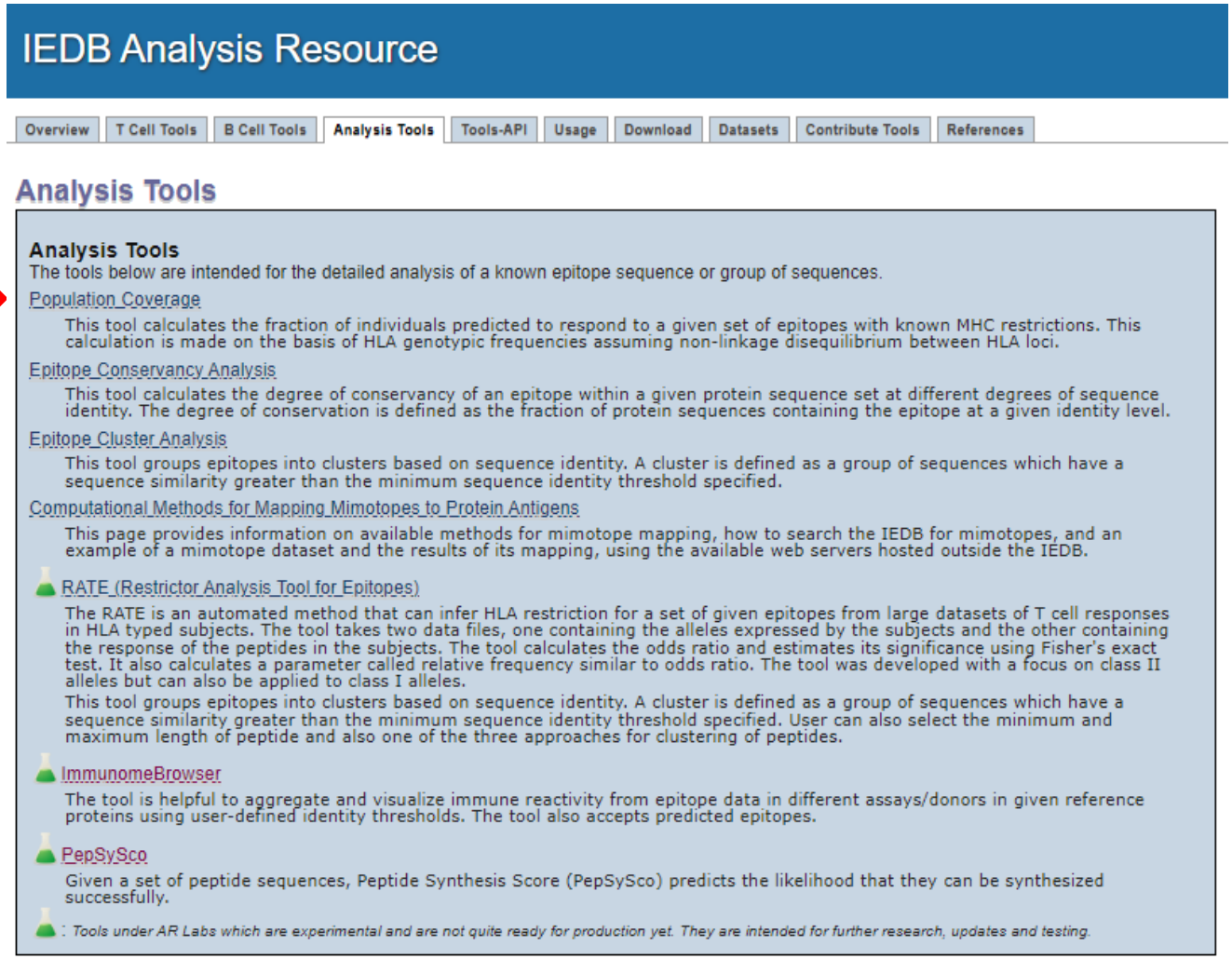
Analysis Tools

tools.iedb.org

Presented by: Alessandro Sette, IEDB Principal Investigator

Analysis tools with broad applications

http://tools.iedb.org/main/analysis-tools/



Population Coverage

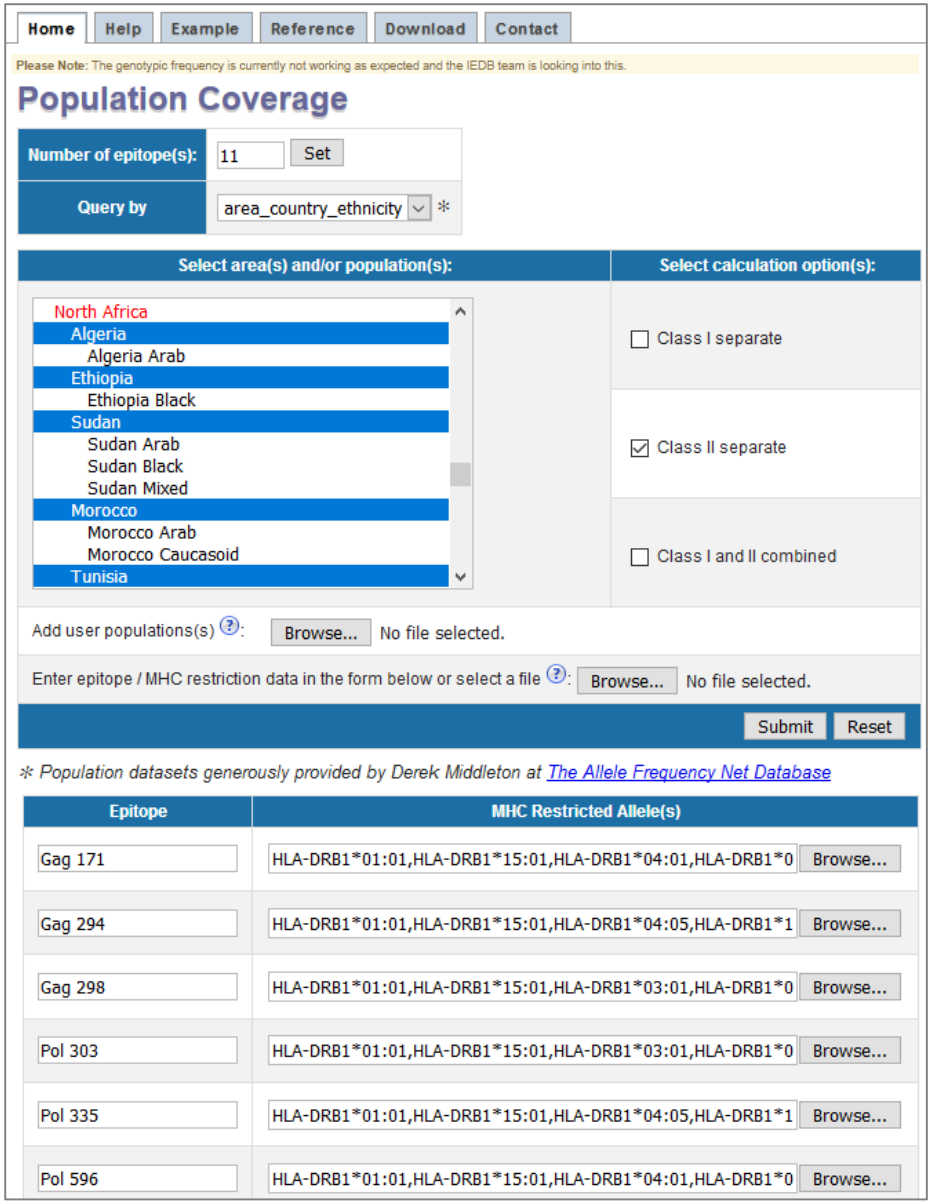
http://tools.iedb.org/population/

- Calculates the fraction of individuals projected to bind and/or respond to a given set of epitopes with defined reactivity
- Based on
  - Epitope known HLA binding/restrictions
  - HLA genotypic frequencies
- HLA genotypic frequencies vary in different ethnicities
  - http://allelefrequencies.net

Population Coverage – example

http://tools.iedb.org/population/

For a set of 11 MHC class II restricted epitopes with promiscuous HLA binding, what is the population coverage in different North African populations?

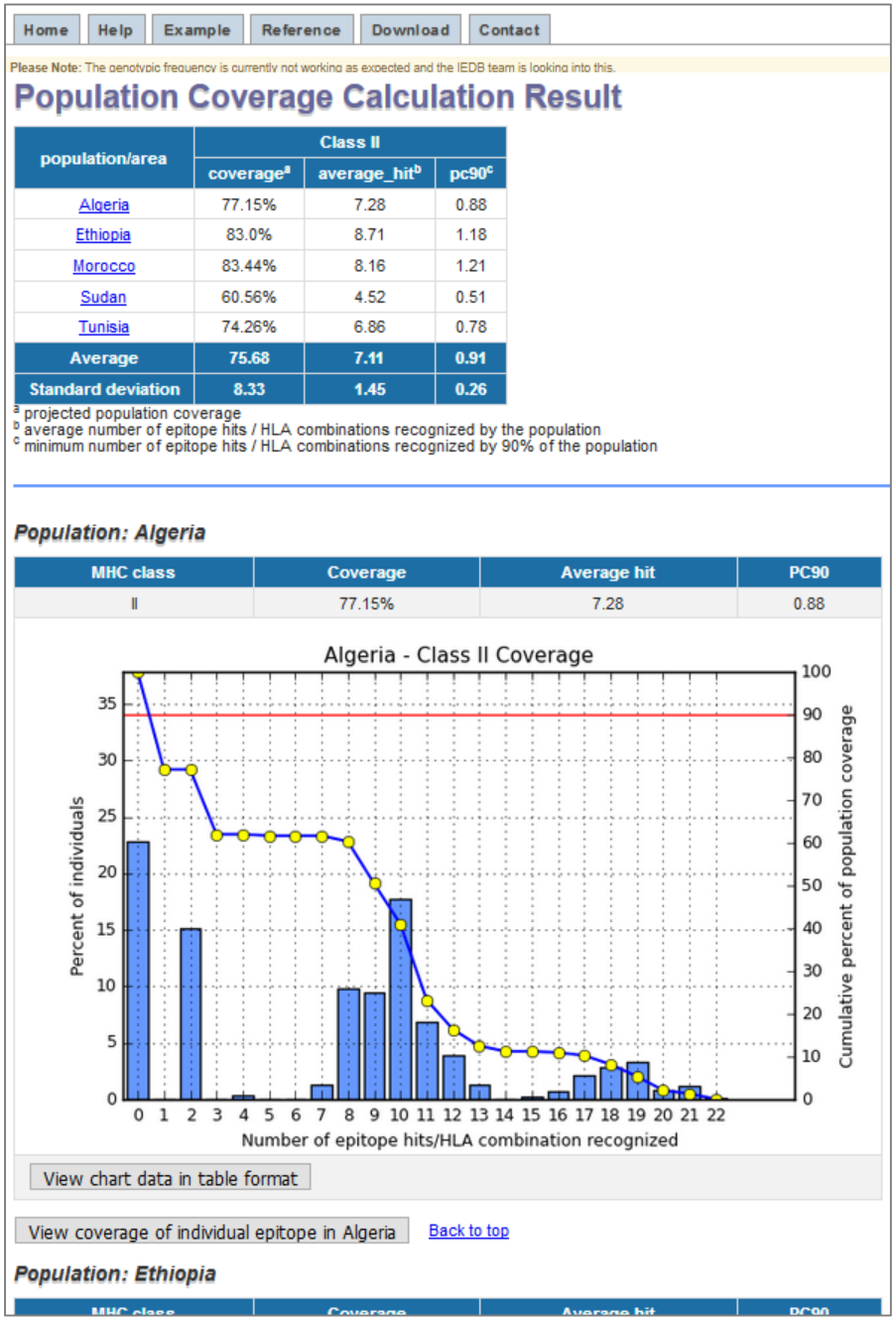


Population Coverage – example

http://tools.iedb.org/population/

Results:

- Summarized in table format
- Plotted per population



Home | Help | Example | Reference | Download | Contact

Please Note: The genotypic frequency is currently not working as expected and the IEDB team is looking into this.

Population Coverage Calculation Result

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
Algeria	77.15%	7.28	0.88
Ethiopia	83.0%	8.71	1.18
Morocco	83.44%	8.16	1.21
Sudan	60.56%	4.52	0.51
Tunisia	74.26%	6.86	0.78
Average	**75.68**	**7.11**	**0.91**
Standard deviation	**8.33**	**1.45**	**0.26**

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

Population: Algeria

MHC class	Coverage	Average hit	PC90
II	77.15%	7.28	0.88

View chart data in table format

View coverage of individual epitope in Algeria | Back to top

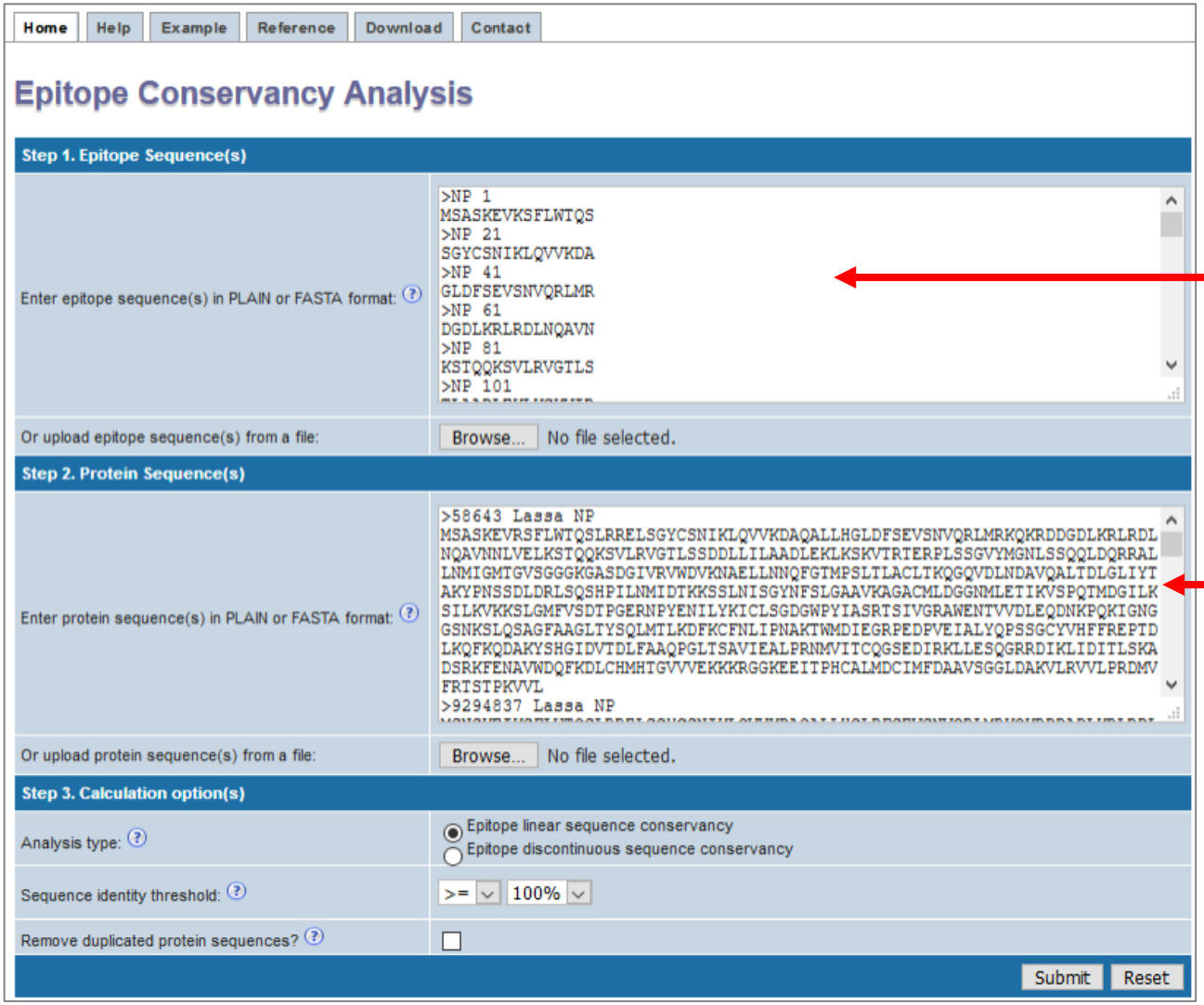
Population: Ethiopia

Epitope Conservancy Analysis

- Calculates the degrees of conservancy of one or more epitopes, within a given set of protein sequences
- Adjustable sequence identity threshold
- “Degree of conservation” = the fraction of protein sequences containing the epitope at a given identity level

Epitope Conservancy – example

http://tools.iedb.org/conservancy/



Known epitopes

Protein sequences

Epitope Conservancy – example

http://tools.iedb.org/conservancy/

Home | Help | Example | Reference | Download | Contact

Epitope Conservancy Analysis Result

Download result

Epitope #	Epitope name	Epitope sequence	Epitope length	Percent of protein sequence matches at identity <= 100%	Minimum identity	Maximum identity	View details
22	NP 421	LKQFKQDAKYSHGID	15	98.44% (63/64)	93.33%	100.00%	Go
20	NP 381	LDPNAKTWMDIEGRP	15	68.75% (44/64)	80.00%	100.00%	Go
17	NP 321	ASRTSITGRAWENTV	15	67.19% (43/64)	86.67%	100.00%	Go
21	NP 401	IALYQPSSGCYIHFF	15	65.62% (42/64)	86.67%	100.00%	Go
25	NP 481	KLIDIALSKTDSRKY	15	54.69% (35/64)	66.67%	100.00%	Go
26	NP 501	DQYKDLCHMHTGVVV	15	54.69% (35/64)	86.67%	100.00%	Go
19	NP 361	FTAGLTYSQLMTLKD	15	50.00% (32/64)	80.00%	100.00%	Go
24	NP 461	TCQGSDDIRKLLESQ	15	50.00% (32/64)	80.00%	100.00%	Go
23	NP 441	AAQPGLTSAVIEALP	15	34.38% (22/64)	86.67%	100.00%	Go
18	NP 341	DGKPQKAGSNNSNKS	15	28.12% (18/64)	60.00%	100.00%	Go
2	NP 21	SGYCSNIKLQVVKDA	15	17.19% (11/64)	26.67%	100.00%	Go
10	NP 181	SLTLACLTKQGQVDL	15	17.19% (11/64)	26.67%	100.00%	Go
11	NP 201	ALTDLGLIYTAKYPN	15	17.19% (11/64)	26.67%	100.00%	Go
3	NP 41	GLDFSEVSNVQRLMR	15	15.62% (10/64)	26.67%	100.00%	Go
13	NP 241	ISGYNFSLGAAVKAG	15	15.62% (10/64)	26.67%	100.00%	Go
16	NP 301	NPYENILYKICLSGD	15	14.06% (9/64)	20.00%	100.00%	Go

Epitope Conservancy – example

http://tools.iedb.org/conservancy/

Show records with identity >= 70% Show records

Protein #	Protein name	Positions	Protein sub-sequence(s)	Identity
1	58643 Lassa NP	401-415	IALYQPSSGCYVHFF	93.33%
2	9294837 Lassa NP	400-414	IALFQPSSGCYIHFF	93.33%
3	9294840 Lassa NP	400-414	IALYQPMSGCYIHFF	93.33%
4	9294844 Lassa NP	82-96	IALYQPMSGCYIHFF	93.33%
5	9294846 Lassa NP	82-96	IALYQPMSGCYIHFF	93.33%
6	9294848 Lassa NP	82-96	IALYQPMSGCYIHFF	93.33%
7	9294850 Lassa NP	82-96	IALYQPISGCYIHFF	93.33%
8	9294852 Lassa NP	82-96	IALYQPISGCYIHFF	93.33%
9	9294854 Lassa NP	82-96	IAIYQPMSGCYIHFF	86.67%
10	9294856 Lassa NP	82-96	IALYQPMSGCYIHFF	93.33%
11	9294858 Lassa NP	82-96	IALYQPNSGCYIHFF	93.33%
12	9294860 Lassa NP	82-96	IALYQPSSGCYIHFF	100.00%
13	9294862 Lassa NP	82-96	IALYQPSSGCYIHFF	100.00%

Epitope Cluster Analysis Tool

- Analyzes how many epitopes in a set have significant sequence homology
- Groups epitopes into clusters based on having sequence identity greater than a specified threshold
- Three different clustering approaches are implemented
- Enables diverse applications such as generating epitope pools, and understanding cross-reactivity

Clustering approaches

1. All connected peptides in a cluster

- All peptides homologous to specified threshold are clustered together (for example, 70%)
- Drawback: members of the cluster might be related by levels of homology lower than threshold (for example, 70%)

2. Fully interconnected clusters (cliques)

- All peptides in a cluster share homology higher than the given threshold
- Drawback: One peptide can be a part of multiple cliques

3. Cluster-break method (recommended method)

- An extension of first approach
- A cluster is broken down into subclusters based on consensus sequence computations

Here is a set of sequences...as an example

TRAPPER

CLAPPER

SNAPPER

RAPPERTIME

DAYTIME

ANYTIME

PERTIME

TIMELESS

TIMER

TWICE

NICEDAY

ICE

CEDAR

HAPPYDAYS

HIPPY

PAYDAY

CALAMARI

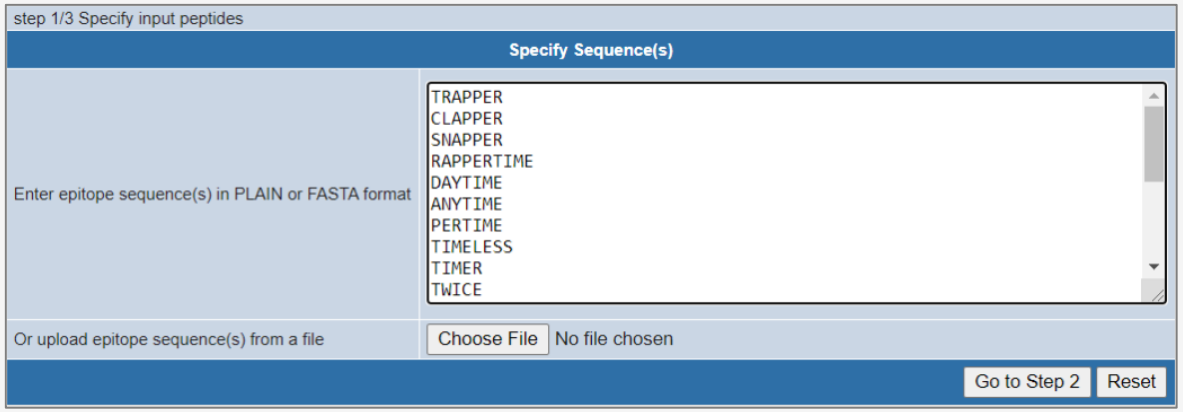
AMA

ISQAVHAAHAEINEAGR

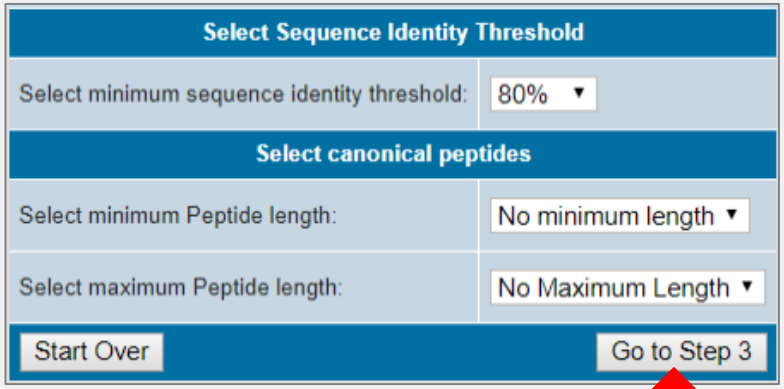
Epitope Clustering – example

http://tools.iedb.org/cluster/

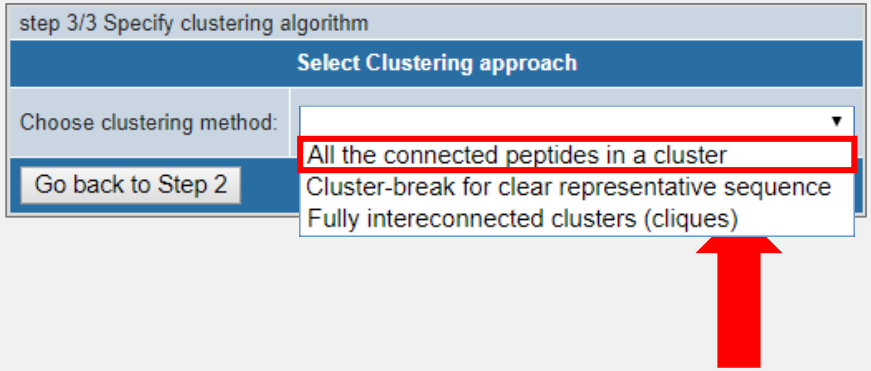
Step 1:
Enter epitope sequences



Step 2:
Specify parameters



Step 3:
Select algorithm



Epitope Clustering – example

Show Table | Graphical Visualization

Cluster Number	Peptide Number	Alignment	Position	Description	Peptide
1	Consensus	TRAPPERTIMEXESS	-	-	-
1	1	TRAPPER--------	1	seq1	TRAPPER
1	2	-RAPPERTIME----	2	seq4	RAPPERTIME
1	3	----DAYTIME----	5	seq5	DAYTIME
1	4	----ANYTIME----	5	seq6	ANYTIME
1	5	----PERTIME----	5	seq7	PERTIME
1	6	-------TIMELESS	8	seq8	TIMELESS
1	7	-------TIMER---	8	seq9	TIMER
2	Consensus	TXICEDAX	-	-	-
2	1	TWICE---	1	seq10	TWICE
2	2	-NICEDAY	2	seq11	NICEDAY
2	3	--ICE---	3	seq12	ICE
2	4	---CEDAR	4	seq13	CEDAR
3	Consensus	HXPPYDAYS	-	-	-
3	1	HAPPYDAYS	1	seq14	HAPPYDAYS
3	2	HIPPY----	1	seq15	HIPPY
3	3	--PAYDAY-	3	seq16	PAYDAY
4	Consensus	CALAMARI	-	-	-
4	1	CALAMARI	1	seq17	CALAMARI
4	2	---AMA--	4	seq18	AMA
5	Singleton	CLAPPER	-	seq2	CLAPPER
6	Singleton	SNAPPER	-	seq3	SNAPPER
7	Singleton	ISQAVHAAHAEINEAGR	-	seq19	ISQAVHAAHAEINEAGR

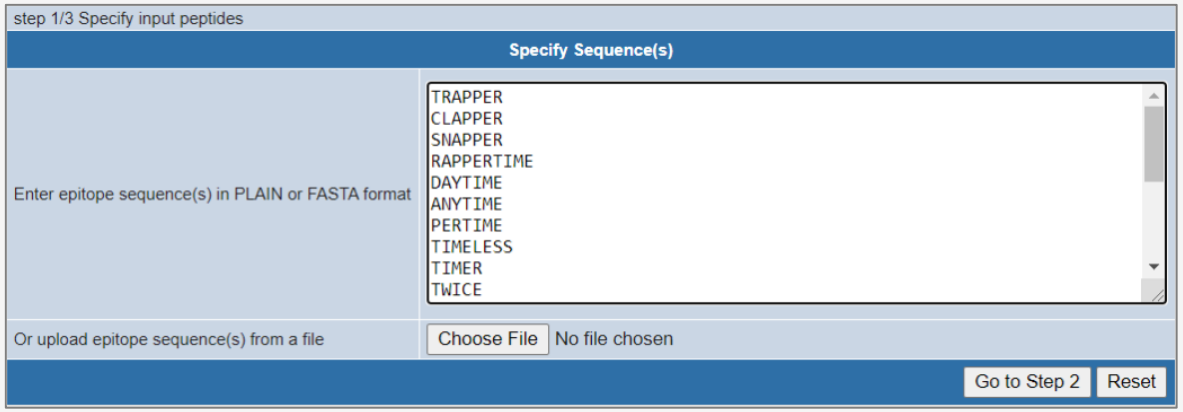
http://tools.iedb.org/cluster/

TRAPPER and TIMELESS do not have 80% homology but are in the same cluster because each have 80% homology to other members of the cluster

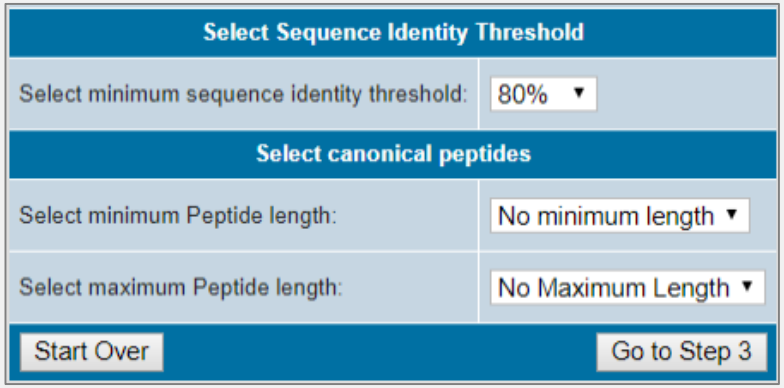
Epitope Clustering – example

http://tools.iedb.org/cluster/

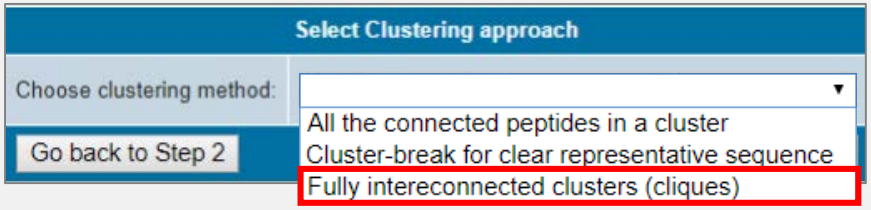
Step 1:
Enter epitope sequences



Step 2:
Specify parameters



Step 3:
Select algorithm



Epitope Clustering – example

Show Table | Graphical Visualization

Clique Number	Peptide Number	Alignment	Position	Description	Peptide
1	Consensus	PERTIMERAPPERTIME	-	-	-
1	1	PERTIME----------	1	seq7	PERTIME
1	2	---TIMER---------	4	seq9	TIMER
1	3	-------RAPPERTIME	8	seq4	RAPPERTIME
2	Consensus	TWICE	-	-	-
2	1	TWICE	1	seq10	TWICE
2	2	--ICE	3	seq12	ICE
3	Consensus	NICEDAY	-	-	-
3	1	NICEDAY	1	seq11	NICEDAY
3	2	-ICE---	2	seq12	ICE
4	Consensus	NICEDAX	-	-	-
4	1	NICEDAY	1	seq11	NICEDAY
4	2	--CEDAR	3	seq13	CEDAR
5	Consensus	CALAMARI	-	-	-
5	1	CALAMARI	1	seq17	CALAMARI
5	2	---AMA--	4	seq18	AMA
6	Consensus	ANYTIMER	-	-	-
6	1	ANYTIME-	1	seq6	ANYTIME
6	2	---TIMER	4	seq9	TIMER
7	Consensus	TIMEXESS	-	-	-
7	1	TIMELESS	1	seq8	TIMELESS
7	2	TIMER---	1	seq9	TIMER
8	Consensus	DAYTIMER	-	-	-
8	1	DAYTIME-	1	seq5	DAYTIME
8	2	---TIMER	4	seq9	TIMER
9	Consensus	HXPPYDAYS	-	-	-
9	1	HAPPYDAYS	1	seq14	HAPPYDAYS

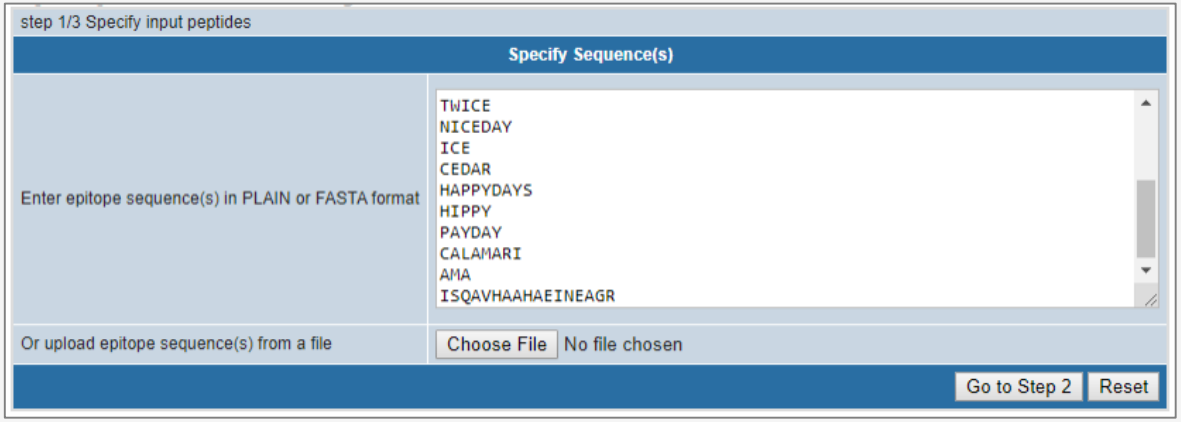
http://tools.iedb.org/cluster/

TIMER (and several others) appear in multiple clusters because they have the specified level of homology to the other members of the clique

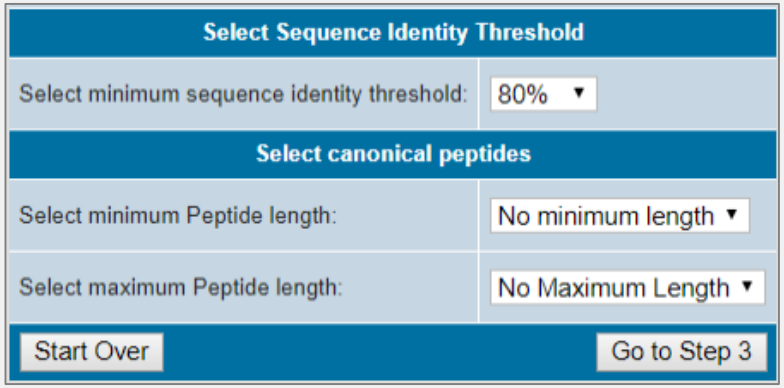
Cluster-break method is the recommended method

http://tools.iedb.org/cluster/

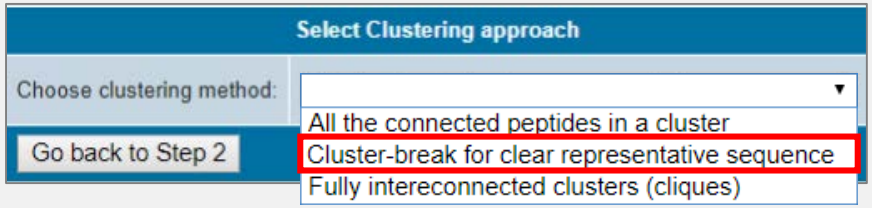
Step 1:
Enter epitope sequences



Step 2:
Specify parameters



Step 3:
Select algorithm

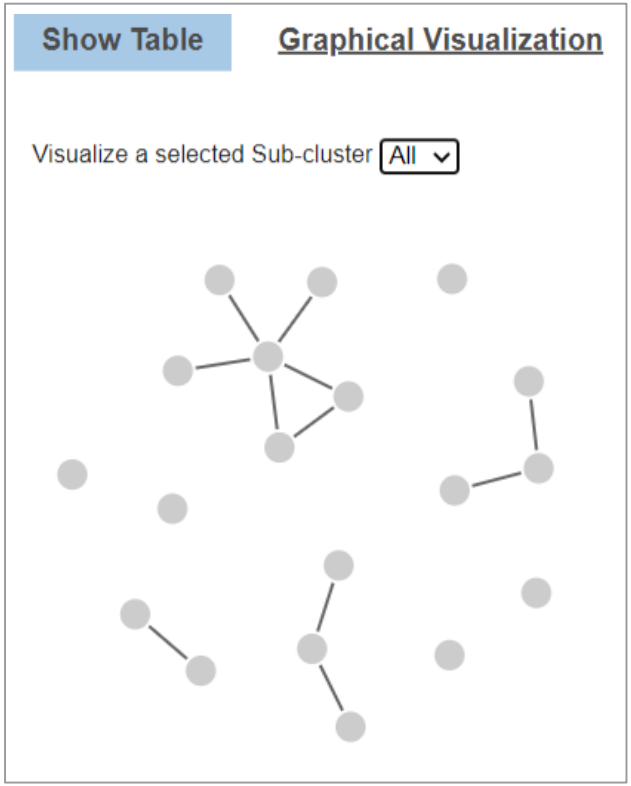


Epitope Clustering – example

Show Table | Graphical Visualization

Cluster.Sub-Cluster Number	Peptide Number	Alignment	Position	Description	Peptide
1.1	Consensus	RAPXXXTIMEXESS	-	-	-
1.1	1	RAPPERTIME----	1	seq4	RAPPERTIME
1.1	2	---ANYTIME----	4	seq6	ANYTIME
1.1	3	---DAYTIME----	4	seq5	DAYTIME
1.1	4	---PERTIME----	4	seq7	PERTIME
1.1	5	------TIMELESS	7	seq8	TIMELESS
1.1	6	------TIMER---	7	seq9	TIMER
1.2	Singleton	TRAPPER	-	seq1	TRAPPER
2.1	Consensus	NICEDAX	-	-	-
2.1	1	NICEDAY	1	seq11	NICEDAY
2.1	2	-ICE---	2	seq12	ICE
2.1	3	--CEDAR	3	seq13	CEDAR
2.2	Singleton	TWICE	-	seq10	TWICE
3.1	Consensus	HXPPYDAYS	-	-	-
3.1	1	HAPPYDAYS	1	seq14	HAPPYDAYS
3.1	2	HIPPY----	1	seq15	HIPPY
3.1	3	--PAYDAY-	3	seq16	PAYDAY
4.1	Consensus	CALAMARI	-	-	-
4.1	1	CALAMARI	1	seq17	CALAMARI
4.1	2	---AMA--	4	seq18	AMA
5.1	Singleton	CLAPPER	-	seq2	CLAPPER
6.1	Singleton	SNAPPER	-	seq3	SNAPPER
7.1	Singleton	ISQAVHAAHAEINEAGR	-	seq19	ISQAVHAAHAEINEAGR

http://tools.iedb.org/cluster/

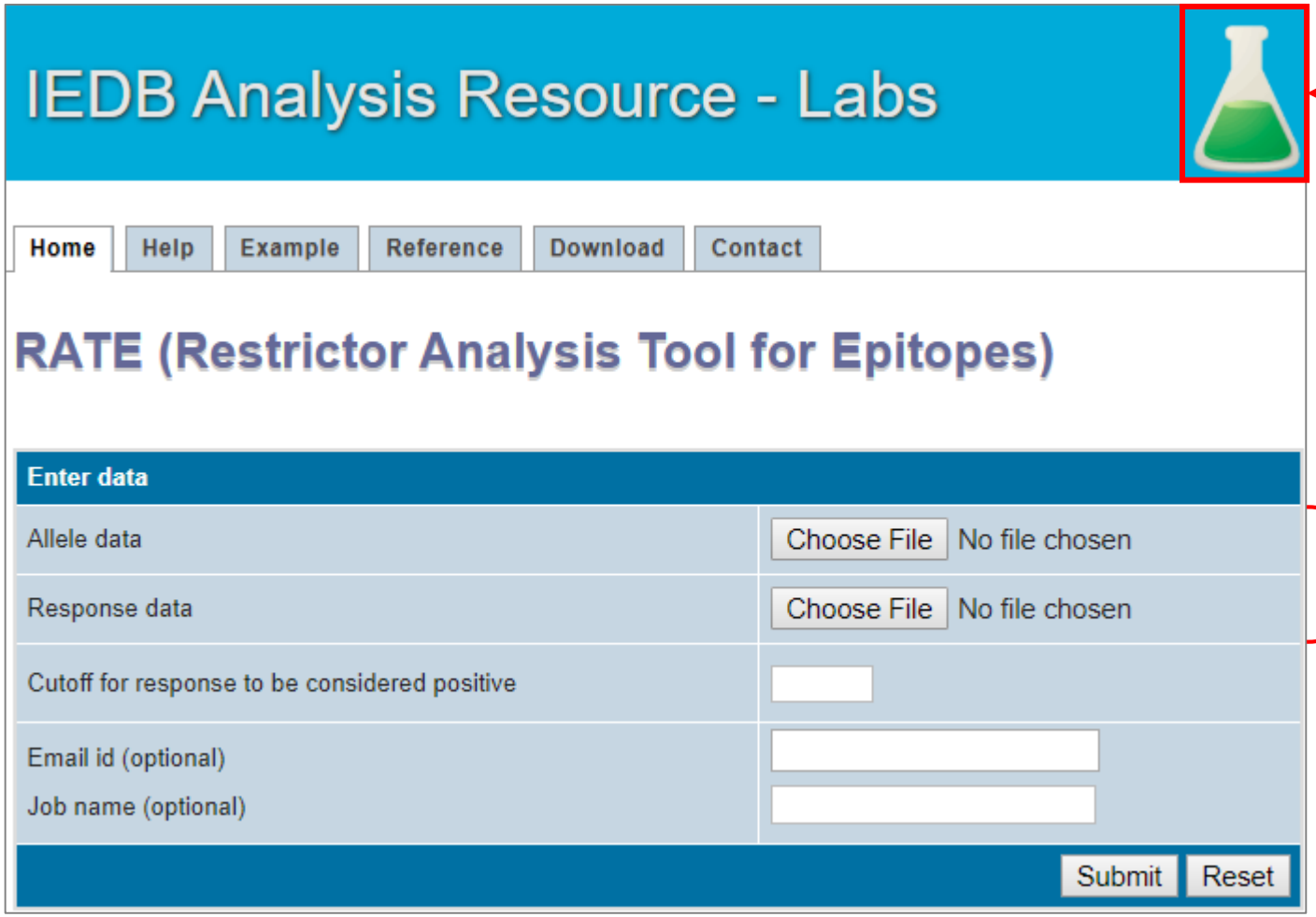


Restrictor Analysis Tool for Epitopes (RATE)

- Automated method to infer HLA restriction of a given epitope set, from immune response data of HLA typed subjects
- The method is based on computing the frequency of all alleles expressed in the population studied in donors who had an immune response to each given epitope
- Compare those frequencies in donors that did not have a response to the same epitope

RATE

http://tools.iedb.org/rate/



Labs designation

Two data input files need to be provided

RATE – Input: Allele data

- Data format = tab separated in plain text file
- Sample data shown here – 12 class II alleles of 6 loci for each donor are listed in separate columns

iedb-rate_sample_input_allele_data - Notepad

File Edit Format View Help

Donor-1	Donor-2	Donor-3	Donor-4	Donor-5	Donor-6	Donor-7	Donor-8	Donor-9	Donor-10	Donor-11	Donor-12	Donor-13	
DRB1*03:01		DRB1*11:01		DRB1*01:01		DRB1*07:01		DRB1*12:01		DRB1*11:02	DRB1*08:04	DRB1*11:01	DRB1*
DRB1*07:01		DRB1*15:03		DRB1*07:01		DRB1*15:03		DRB1*15:03		DRB1*13:02	DRB1*15:02	DRB1*13:02	DRB1*
DRB3*02:02		DRB3*02:02		DRB3*02:02		DRB4*01:03		DRB3*01:01		DRB3*02:02	DRB5*01:02	DRB3*02:02	DRB3*
DRB4*01:03		DRB5*01:01		DRB4*01:03		DRB5*01:01		DRB5*01:01		DRB3*03:01	n/a	DRB3*03:01	DRB3*02:02
DQA1*02:01		DQA1*01:02		DQA1*02:01		DQA1*01:02		DQA1*01:01		DQA1*01:02	DQA1*01:03	DQA1*01:02	DQA1*
DQA1*05:01		DQA1*05:05		DQA1*05:01		DQA1*02:01		DQA1*01:02		DQA1*05:05	DQA1*04:01	DQA1*05:05	DQA1*
DQB1*02:01		DQB1*06:02		DQB1*03:02		DQB1*02:02		DQB1*05:01		DQB1*03:19	DQB1*03:19	DQB1*06:09	DQB1*
DQB1*05:03		DQB1*06:02		DQB1*03:02		DQB1*06:02		DQB1*06:02		DQB1*06:09	DQB1*06:01	DQB1*06:09	DQB1*
DPA1*01:03		DPA1*01:03		DPA1*01:03		DPA1*01:03		DPA1*01:03		DPA1*01:03	DPA1*02:01	DPA1*01:03	DPA1*
DPA1*02:01		DPA1*03:01		DPA1*02:01		DPA1*02:01		DPA1*03:01		DPA1*01:03	DPA1*02:02	DPA1*01:03	DPA1*
DPB1*13:01		DPB1*03:01		DPB1*17:01		DPB1*01:01		DPB1*18:01		DPB1*34:01	DPB1*01:01	DPB1*02:01	DPB1*
DPB1*17:01		DPB1*105:01		DPB1*01:01		DPB1*02:01		DPB1*105:01		DPB1*02:01	DPB1*13:01	DPB1*105:01	DPB1*

RATE – Input: Response data

- Data format = tab separated in plain text file
- The response data (here SFC values) for each epitope in each of the donors are provided

iedb-rate_sample_input_response_data - Notepad

File Edit Format View Help

Peptide #	Peptide_ID	Peptide_Seq	Donor-1	Donor-2	Donor-3	Donor-4	Donor-5	Donor-6	Donor-7	Donc
1	3531.0365	GINTIPIAINEAEYV	98	0	0	0	0	0	40	0
2	3531.0367	AAFQAAHARFVAAAA	68	0	50	0	0	0	0	0
3	3531.0514	AAVVRFQEAANKQKQ	0	55	0	0	0	0	0	0
4	3531.0494	ELFVAAYVPYVAWLV	0	0	0	0	n/a	n/a	0	0
5	3531.037	AAGTYVAADAAAAST	0	0	83	0	0	0	0	0

RATE – how it works

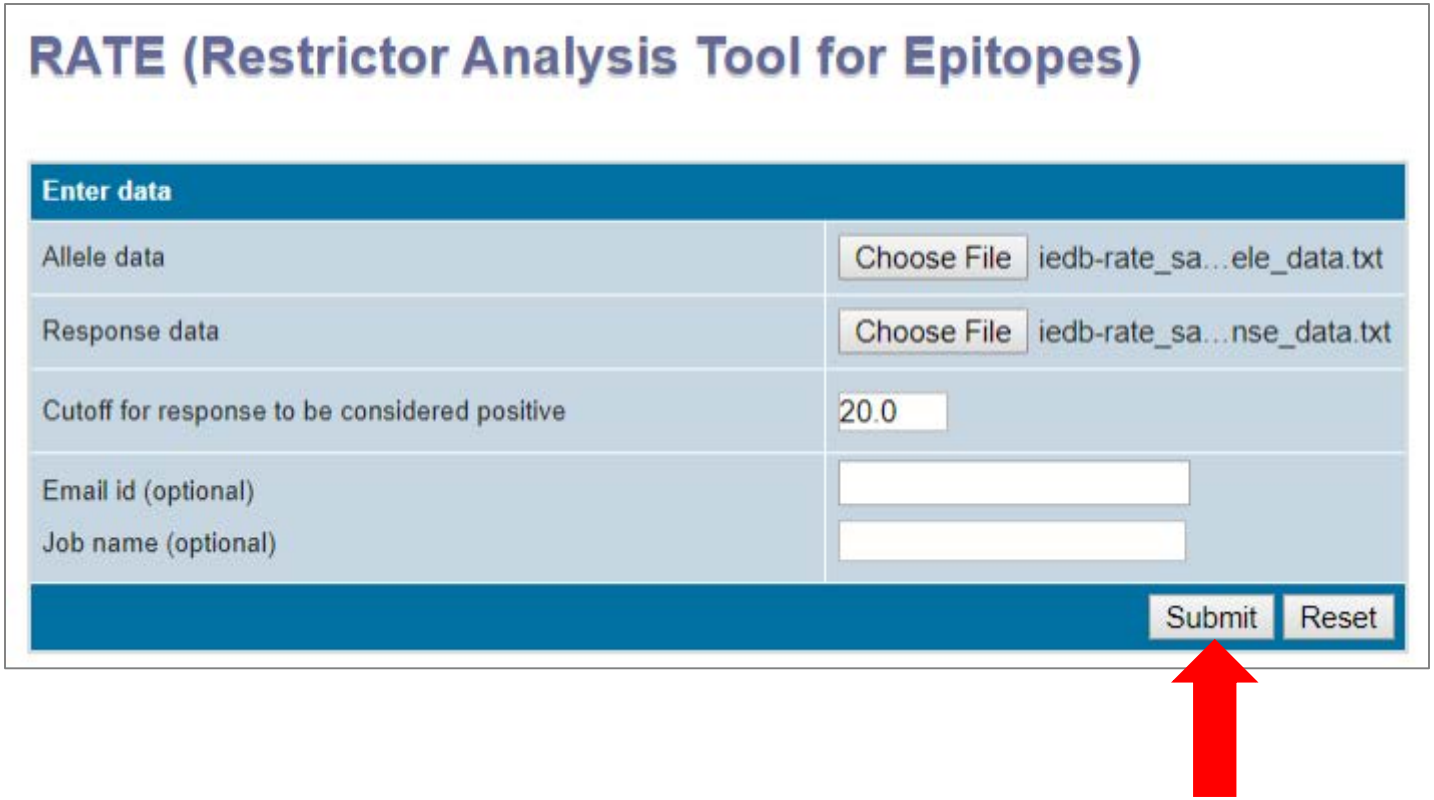
- Restrictions are determined based on
  - "Odds Ratio" and significance estimated using Fisher's exact test.

$$OR = \frac{(A^{+}R^{+}) \times (A^{-}R^{-})}{(A^{-}R^{+}) \times (A^{+}R^{-})}$$

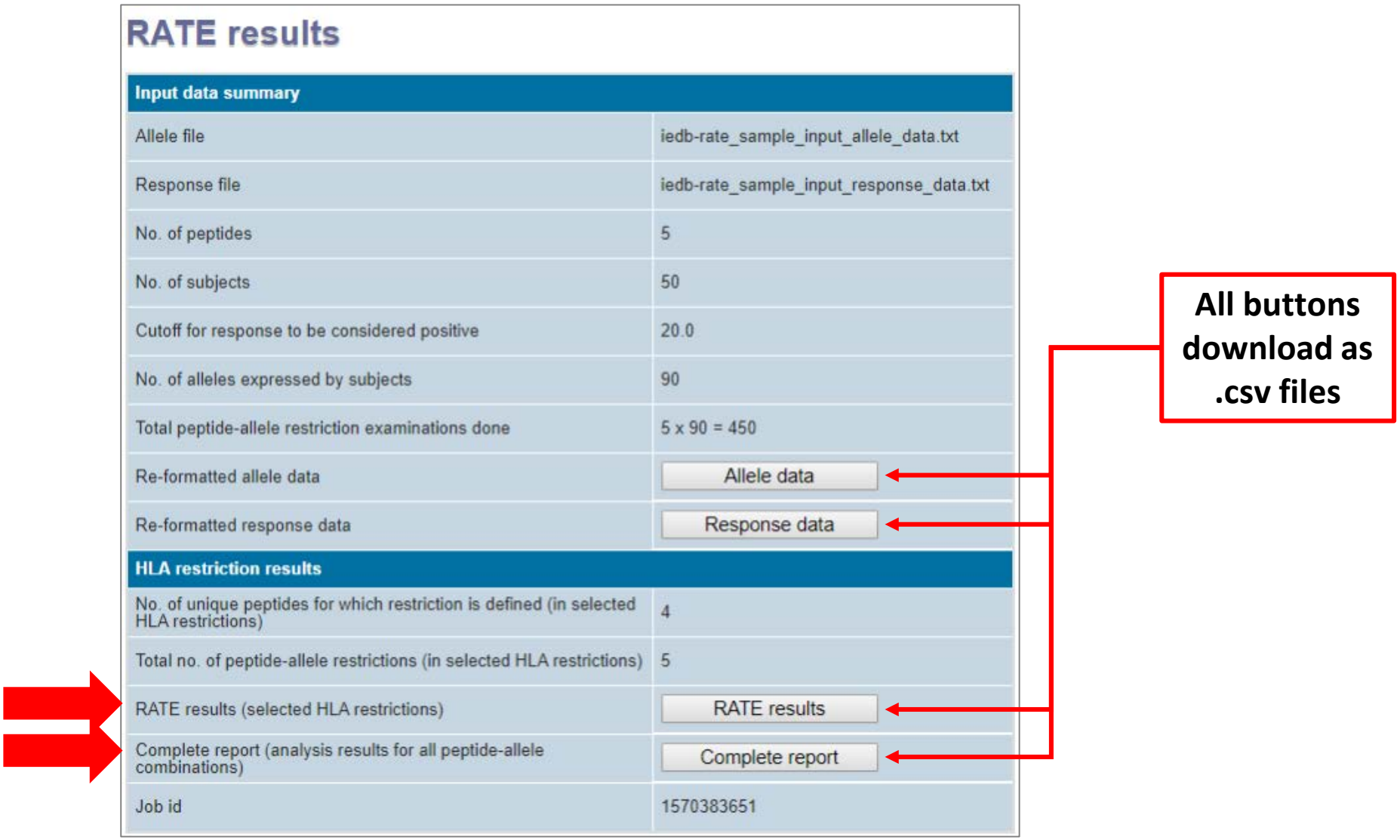
  - "Relative Frequency", a parameter estimated by the tool based on the frequency of alleles and donors responded

$$RF = \frac{A^{+}R^{+} / (A^{+}R^{+} + A^{+}R^{-})}{(A^{+}R^{+} + A^{-}R^{+}) / \text{Total donors}}$$

RATE – example



RATE – example



RATE results

Input data summary	
Allele file	iedb-rate_sample_input_allele_data.txt
Response file	iedb-rate_sample_input_response_data.txt
No. of peptides	5
No. of subjects	50
Cutoff for response to be considered positive	20.0
No. of alleles expressed by subjects	90
Total peptide-allele restriction examinations done	5 x 90 = 450
Re-formatted allele data	Allele data
Re-formatted response data	Response data
HLA restriction results	
No. of unique peptides for which restriction is defined (in selected HLA restrictions)	4
Total no. of peptide-allele restrictions (in selected HLA restrictions)	5
RATE results (selected HLA restrictions)	RATE results
Complete report (analysis results for all peptide-allele combinations)	Complete report
Job id	1570383651

RATE – results (complete report)

- All epitopes

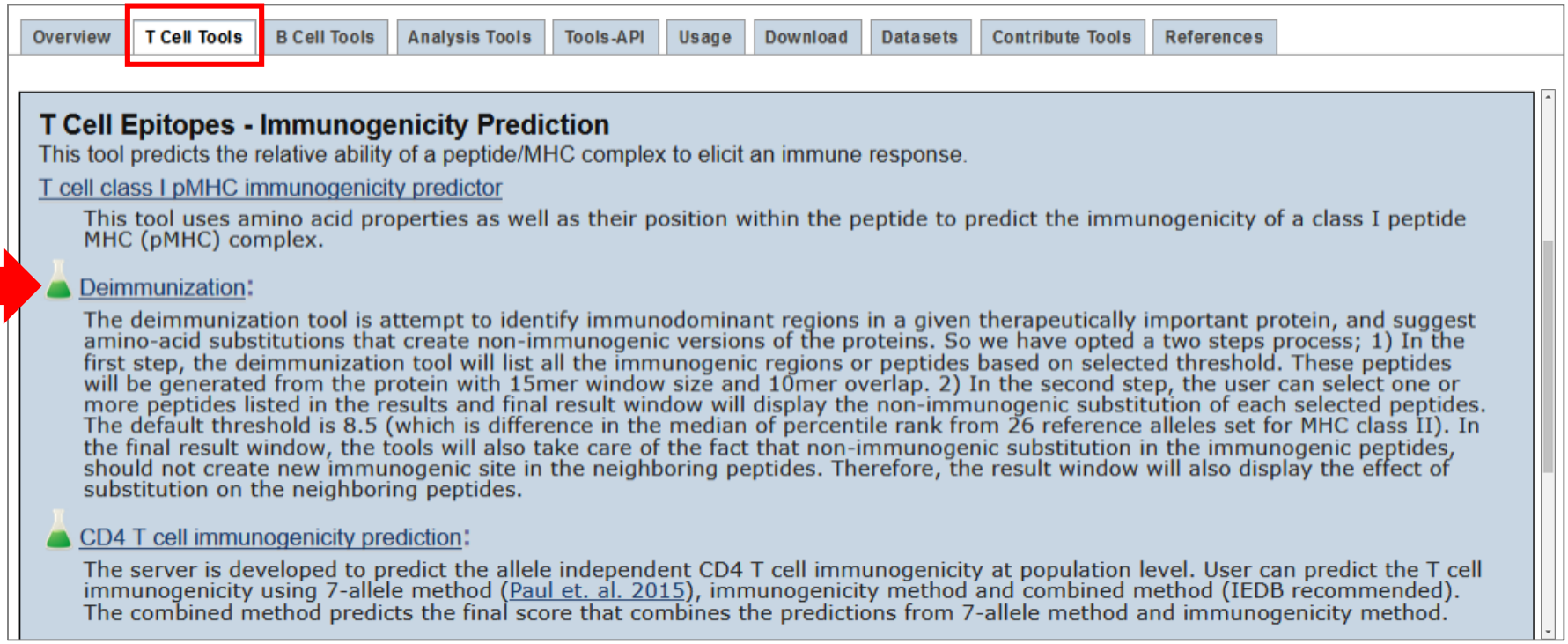
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Peptide#	Peptide_id	peptide_seq	Allele#	Allele	A+R+	A-R+	A+R-	A-R-	No_of_Donors	Response_n/a	Relative_freq	Odds_ratio	P-value
2	1	3531.037	GINTIPIAINEAEYV	1	DPA1*01:03	5	1	34	10	50	0	1.06838	1.47059	1
3	2	3531.037	AAFQAAHARFVAAAA	1	DPA1*01:03	10	2	29	9	50	0	1.06838	1.55172	1
4	3	3531.051	AAVVRFQEAANKQKQ	1	DPA1*01:03	8	1	28	10	47	3	1.16049	2.85714	0.6631
5	4	3531.049	ELFVAAYVPYVAWLV	1	DPA1*01:03	1	0	36	11	48	2	1.2973	inf	1
6	5	3531.037	AAGTYVAADAAAAST	1	DPA1*01:03	5	1	34	10	50	0	1.06838	1.47059	1
7	1	3531.037	GINTIPIAINEAEYV	2	DPA1*01:04	0	6	1	43	50	0	0	0	1
8	2	3531.037	AAFQAAHARFVAAAA	2	DPA1*01:04	0	12	1	37	50	0	0	0	1
9	3	3531.051	AAVVRFQEAANKQKQ	2	DPA1*01:04	0	9	1	37	47	3	0	0	1
10	4	3531.049	ELFVAAYVPYVAWLV	2	DPA1*01:04	0	1	1	46	48	2	0	0	1
11	5	3531.037	AAGTYVAADAAAAST	2	DPA1*01:04	0	6	1	43	50	0	0	0	1
12	1	3531.037	GINTIPIAINEAEYV	3	DPA1*02:01	3	3	20	24	50	0	1.08696	1.2	1
13	2	3531.037	AAFQAAHARFVAAAA	3	DPA1*02:01	5	7	18	20	50	0	0.9058	0.79365	1
14	3	3531.051	AAVVRFQEAANKQKQ	3	DPA1*02:01	3	6	18	20	47	3	0.74603	0.55556	0.7111
15	4	3531.049	ELFVAAYVPYVAWLV	3	DPA1*02:01	0	1	23	24	48	2	0	0	1
16	5	3531.037	AAGTYVAADAAAAST	3	DPA1*02:01	2	4	21	23	50	0	0.72464	0.54762	0.674
17	1	3531.037	GINTIPIAINEAEYV	4	DPA1*02:02	1	5	10	34	50	0	0.75758	0.68	1
18	2	3531.037	AAFQAAHARFVAAAA	4	DPA1*02:02	2	10	9	29	50	0	0.75758	0.64444	1
19	3	3531.051	AAVVRFQEAANKQKQ	4	DPA1*02:02	1	8	10	28	47	3	0.47475	0.35	0.6631
20	4	3531.049	ELFVAAYVPYVAWLV	4	DPA1*02:02	0	1	11	36	48	2	0	0	1
21	5	3531.037	AAGTYVAADAAAAST	4	DPA1*02:02	1	5	10	34	50	0	0.75758	0.68	1
22	1	3531.037	GINTIPIAINEAEYV	5	DPA1*03:01	0	6	9	35	50	0	0	0	0.5756
23	2	3531.037	AAFQAAHARFVAAAA	5	DPA1*03:01	2	10	7	31	50	0	0.92593	0.88571	1
24	3	3531.051	AAVVRFQEAANKQKQ	5	DPA1*03:01	2	7	6	32	47	3	1.30556	1.52381	0.6388
25	4	3531.049	ELFVAAYVPYVAWLV	5	DPA1*03:01	0	1	8	39	48	2	0	0	1
26	5	3531.037	AAGTYVAADAAAAST	5	DPA1*03:01	1	5	8	36	50	0	0.92593	0.9	1
27	1	3531.037	GINTIPIAINEAEYV	6	DPA1*04:01	1	5	0	44	50	0	8.33333	inf	0.12
28	2	3531.037	AAFQAAHARFVAAAA	6	DPA1*04:01	0	12	1	37	50	0	0	0	1
29	3	3531.051	AAVVRFQEAANKQKQ	6	DPA1*04:01	0	9	1	37	47	3	0	0	1
30	4	3531.049	ELFVAAYVPYVAWLV	6	DPA1*04:01	0	1	1	46	48	2	0	0	1

RATE – results (selected HLA restrictions)

- Epitope-allele combinations with RF ≥ 1.3 and p-value < 0.01 are reported (not Bonferroni corrected)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	**Peptide#**	**Peptide_id**	**peptide_seq**	**Allele#**	**Allele**	**A+R+**	**A-R+**	**A+R-**	**A-R-**	**No_of_Donors**	**Response_n/a**	**Relative_freq**	**Odds_ratio**	**P-value**
2	1	3531.0365	GINTIPIAINEAEYV	84	DRB3*02:02	6	0	18	26	50	0	2.08333	inf	0.00847
3	2	3531.0367	AAFQAAHARFVAAAA	54	DRB1*01:01	4	8	0	38	50	0	4.16667	inf	0.00215
4	2	3531.0367	AAFQAAHARFVAAAA	65	DRB1*07:01	5	7	2	36	50	0	2.97619	12.85714	0.00593
5	3	3531.0514	AAVVRFQEAANKQKQ	89	DRB5*01:01	7	2	8	30	47	3	2.43704	13.125	0.0025
6	5	3531.037	AAGTYVAADAAAAST	54	DRB1*01:01	4	2	0	44	50	0	8.33333	inf	7.00E-05

Deimmunization



Deimmunization – background

- Wild type and engineered proteins are widely used as drugs
- Immunogenicity of protein drugs is associated with serious potency and safety issues
- A potential approach to reducing immunogenicity is based on removal of T cell epitopes (de-immunization)

Deimmunization – approach

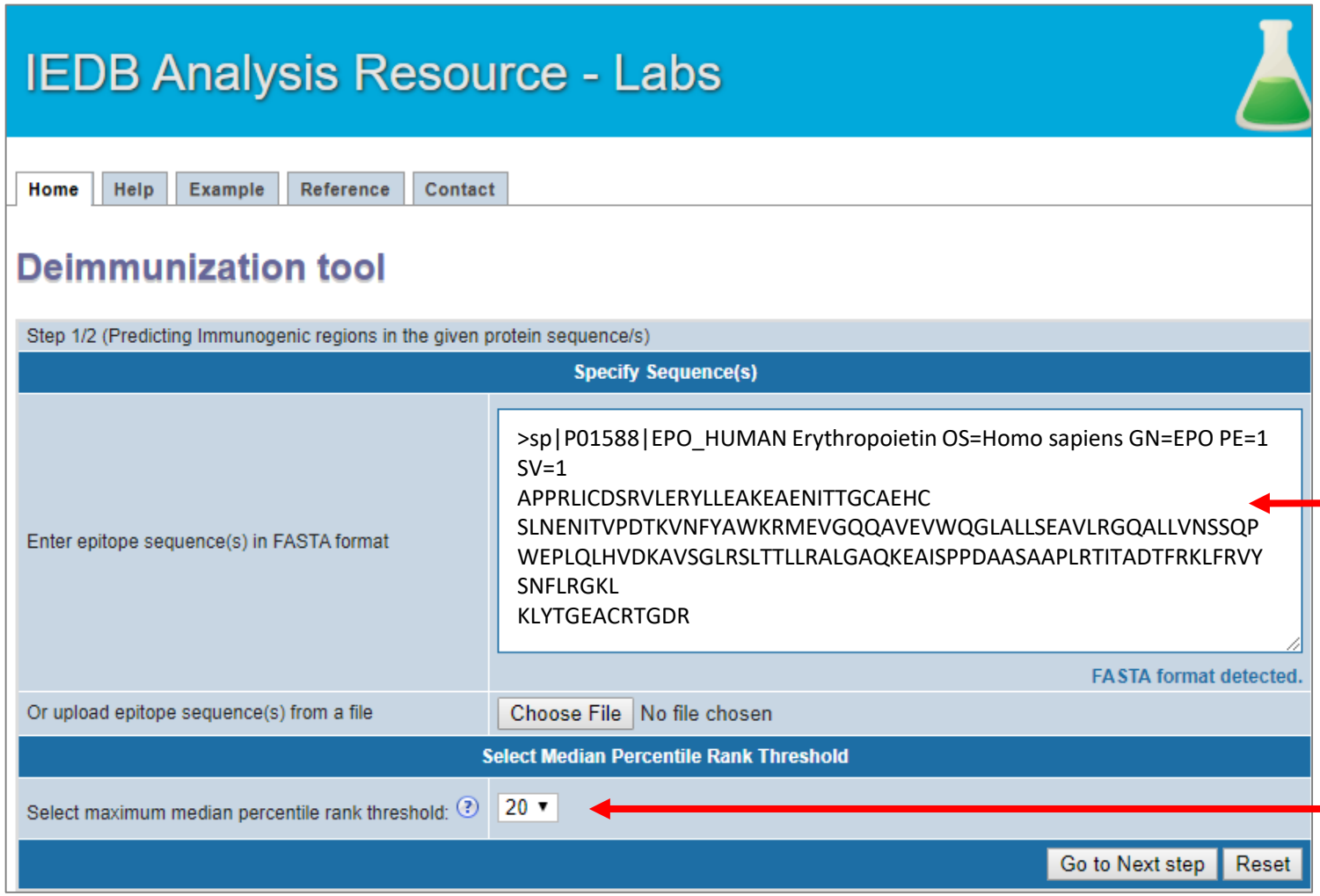
- Generate overlapping peptides from protein sequence
- Predict HLA class II binding regions

APPRLICDSRVLERY	1-15	38.89
ICDSRVLERYLLEAK	6-20	60.39
VLERYLLEAKEAENI	11-25	23.46
LLEAKEAENITTGCA	16-30	21.76
. . .		
DTFRKLFRVYSNFLR	**136-150**	**6.08**

- Suggest amino acid substitutions that are predicted to decrease binding
  - Also consider the effect of substitutions on neighboring peptides

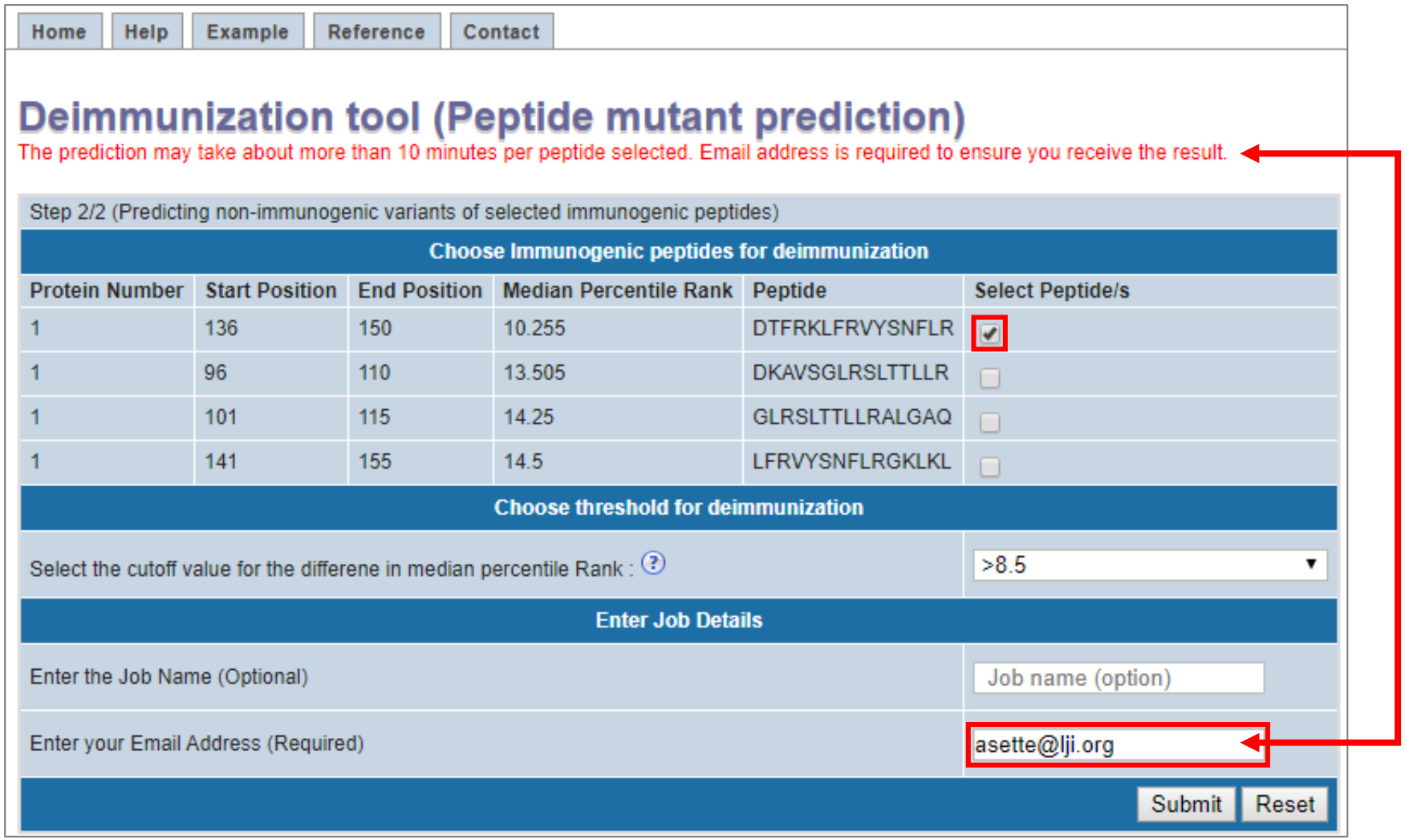
Deimmunization – example

http://tools.iedb.org/deimmunization/



Deimmunization – example

http://tools.iedb.org/deimmunization/



Deimmunization Score

Immunogenicity for Neighboring peptide (1)	Immunogenicity for Neighboring peptide (2)	Score
Absent	Absent	1
Reduced	Absent	2
Reduced	Reduced	3
Neutral	Absent	4
Reduced	Neutral	5
Neutral	Neutral	6
Increased	Absent	7
Reduced	Increased	8
Increased	Neutral	9
Increased	Increased	10

Deimmunization – example

	A	B	C	D	E	F	G	H	I	J	K	L
1	Protein Number	Peptide	Peptide ID	Start Position	End Position	Median Percentile Rank	Median Difference	C terminal Neighbor 1 (Median)	C terminal Neighbor 2 (Median)	N terminal Neighbor 1 (Median)	N terminal Neighbor 2 (Median)	Deimmunization Score
2	1	DTFRKLFRVYSNFLR	wild	136	150	10.255	0	14.5	49.75	47	44.25	NA
3	1	DTFRKLFRVYSNFDR	L149D	136	150	32	21.745	41	68.5	NA	NA	3
4	1	DTFRKLFRVYSNFGR	L149G	136	150	27.5	17.245	31.5	68.75	NA	NA	3
5	1	DTFRKEFRVYSNFLR	L141E	136	150	26.5	16.245	26.5	NA	60.75	NA	3
6	1	DTFRKPFRVYSNFLR	L141P	136	150	26.35	16.095	26.5	NA	52.5	NA	3
7	1	DTFRKQFRVYSNFLR	L141Q	136	150	26	15.745	25.5	NA	53.25	NA	3
8	1	DTFRKLFRVYSNFNR	L149N	136	150	26	15.745	21.75	61.75	NA	NA	3
9	1	DTFRKLGRVYSNFLR	F142G	136	150	25.075	14.82	31	NA	50.75	NA	3
10	1	DTFRKLFDVYSNFLR	R143D	136	150	24.75	14.495	35.5	NA	49.25	NA	3
11	1	DTFRKKFRVYSNFLR	L141K	136	150	24.525	14.27	23	NA	59	NA	3
12	1	DTFRKLFRVYSNFCR	L149C	136	150	24.25	13.995	36	64	NA	NA	3
13	1	DTFRKCFRVYSNFLR	L141C	136	150	23.85	13.595	23.5	NA	73	NA	3
14	1	DTFRKLFRVYSCFLR	N147C	136	150	23.75	13.495	39.75	50	NA	NA	3
15	1	DTFRKDFRVYSNFLR	L141D	136	150	23.5	13.245	27.5	NA	62.5	NA	3
16	1	DTFRKGFRVYSNFLR	L141G	136	150	23.5	13.245	27	NA	57.5	NA	3
17	1	DTFRKLFRVYSNFKR	L149K	136	150	23.5	13.245	28	59	NA	NA	3
18	1	DTFRKLFRVYSNFER	L149E	136	150	22.75	12.495	34	62	NA	NA	3
19	1	DTFRKLERVYSNFLR	F142E	136	150	22.7	12.445	31	NA	48	NA	3
20	1	DTFRKLCRVYSNFLR	F142C	136	150	22.4	12.145	31	NA	50.25	NA	3
21	1	DTFRKLTRVYSNFLR	F142T	136	150	22.175	11.92	30	NA	48.75	NA	3

Analysis Tools Recap

- Help to examine existing sets of epitopes and gain new knowledge across a broad array of applications

Population Coverage	Analyze T cell epitopes with known HLA restriction that are recognized in a population based on HLA frequencies
Conservancy	Investigate epitope conservancy across different protein sequences.
Cluster	Cluster epitopes on the basis of homology
RATE (Restrictor Analysis)	Infer HLA restrictions for epitopes of T cell response frequency in HLA typed subjects
Deimmunization	Identify immunodominant regions in a given protein, and suggest amino-acid substitutions that create non-immunogenic versions of the protein.