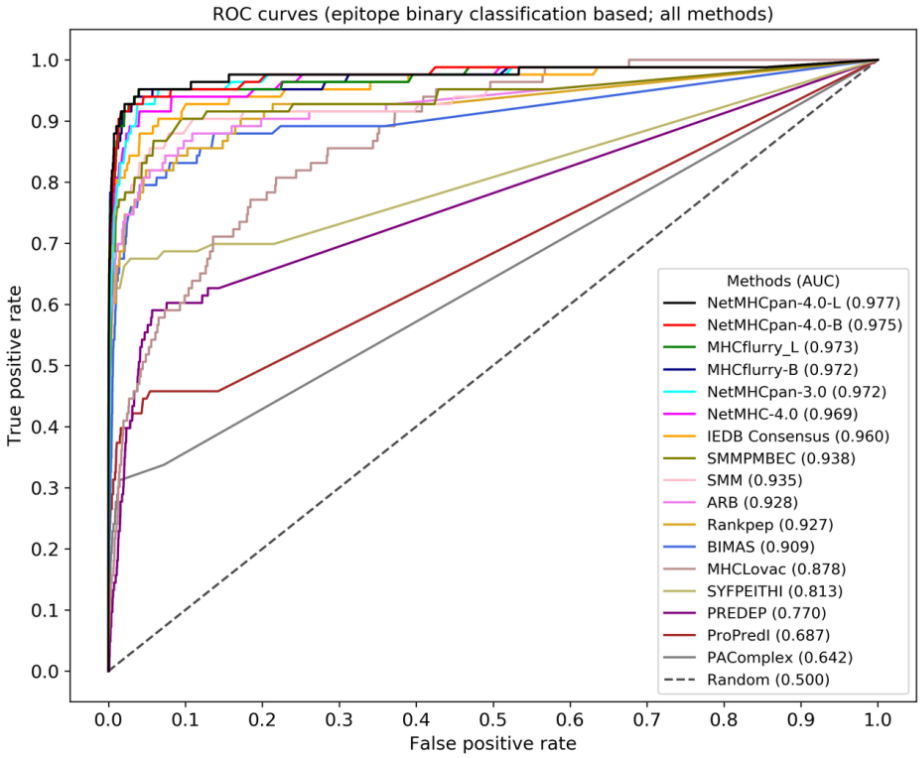


Results

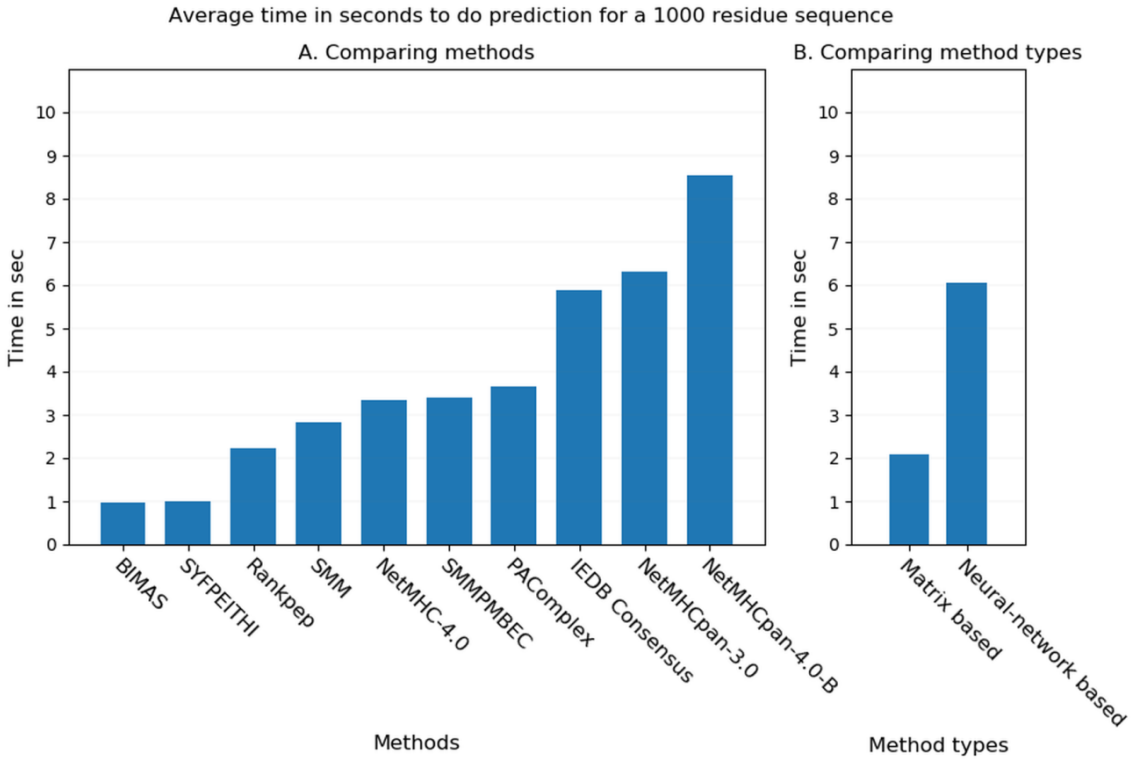
- Binary classification (epitope/non-epitope) & T cell response based
- In terms of AUC (Area under curve of ROC curves)

Method	Binary classification (epitope/non-epitope) based		T cell response based	
	AUC	Rank	AUC	Rank
NetMHCpan-4.0-L	0.977	1	0.979	1
NetMHCpan-4.0-B	0.975	2	0.978	2
MHCflurry-L	0.973	3	0.977	3
MHCflurry-B	0.972	4	0.976	4
NetMHCpan-3.0	0.972	5	0.975	5
NetMHC-4.0	0.969	6	0.974	6
IEDB Consensus	0.960	7	0.961	7
SMMPMBEC	0.938	8	0.940	8
SMM	0.935	9	0.938	10
ARB	0.928	10	0.939	9
Rankpep	0.927	11	0.894	12
BIMAS	0.909	12	0.918	11
MHCLovac	0.878	13	0.863	13
SYFPEITHI	0.813	14	0.778	14
PREDEP	0.770	15	0.737	15
ProPred-I	0.687	16	0.651	17
PAComplex	0.642	17	0.652	16



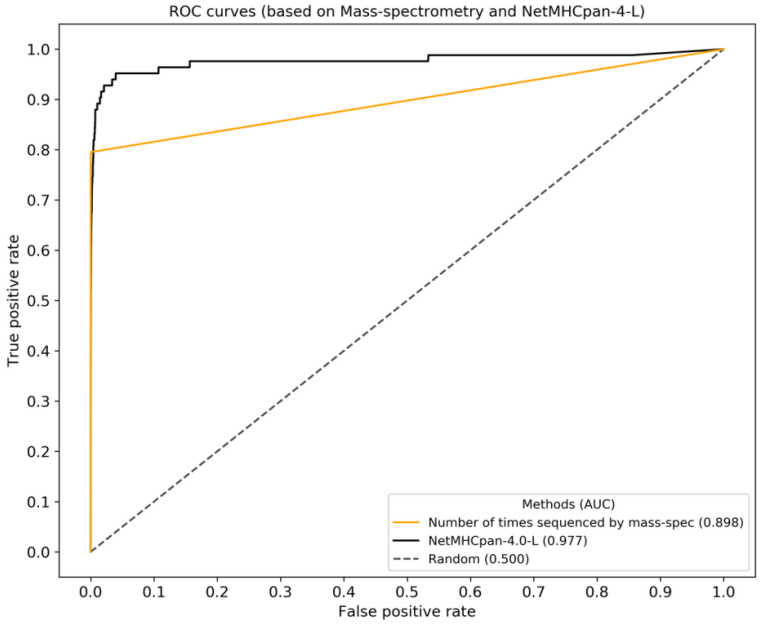
Prediction speed comparison

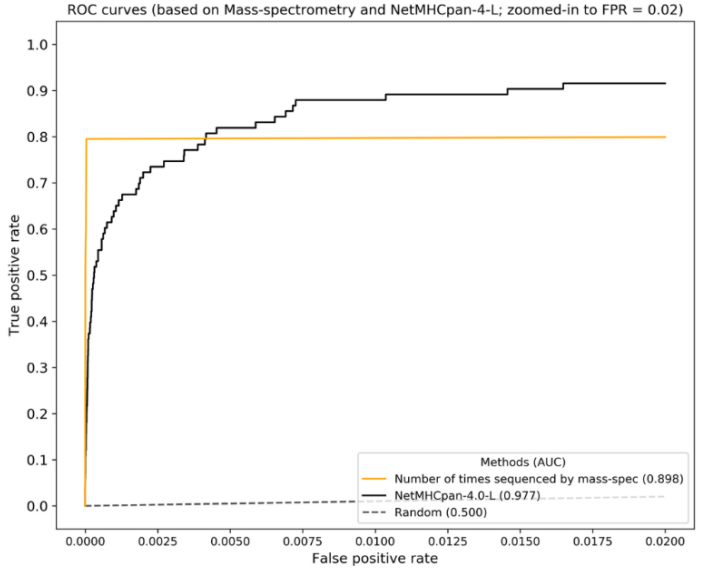
- 5 random sequences of 1000 residues each
- Matrix-based methods faster compared to Neural network-based methods



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





Summary

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