Genome-wide prediction of vaccine targets for human herpes simplex viruses using Vaxign reverse vaccinology

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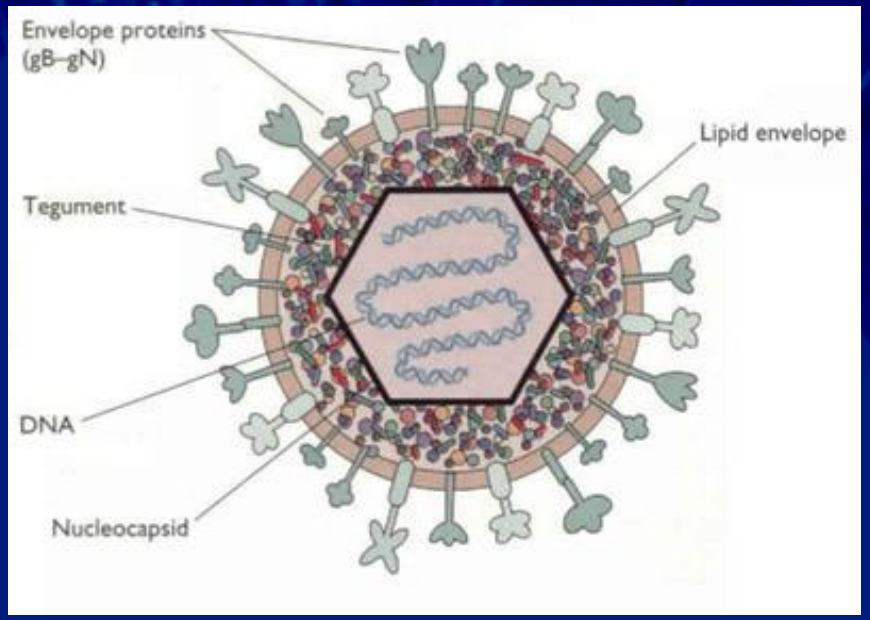
Human Herpes Simplex (HSV) Viruses

- Herpesviruses are a family of DNA viruses that cause diseases in humans and various animals.
- All herpesviruses are species-specific.
- Human herpesviruses (HHVs) have eight members, including: HSV-1 and HSV-2, the most common infectious agents of humans.
- Infectious virions are spherical.



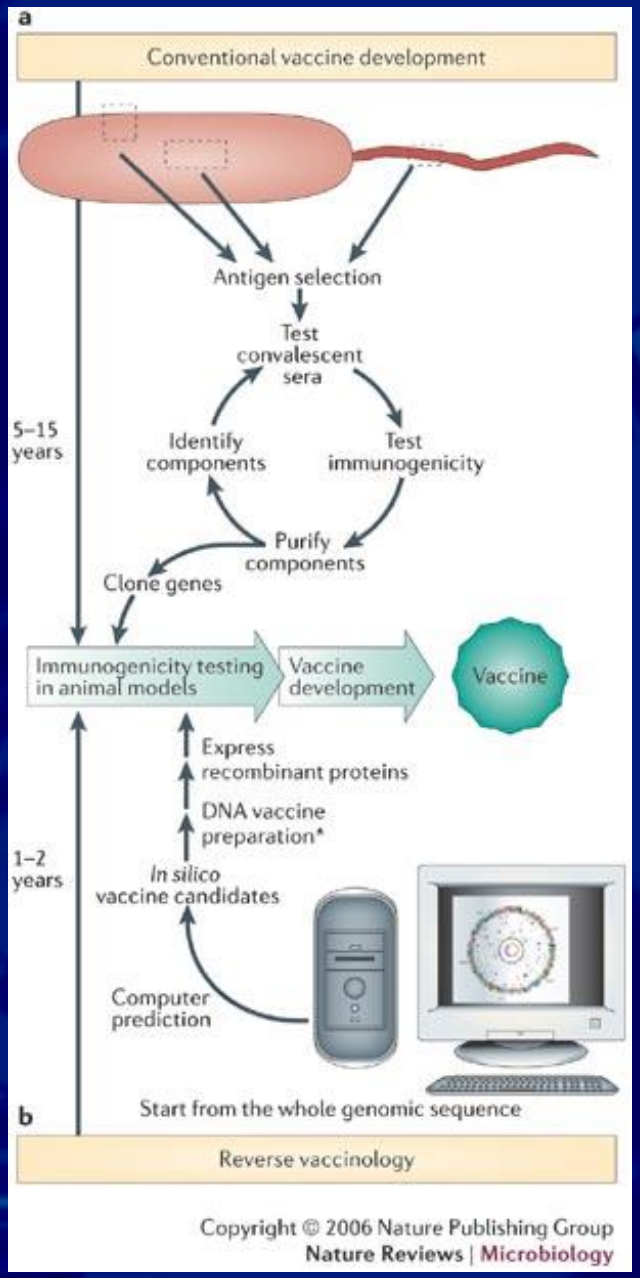
Virion structure: a linear, double-stranded DNA molecule densely packaged into a protein cage called **capsid**. The capsid is surrounded by an amorphous protein layer, called the **tegument**, consisting of viral proteins and viral mRNAs and a lipid bilayer membrane (envelope).

Human Herpes Simplex (HSV) Viruses

- HSV-1 and -2 are the most common infectious agents of humans.
- USA: Seroprevalence of HSV-1 and HSV-2 in adults is 68% and 21%, respectively
- USA: ~700-2000 cases of neonatal HSV infections per year occur in the US.
- No safe and effective HSV vaccines are available.

- Herpesvirus genomes available:
  - 52 herpesvirus genomes
  - 3 HHV-1 genomes: HHV-1 genome has 77 proteins
  - 12 HHV genomes
- Question: Can we predict vaccine candidates using these genome data?

Reverse Vaccinology (RV)



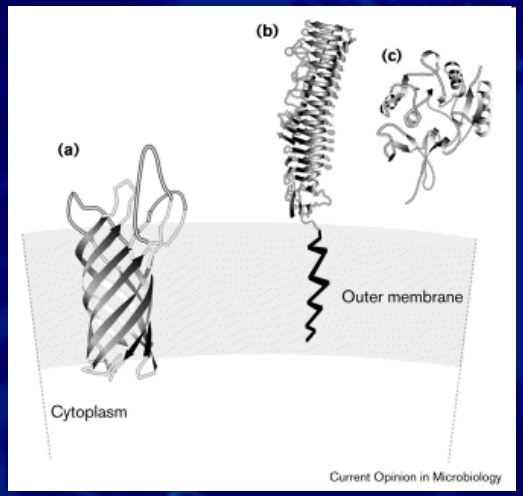
Dr. Rino Rappuoli
Pioneer of
Reverse Vaccinology

Definition: RV is a vaccine development strategy that starts with bioinformatics analysis to find potential vaccine candidates from pathogenic genomes. These candidate genes can then be tested in normal wet lab for protective immune responses.

- Johri *et al. Nat Rev Microbiol.* 2006 Dec; 4(12):932–42.
- Rappuoli R. *Curr Opin Microbiol.* 2000 Oct;3(5):445-50.

The First RV Success: MenB

MenB: Serogroup B meningococcus



First success: MenB

1) Genome sequence of *Neisseria meningitidis* serogroup B strain MC58 was obtained and used.
2) 570 genes predicted to code for surface-exposed or exported proteins.
3) 350 were successfully cloned to *E. coli*, expressed, and purified.
4) Mice were immunized.
5) 25 proteins induced bactericidal antibodies, which correlate with vaccine efficacy in humans.

Reference: Pizza M, *et al. Science.* 2000 Mar 10;287(5459):1816-20.

Reverse Vaccinology Criteria

Original Criteria:

1. Subcellular localization
   - ✓ Outer membrane proteins
   - ✓ Secreted proteins

New Criteria:

1. Transmembrane domains
   - ✓ >2 α–helix domains → difficult to isolate
2. Adhesin probability
   - ✓ Adhesin is important for pathogen invasion
3. MHC-Epitope binding
   - ✓ MHC class I epitope → cell-mediated immunity
   - ✓ MHC class II epitope → antibody response
4. Sequence conservation and exclusion
   - ✓ Shared genes in pathogens but not in avirulent strains
5. Similarity to host proteins
   - ✓ Avoid autoimmunity or immune tolerance

It is challenging to apply reverse vaccinology without a comprehensive pipeline

→ To address this challenge, we developed

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

- Vaxign vaccine design 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.

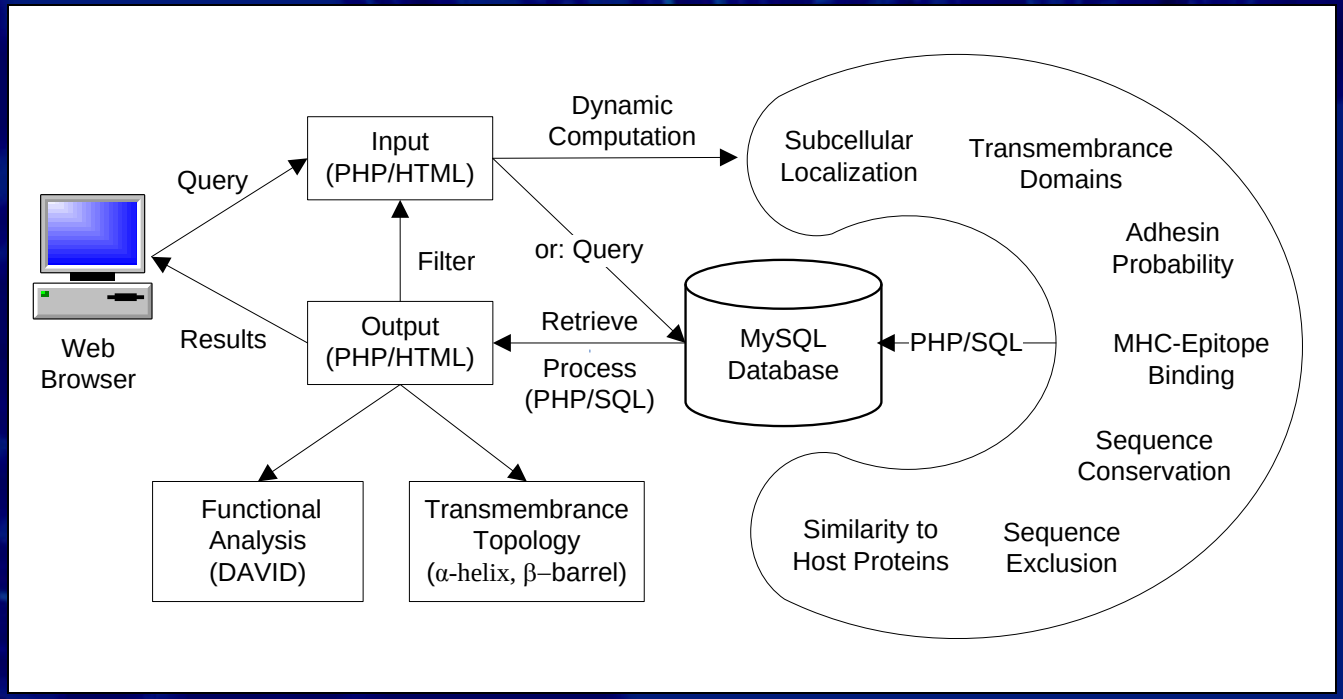
- Vaxign vaccine design for uropathogenic *E. coli:*

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.

Many have cited Vaxign, esp. this year!

Vaxign: Vaccine Design System

- Aim: Vaccine target prediction for reverse vaccinology

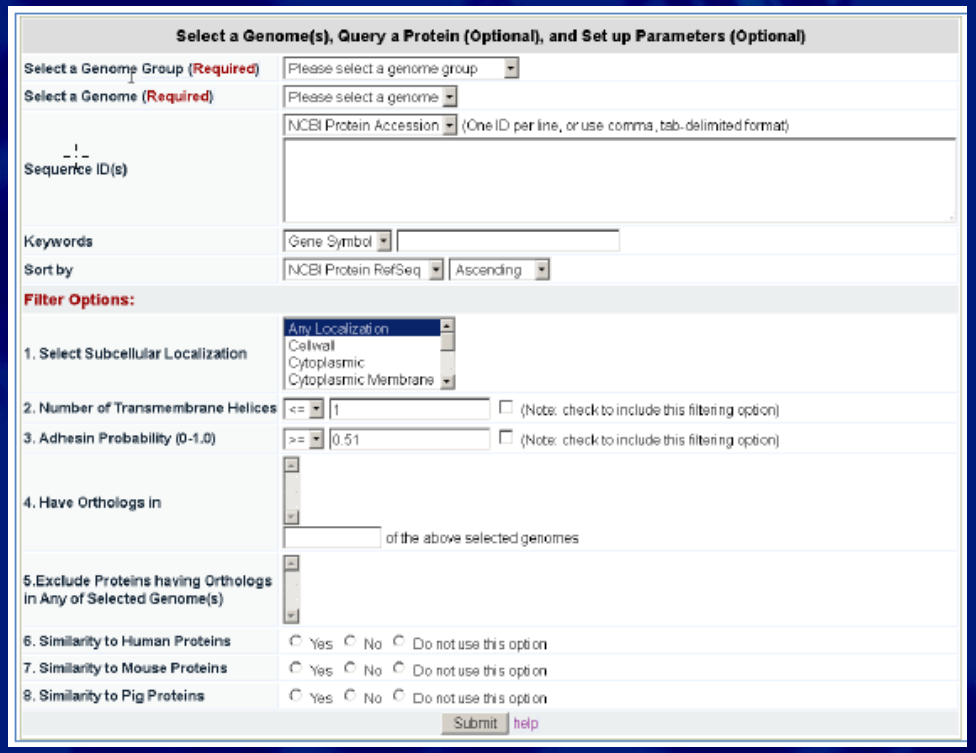


- The 1st web-based reverse vaccinology system
- Freely available: http://www.violinet.org/vaxign

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]

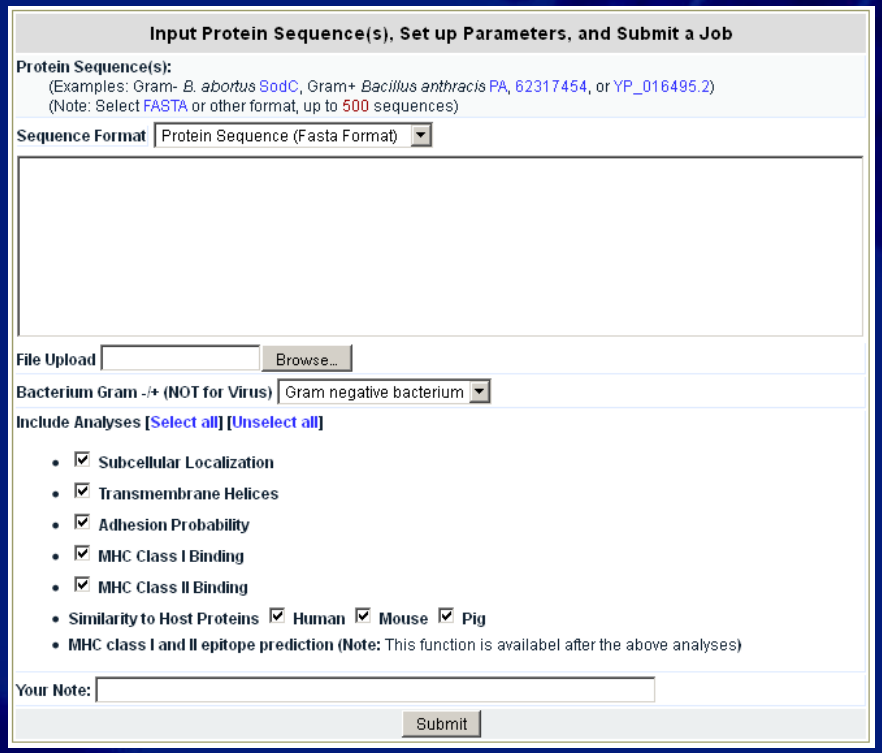
Two Forms of Vaxign Usage

Pre-computed data query



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

Dynamic analysis



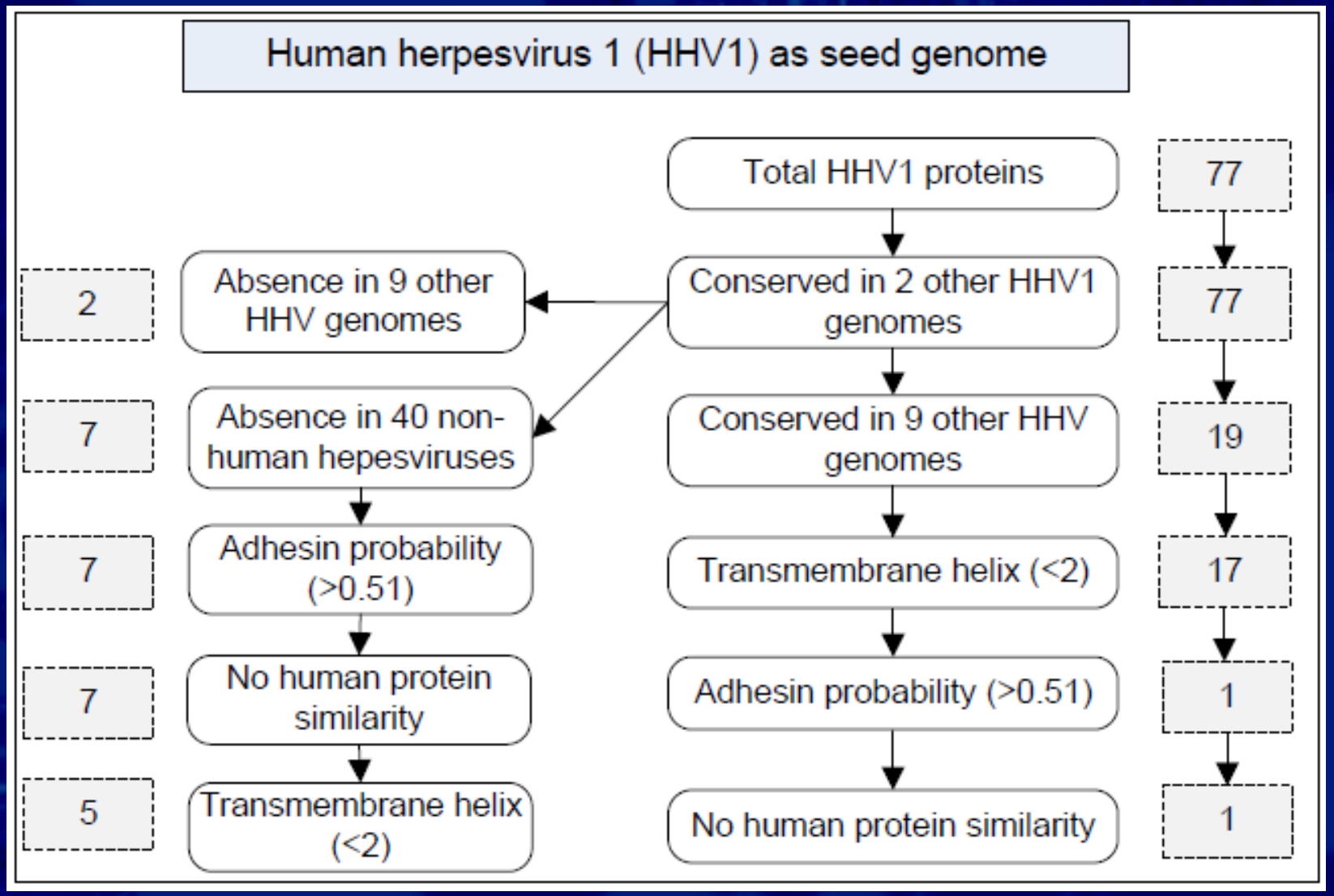
- Runtime execution
- Can analyze up to 500 proteins at one time

Vaxign
VACCINE DESIGN

Human Herpesvirus Vaccine Design

- Downloaded from NCBI RefSeq database:
  - Three HHV-1 genomes:
    - ✓ 77 proteins in human herpsevirus 1
  - 12 HHV genomes
  - 52 herpesvirus genomes
- Vaxign pre-computation and results saved in Vaxign database
- User-friendly web interface for result query

Workflow and Result Summary



Seven HSV-1 proteins having No orthologs in all 40 non-human herpesviruses

#	*Protein Accession*	*Protein Note*	*Adhesin Probability*	*Trans-membrane helices*
1	NP_044675.1	TAP transporter inhibitor ICP47	0.079	0
2	NP_044674.1	tegument protein US11	0.245	0
3	NP_044667.1	envelope glycoprotein gJ	0.176	2
4	NP_044666.1	envelope glycoprotein gG	0.419	2
5	NP_044659.1	membrane protein UL56	0.238	1
6	NP_044661.1	neurovirulence protein ICP34.5	0.228	0
7	NP_044600.1	neurovirulence protein ICP34.5	0.228	0

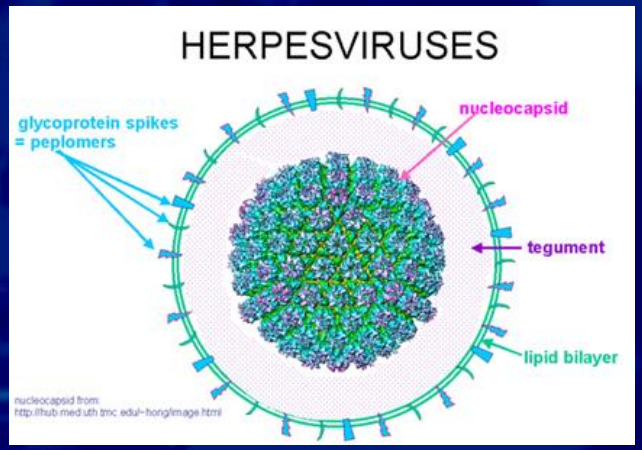
So these proteins are human-specific.
Not good for animal test.

19 HSV-1 proteins conserved in human herpesviruses

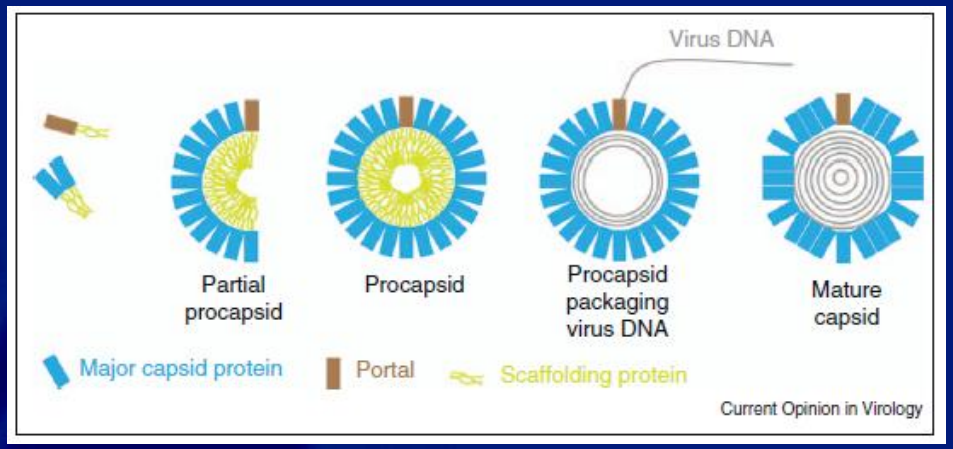
#	*Protein Accession*	*Protein GI*	*Protein Note*	*Adhesin Probability*	*Trans-membrane helices*
1	NP_044603.1	9629382	uracil-DNA glycosylase	0.262	0
2	NP_044606.1	9629385	helicase-primase helicase subunit	0.115	0
3	NP_044655.1	9629434	helicase-primase primase subunit	0.163	0
4	NP_044607.1	9629386	capsid portal protein	0.241	0
5	NP_044620.1	9629399	major capsid protein	0.113	0
6	NP_044627.1	9629406	capsid maturation protease (UL26)	0.386	0
7	NP_044628.1	9629407	capsid scaffold protein UL26.5	**0.675**	0
8	NP_044611.1	9629390	envelope glycoprotein gM	0.244	8
9	NP_044629.1	9629408	envelope glycoprotein gB	0.229	3
10	NP_044613.1	9629392	deoxyribonuclease	0.203	0
11	NP_044616.1	9629397	DNA packaging terminase subunit 1	0.165	0
12	NP_044630.1	9629409	DNA packaging terminase subunit 2	0.188	0
13	NP_044626.1	9629405	DNA packaging tegument protein UL25	0.210	0
14	NP_044634.1	9629413	DNA packaging protein UL32	0.185	0
15	NP_044635.1	9629414	DNA packaging protein UL33	0.264	0
16	NP_044625.1	9629404	nuclear protein UL24	0.195	0
17	NP_044631.1	9629410	single-stranded DNA-binding protein	0.168	0
18	NP_044632.1	9629411	DNA polymerase catalytic subunit	0.101	0
19	NP_044641.1	9629420	ribonucleotide reductase subunit 1	0.193	0

UL26.5 for HHV-1 Vaccine Development?

- U26.5 capsid scaffold protein is important for virus capsid formation
- Has not been reported for vaccine development
- U26.5 capsid scaffold protein has adhesin-like characteristics? Why?



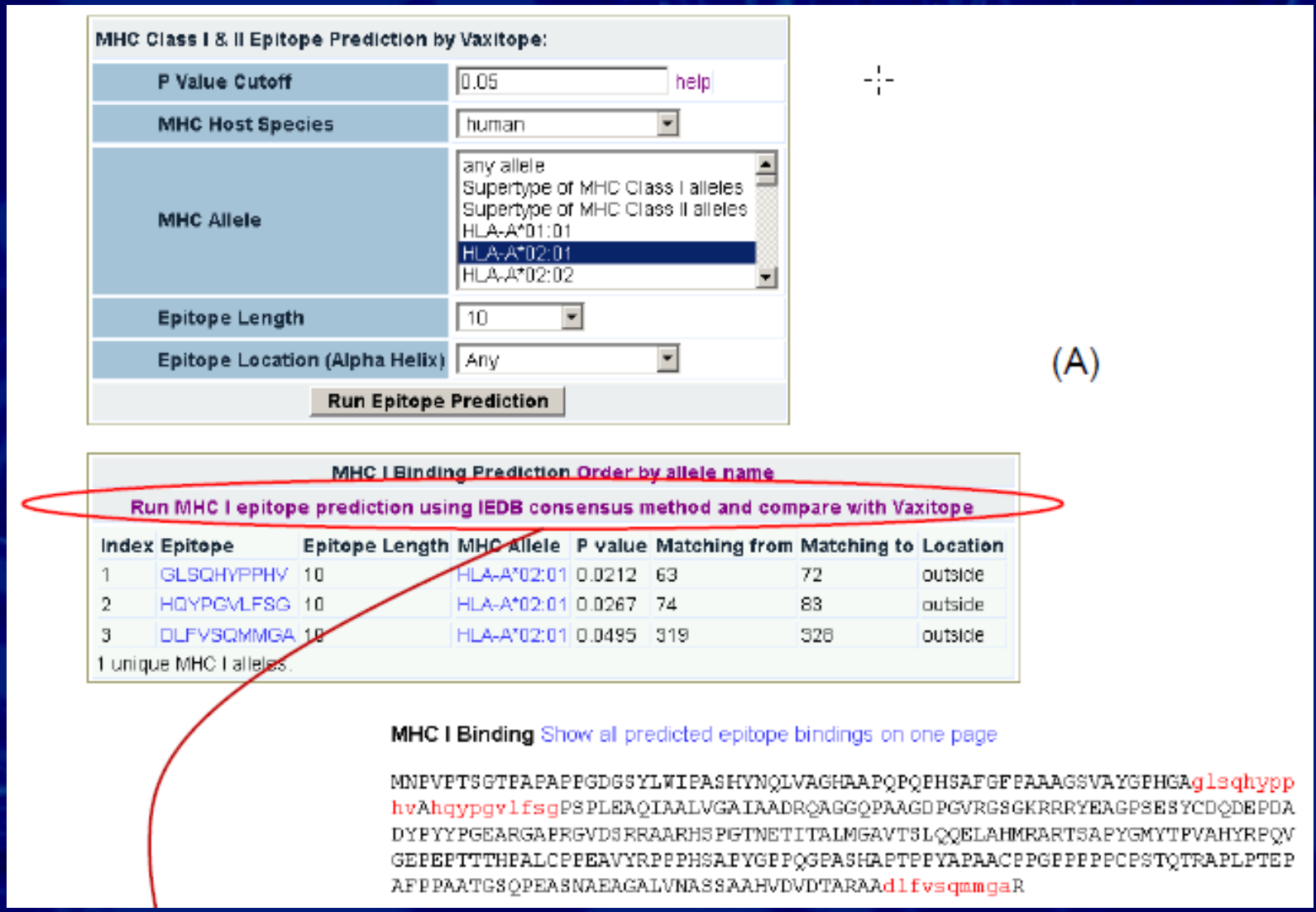
http://microbiologybook.org/mhunt/dna1.htm



http://www.ncbi.nlm.nih.gov/pubmed/21927635

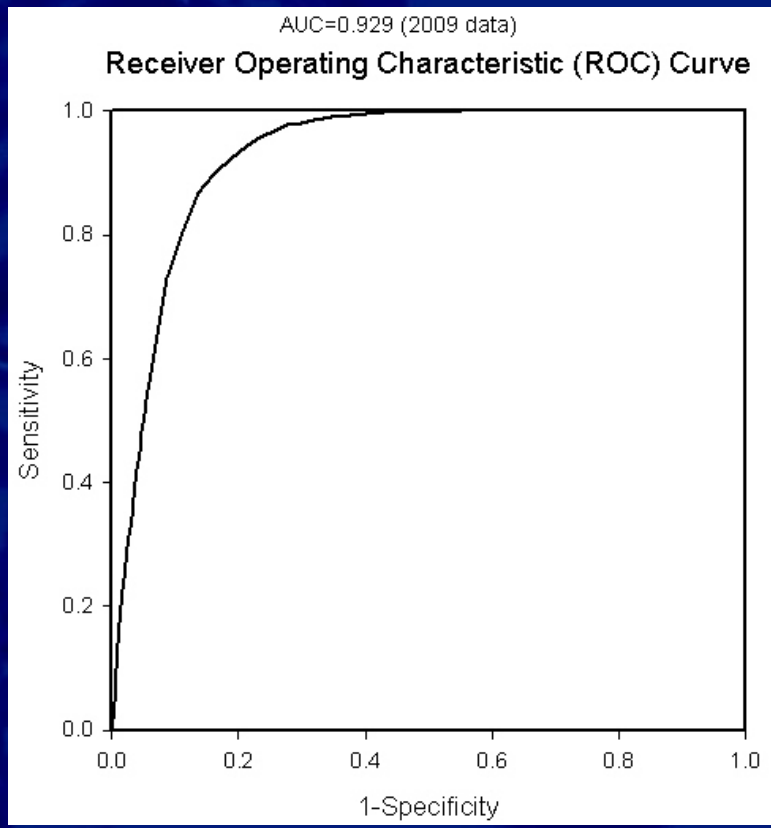
Scaffold protein lost in mature virus

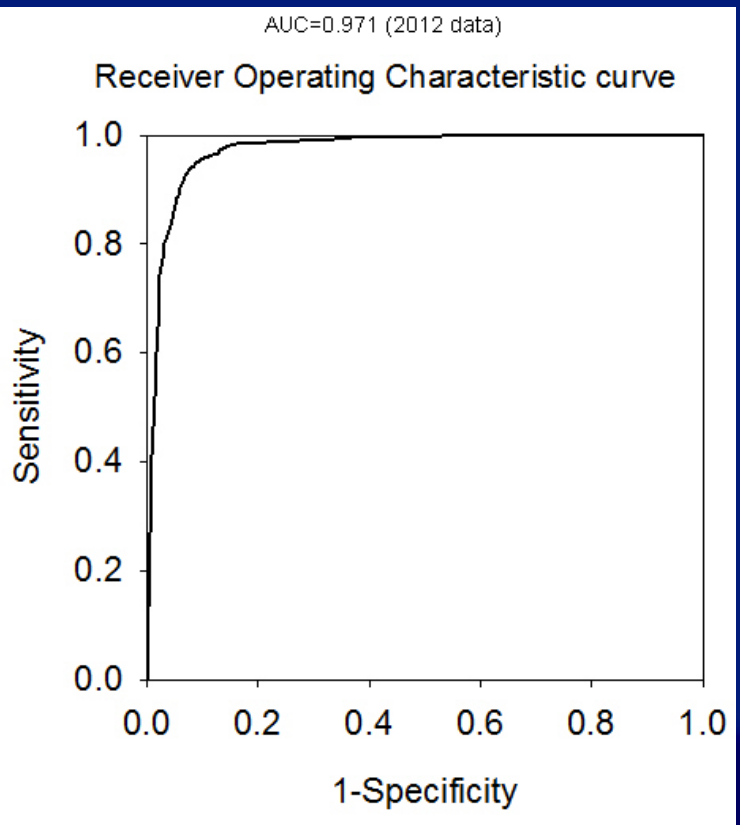
MHC Class I Epitope Prediction Example



Vaxign-Vaxitop MHC Class I and II Epitope Predictions

- Internally developed project
- Based on position specific scoring matrices (PSSM)
- Unique: Calculate statistical P-value for each prediction





Vaxign and IEDB preidiction comparison on MHC Class I Epitope Prediction

Epitope predicted by IEDB consensus method

Index	Epitope	Epitope length	MHC allele	Matching from	Matching to	IC50 (IEDB consensus)	Vaxitope P-value
1	GLSQHYPPHV	10	HLA-A*02:01	63	72	1.05	0.0212
2	DLFYSQMMGA	10	HLA-A*02:01	319	328	4.45	0.0495
3	HQYPGVLFSG	10	HLA-A*02:01	74	83	5.4	0.0267
4	ALMGAVTSLQ	10	HLA-A*02:01	174	183	5.4	>0.1
5	FGFPAAAGSV	10	HLA-A*02:01	46	55	6.95	>0.1
6	TALMGAVTSL	10	HLA-A*02:01	173	182	7.05	0.087
7	YLWIPASHYN	10	HLA-A*02:01	20	29	7.2	>0.1
8	SAPYGMYTPV	10	HLA-A*02:01	194	203	7.4	0.0641
9	VLFSGPSPLE	10	HLA-A*02:01	79	88	8.95	>0.1
10	GMYTPVAHYR	10	HLA-A*02:01	198	207	9.45	>0.1
11	DTARAADLFV	10	HLA-A*02:01	313	322	9.45	>0.1
12	GVLFSGPSPL	10	HLA-A*02:01	78	87	9.9	>0.1

consistent

Epitope predicted by Vaxitop method

Index	Epitope	Epitope Length	MHC Allele	Matching from	Matching to	P-value	IC50 (IEDB consensus)
1	GLSQHYPPHV	10	HLA-A*02:01	63	72	0.0212	1.05
2	HQYPGVLFSG	10	HLA-A*02:01	74	83	0.0267	5.4
3	DLFYSQMMGA	10	HLA-A*02:01	319	328	0.0495	4.45
4	SAPYGMYTPV	10	HLA-A*02:01	194	203	0.0641	7.4
5	GGPAAGDPGV	10	HLA-A*02:01	106	115	0.0762	>50
6	TALMGAVTSL	10	HLA-A*02:01	173	182	0.087	7.05

inconsistent

- Results overlap
- Vaxitop is more conservative in predicting positive results.

Conclusion

- Vaxign is a specific and sensitive predictor of vaccine targets.
- It is web-based, user-friendly, and free.
- More information in next demo and hands-on training

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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