Vaxign Reverse Vaccinology Software Demo Introduction

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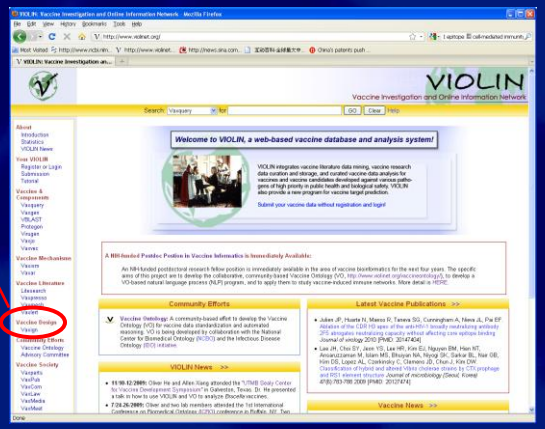
Vaxign is a program in VIOLIN

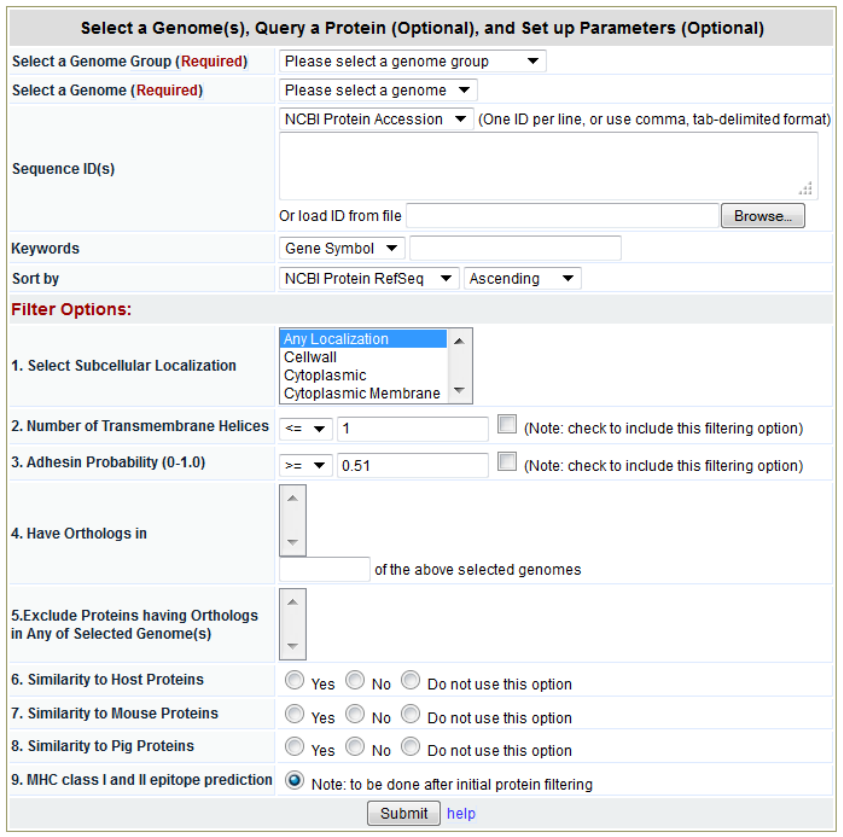
- VIOLIN: Vaccine Investigation and Online Information Network
- A vaccine research database and vaccine data analysis system
- Key tasks:
  - Build a database for vaccines and vaccine candidates in use, clinical trials, and research
  - Vaccine literature mining and curation
  - Vaccine design → **Vaxign**
  - Development and applications of community-based Vaccine Ontology (VO)



Publically available:
http://www.violinet.org/

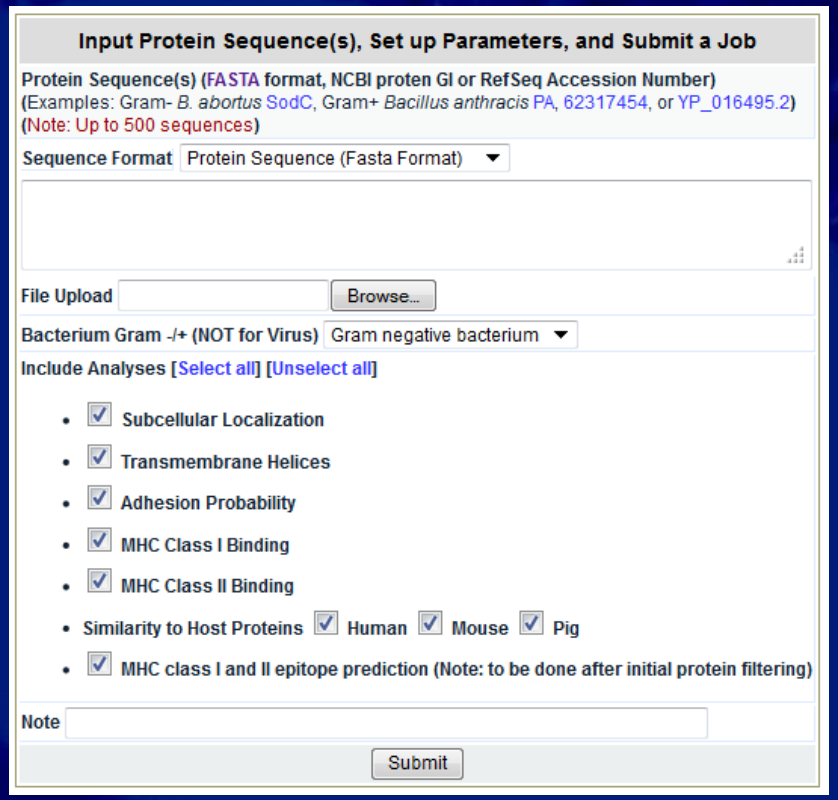
Two Forms of Vaxign Usage

Pre-computed data query



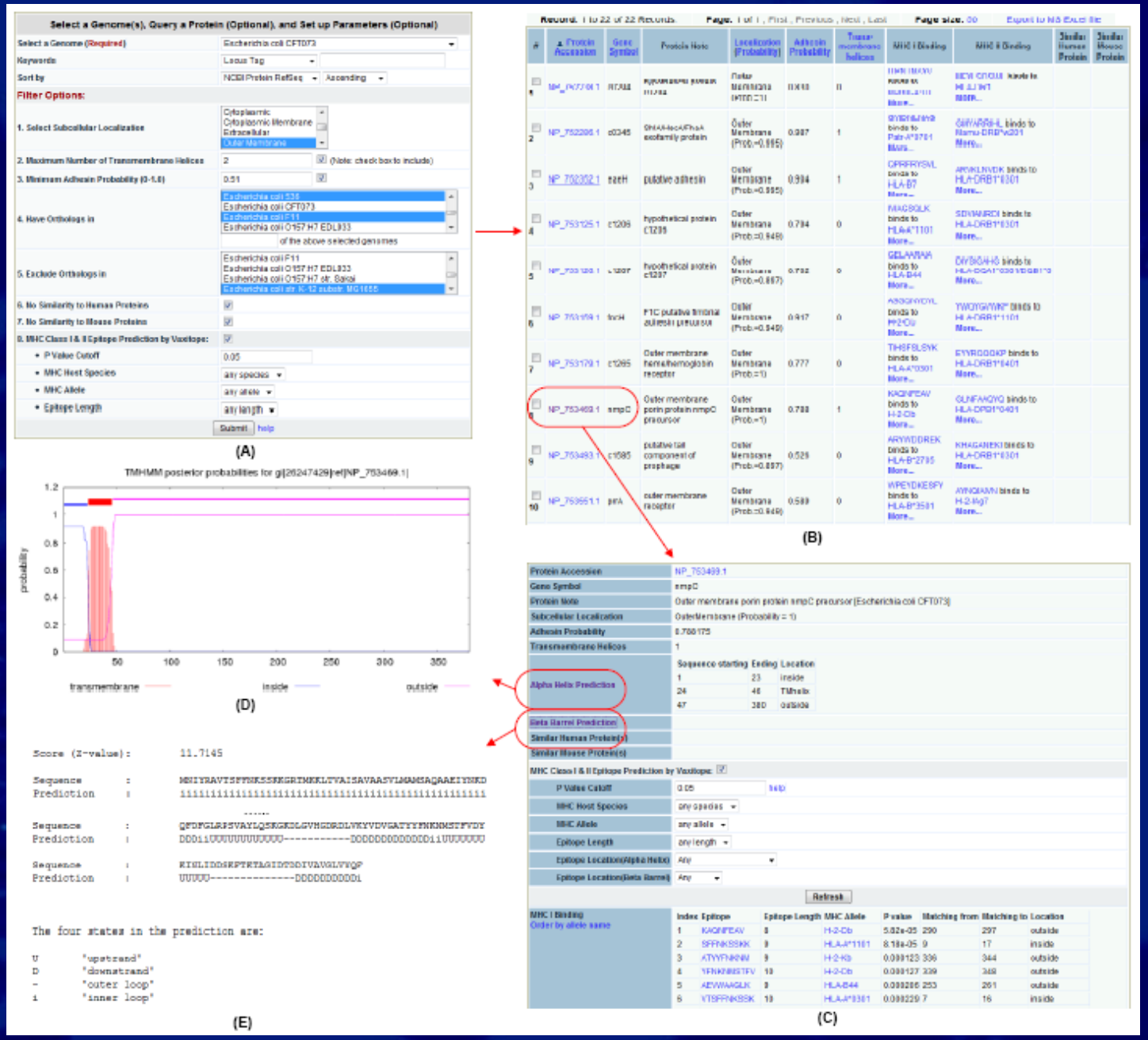
- Pre-computed results
- > 200 genomes in database
- Easy to query

Dynamic analysis



- Runtime execution
- Can analyze up to 500 proteins at one time

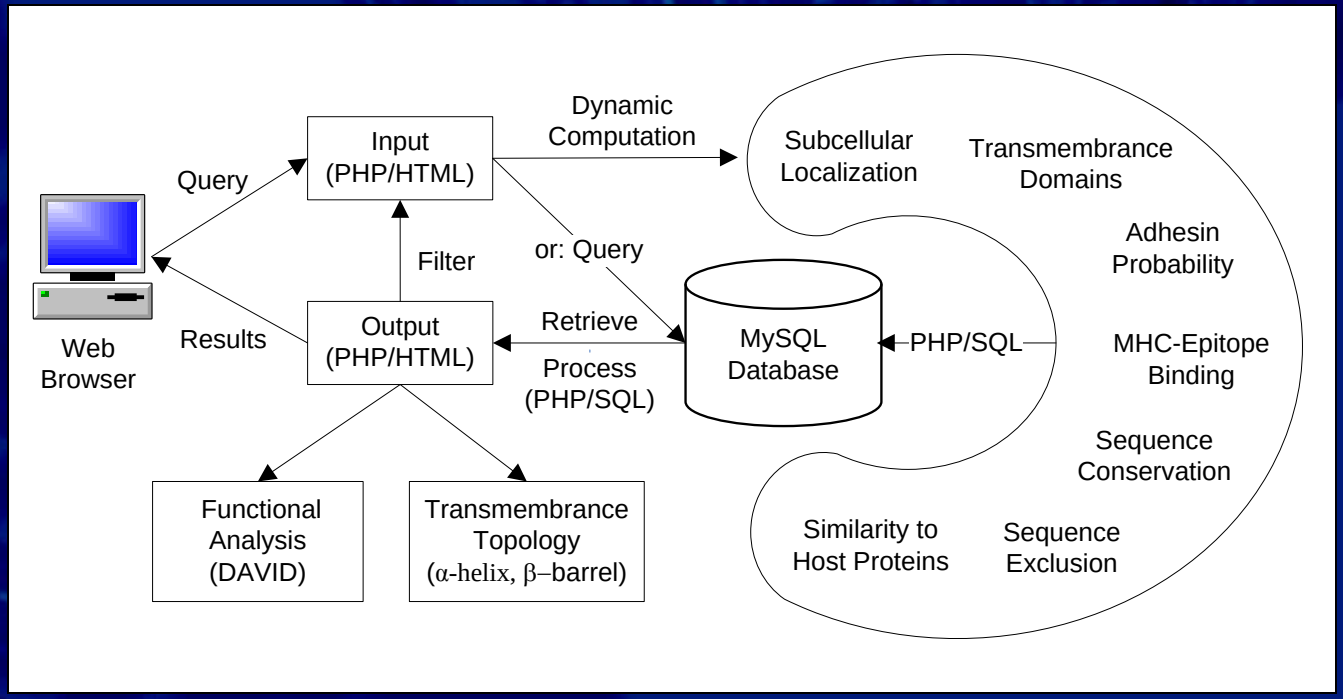
Vaxign analysis pipeline demo



Vaxign
VACCINE DESIGN

Vaxign: Vaccine Design System

- Aim: Vaccine target prediction for reverse vaccinology



- The 1st web-based reverse vaccinology system
- Freely available: http://www.violinet.org/vaxign

Reference: He Y, Xiang Z, Mobley HLT. Vaxign: the first web-based vaccine design program for reverse vaccinology and an application for vaccine development. *Journal of Biomedicine and Biotechnology*. Volume 2010 (2010), Article ID 297505, 15 pages. [PMID: 20671958]

Vaxign: how to predict subcellular localization?

- Using customized PSORTb 3.0:
  - Source: http://www.psort.org/psortb/
  - Has overall precision (specificity) of 96%
  - GNU General Public Licence (GNU GPL)

- Vaxign customizations:
  - Automatically generate PSORTb input
  - Parse PSORTb output and store in Vaxign relational database

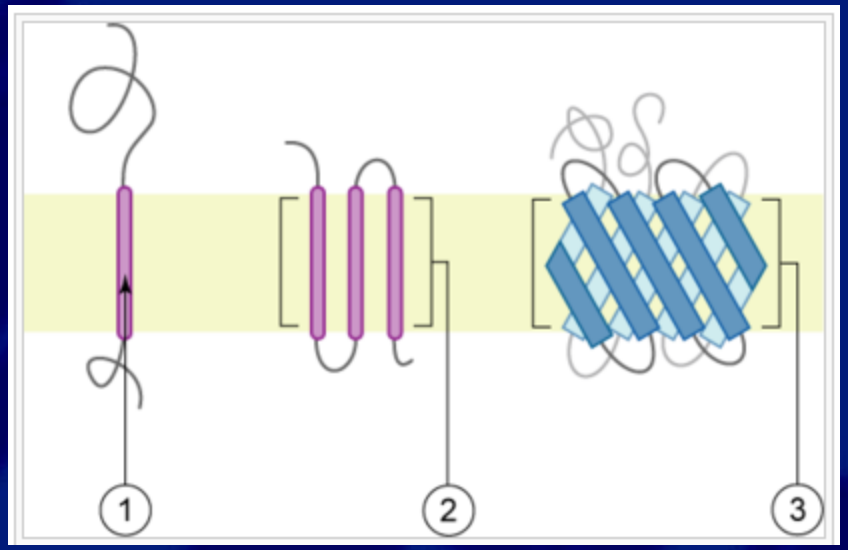
Vaxign: how to predict transmembrane helices?

- Using customized TMHMM:
  - Source: http://www.cbs.dtu.dk/services/TMHMM-2.0/
  - based on a hidden Markov model
  - Academic Software License Agreement

- Vaxign customizations:
  - Automatically generate TMHMM input
  - Parse TMHMM output and store in Vaxign relational database

Vaxign: how to predict β-barrel?

- Using customized PROFtmb:
  - http://www.ncbi.nlm.nih.gov/pubmed/16844988
  - Four-state (up-, down-strand, inner loop, outer loop) accuracy as high as 86%
  - GNU General Public License



http://en.wikipedia.org/wiki/Transmembrane_protein

Vaxign: how to predict adhesin probability?

- Using customized SPAAN:
  - Adhesin: cell-surface components that facilitate bacterial adhesion or adherence to other cells.
  - Source: http://www.ncbi.nlm.nih.gov/pubmed/15374866
  - Has optimal sensitivity of 89% and specificity of 100% on a defined test set
  - Default value is 0.51
  - Freely available

Vaxign: how to predict sequence conservation?

- Using customized BLAST-based OrthoMCL:
  - Source: http://www.orthomcl.org/
  - E-value of 10^{-5} is set as cutoff
  - GNU General Public License

- Alternative internal BLAST method: based on reciprocal best fit principle.

Vaxign: how to do gene functional analysis?

- Link to DAVID:
  - Source: http://david.abcc.ncifcrf.gov/
  - What Vaxign does: parse data in DAVID format and send to DAVID website.

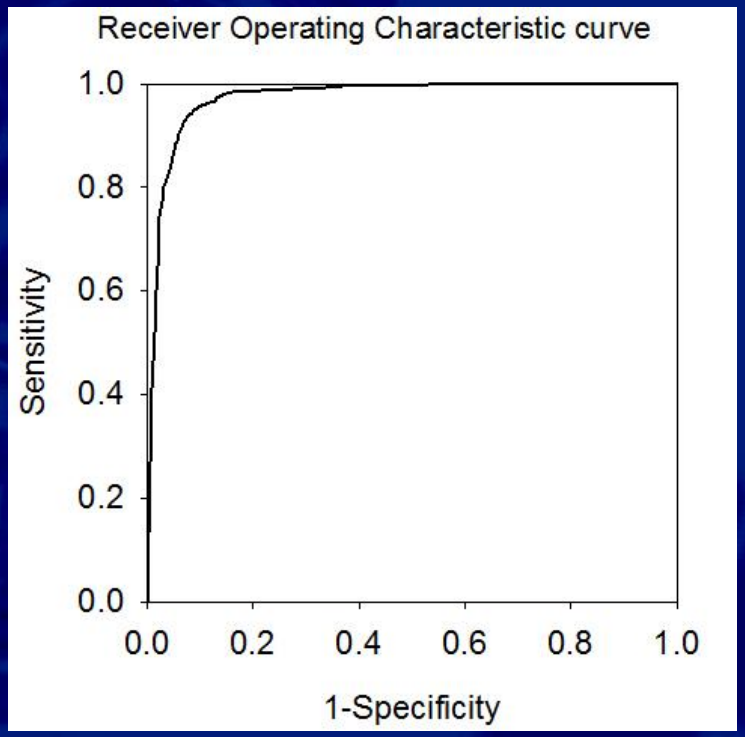
- Develop our own COG-based analysis:
  - Clusters of Orthologous Groups of proteins (COGs): http://www.ncbi.nlm.nih.gov/COG/
  - Classify proteins
  - TO DO: Fisher exact test to find significantly enriched COG groups.

Vaxign Vaxitop program: Epitope Prediction

- An MHC Class I & II binding epitope prediction tool developed internally
- Based on: position specific scoring matrix (PSSM)
- Uniqueness: calculate statistical P value (instead of a percentage or top number) as the cutoff

Vaxitop performance

- Vaxitop is specific and sensitive
- Comparable to known epitope prediction tools
- Example:



Receiver operating characteristic (ROC)

For human MHC class I allele: HLA A*0201

ROC Area Under Curve (AUC)
AUC value: 0.971

Vaxitop P-value of 0.05 has a balanced sensitivity and specificity

#	MHC allele	length	AUC	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
				(P = 0.01)		(P = 0.05)		(P = 0.1)	
1	HLA-A*01:01	9	0.984	0.962	0.9	1	0.791	1	0.699
2	HLA-A*02:01	9	0.971	0.647	0.979	0.961	0.889	0.986	0.776
3	HLA-A*02:01	10	0.943	0.695	0.961	0.933	0.803	0.978	0.559
4	HLA-B*15:17	9	0.976	0.597	0.996	0.925	0.901	0.984	0.755
5	HLA-B*35:01	9	0.96	0.687	0.978	0.942	0.799	0.987	0.674
6	HLA-A*02:02	9	0.946	0.624	0.96	0.95	0.805	0.988	0.565
7	HLA-A*02:02	10	0.909	0.542	0.946	0.908	0.731	0.988	0.45
8	HLA-A*02:03	9	0.959	0.558	0.99	0.908	0.87	0.981	0.689
9	HLA-A*02:03	10	0.878	0.553	0.941	0.878	0.671	0.97	0.392
10	HLA-A*02:06	9	0.918	0.539	0.968	0.886	0.796	0.964	0.641
11	HLA-A*03:01	9	0.97	0.745	0.974	0.977	0.823	0.994	0.638
12	HLA-A*03:01	10	0.896	0.66	0.909	0.971	0.469	0.996	0.22
13	HLA-A*11:01	9	0.98	0.664	0.991	0.98	0.867	0.998	0.69
14	HLA-A*11:01	10	0.9	0.6	0.942	0.971	0.448	1	0.2
15	HLA-A*24:03	9	0.995	0.799	1	0.991	0.895	0.995	0.763
16	HLA-A*29:02	9	0.932	0.651	0.953	0.929	0.764	0.953	0.608
17	HLA-A*30:01	9	0.964	0.694	0.989	0.91	0.892	0.95	0.806
18	HLA-A*31:01	9	0.968	0.644	0.981	0.957	0.849	0.993	0.725
19	HLA-A*68:01	9	0.837	0.561	0.903	0.966	0.451	0.997	0.11
20	HLA-A*68:01	10	0.875	0.572	0.937	0.955	0.472	0.996	0.141

New feature: Perform IEDB MHC class I epitope prediction and compare with Vaxitop results.

Vaxign Use Case Studies

Two use cases published:

- For *Brucella*:

Citations: -- Xiang Z, He Y. 2009. Vaxign: a web-based vaccine target design program for reverse vaccinology. *Procedia in Vaccinology*. Volume 1, Issue 1, Pages 23-29.

-- He Y, Xiang Z. Bioinformatics analysis of *Brucella* vaccines and vaccine targets using VIOLIN. *Immunome Res*. 2010 Sep 27;6 Suppl 1:S5.

- For uropathogenic *E. coli:*

Citation: He Y, Xiang Z, Mobley HLT. Vaxign: the first web-based vaccine design program for reverse vaccinology and an application for vaccine development. *Journal of Biomedicine and Biotechnology*. Volume 2010 (2010), Article ID 297505, 15 pages.

Two use cases for this afternoon's exercises:

- For human herpesvirus 1 (*i.e.*, Human simplex virus 1)
- For enterohemorrhagic *E. coli* strain O157:H7

Use Case 1: Human Herpesvirus 1 Vaccine Design

- Downloaded from NCBI RefSeq database:
  - Three HHV-1 genomes
  - 12 HHV genomes
  - 52 herpesvirus genomes

- Vaxign pre-computed analysis ready for query
  1) 77 proteins in human hepsevirus 1
  2) All present in three human hepsevirus 1 genomes
  3) Only 19 conserved in 12 human hepsevirus genomes
  4) 17 removed due to high transmembrane domains
  5) One with high adhesin probability: NP_044628.1, which is a capsid scaffold protein (UL26.5)

Results and discussion:

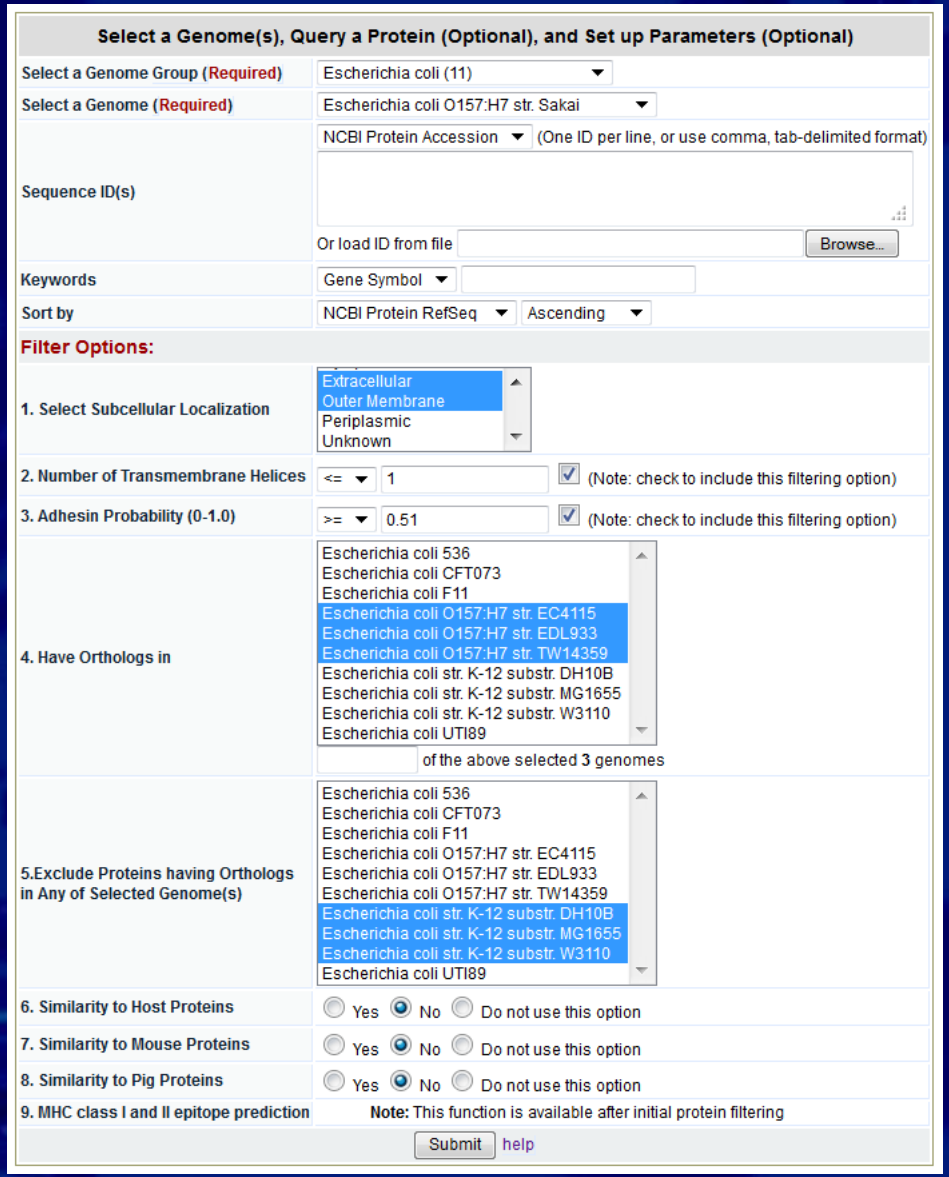
See Oliver’s presentation for details.

Use Case 2: enterohemorrhagic *E. coli* strain O157:H7

- Genomes available for four pathogenic O157:H7 strains:
  - str. EC4115
  - str. EDL933
  - str. Sakai (NOTE: used as seed genome since it comes from RefSeq database)
  - str. TW14359

- Control: three *E. coli* str. K-12 genomes
  - substr. DH10B
  - substr. W3110
  - substr. MG1655

A Vaxign setting for *E. coli* strain O157:H7 vaccine design



- Choose str. Sakai as seed genome
- Outer membrane or secreted proteins
- Conserved in all O157:H7 strains
- Absent from nonpathogenic K-12 strains
- Others …

Vaxign results for *E. coli* strain O157:H7 vaccine design

- 35 genes found
- DAVID gene functional enrichment results

Rerun Using Options | Create Sublist

30 chart records

Download File

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
☐	GOTERM_CC_FAT	outer membrane	RT		30	50.0	1.1E-17	1.6E-16
☐	GOTERM_CC_FAT	cell outer membrane	RT		24	40.0	2.5E-14	1.8E-13
☐	GOTERM_BP_FAT	biological adhesion	RT		13	21.7	5.4E-14	7.0E-13
☐	GOTERM_BP_FAT	cell adhesion	RT		13	21.7	5.4E-14	7.0E-13
☐	GOTERM_CC_FAT	external encapsulating structure part	RT		24	40.0	3.5E-10	1.7E-9
☐	GOTERM_CC_FAT	cell envelope	RT		24	40.0	5.0E-8	1.8E-7
☐	GOTERM_BP_FAT	pathogenesis	RT		7	11.7	5.6E-8	3.6E-7
☐	COG_ONTOLOGY	Intracellular trafficking and secretion / Extracellular structures	RT		4	6.7	1.8E-6	7.1E-6
☐	GOTERM_CC_FAT	pilus	RT		13	21.7	1.7E-5	4.8E-5
☐	GOTERM_CC_FAT	envelope	RT		24	40.0	3.3E-5	7.7E-5
☐	KEGG_PATHWAY	Pathogenic Escherichia coli infection	RT		3	5.0	6.1E-5	3.0E-4
☐	KEGG_PATHWAY	Pathogenic Escherichia coli infection	RT		3	5.0	7.3E-5	1.8E-4
☐	GOTERM_CC_FAT	external encapsulating structure	RT		24	40.0	8.5E-5	1.7E-4
☐	GOTERM_CC_FAT	cell projection	RT		13	21.7	2.4E-4	4.1E-4

Conclusion

- Vaxign is a specific and sensitive predictor of vaccine targets.
- It is web-based, user-friendly, and free.

http://www.violinet.org/vaxign/

Discussion

Challenges:

How to better rank predicted vaccine targets?

What's unique about protective antigens?

Directions: integrated with microarray, proteomics, literature mining, 3D structure, and VIOLIN components.

Acknowledgements

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