iVAX: A Sophisticated Suite of Online Vaccine Design Tools

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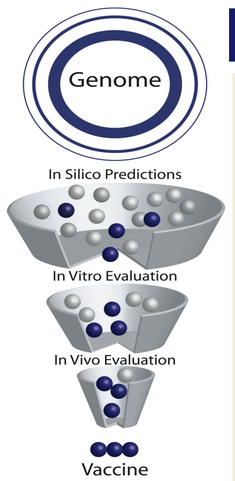
Abstract

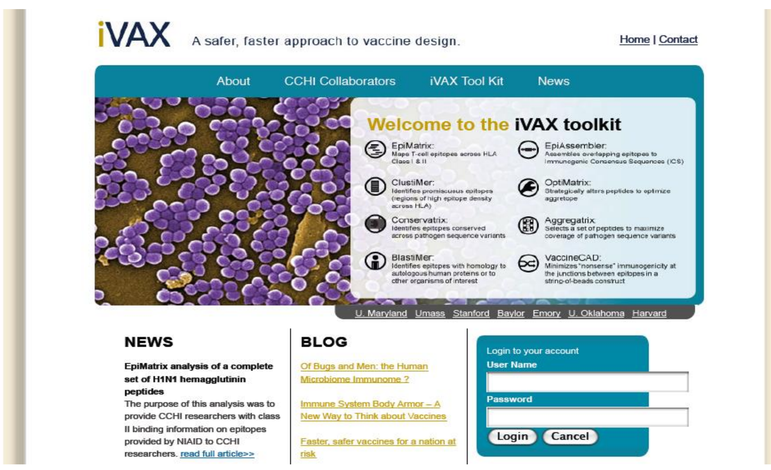
Emerging and re-emerging infectious diseases represent a significant challenge for next-generation vaccine design and bioterror preparedness. We have composed a suite of online immunoinformatics tools for accelerated design of genome-derived, epitope-driven vaccines generated from protein sequences. Using the Conservatrix algorithm, even the most mutable pathogenic genomes may be probed for highly conserved segments, which are then mapped for T cell epitopes and regions of high epitope density using EpiMatrix and ClustiMer. JanusMatrix, an improved homology analysis tool examining pathogen/host sequence similarity with respect to the HLA and TCR faces of an epitope, is used to screen out sequences which could potentially elicit an undesired autoimmune or regulatory T cell response due to homology with sequences encoded by the human genome. Immunogenic Consensus Sequences are created by EpiAssembler, a tool which optimizes the balance between pathogen and population coverage. VaccineCAD links candidate epitopes into a string-of-beads design while minimizing non-specific junctional epitopes that may be created in the linking process.

With proof of principle established in animal models for vaccines against tularemia, Vaccinia and *H. pylori*, the iVAX toolkit exemplifies a rapid, efficient, easily accessible and broadly applicable solution to accelerate the development of critically important vaccines for human health and biodefense.



Genome to Vaccine Pipeline

Online Access



iVAX:

Web-accessible immunoinformatic analysis and data management

Web Based Tools

Data Management

The iVAX database allows for storage and organization of large sequence files. Users may upload genomes, proteins, peptides or archives of previous analyses, all of which are stored securely on an Oracle 11g database.

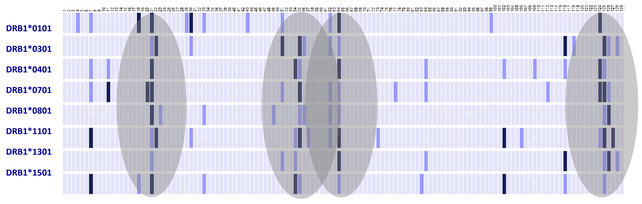
Epitope Discovery

EpiMatrix

EpiMatrix maps input proteins for putative T cell epitopes restricted by Class I and Class II HLA alleles. Based on overall epitope content, a Protein Immunogenicity Score can be calculated and then normalized for protein length. Immunogenicity scores of different proteins are thus directly comparable.

ClustiMer

Class II T cell epitopes tend to cluster within specific protein sub-regions (shown below). These T cell epitope clusters are high priority targets for vaccine design. ClustiMer identifies these regions and calculates a Cluster Immunogenicity Score for each peptide. Users may select specific HLA alleles to evaluate, and may sort input proteins or peptides by score to create prioritized lists of potential targets.



Conservation Analysis

Conservatrix

Conservatrix identifies conserved peptides among protein sequences from even the most mutable of pathogens. For any given sequence file, users may view the number of times a 9-mer or 10-mer occurs within the input file, the percent conservation of each peptide among input proteins, and the predicted HLA binding profile for each peptide.

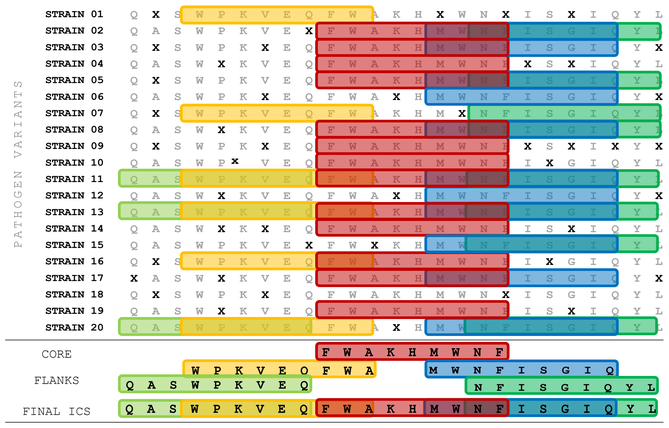
Conservatrix – conservation analysis without alignment conserved peptides scored for epitope content

Peptide #	AA Sequence	Count	Percent	A0101 Z-Score	A0201 Z-Score	A0301 Z-Score	A1101 Z-Score	A2402 Z-Score	A6801 Z-Score	B0702 Z-Score	B0801 Z-Score	B2705 Z-Score	B3501 Z-Score	B4403 Z-Score	Hits
1	LTVWGIKQL	1344	0.99	0.97	1.67	0.23	0.17	1.29	0.54	1.02	0.16	1.33	0.96	0.66	1
2	LLSGIVQQQ	1339	0.99	0.96	1.21	0.26	0.18	0	0.31	-0.57	0.12	-0.27	0.12	-1.69	0
3	STQLLLNGS	1318	0.98	0.86	0.35	0.21	0.64	-0.19	0.18	-0.36	-1.09	-0.59	-0.75	0.8	0
4	TQLLLNGSL	1313	0.97	-0.6	1.3	0.51	0.64	2.02	0.02	0.91	0.27	0.9	1.46	0.88	1
5	VSTQLLLNG	1311	0.97	0.77	-0.78	0.55	0.68	-0.25	0.49	-0.77	0.19	-0.58	-0.17	0.32	0
6	WATHACVPT	1309	0.97	-1.66	0.84	0.16	0.21	-0.17	0.08	0.57	0.42	-0.62	0.26	0.39	0
7	THACVPTDP	1306	0.97	-0.77	-0.94	-0.55	-0.64	0.57	-0.58	-0.52	-3.55	-0.8	-0.96	-0.83	0
8	ATHACVPTD	1305	0.97	0.29	0.45	0.89	1.02	0.06	0.46	0.1	0.07	-0.62	-1.63	0.51	0
9	NWRSELYKY	1305	0.97	1.05	-0.98	0.74	0.65	1.83	0.63	0.44	0.38	1.13	1.14	0.31	1
10	WRSELYKYK	1305	0.97	-0.32	-0.21	1.72	1.57	-0.18	1.68	-1.03	0.5	2.46	-0.36	-0.13	3
•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
61247	NLWNWFDIX	1	0	1.27	2.41	1.4	0.78	1.89	1.84	0.28	0.62	0.47	1.57	0.05	3
61248	FDIXNWLWY	1	0	2.87	0.05	1.24	1.27	0.77	1.32	1.61	-0.07	1.46	1.78	2.5	3
61249	LFLFSYRQL	1	0	0.12	0.41	0.42	0.36	3.48	0.52	0.56	0.57	0.81	1.16	0.71	1
61250	DLLLIAARV	1	0	-0.44	1.91	-0.24	-0.22	-0.17	0.06	0.01	1.46	-0.2	0.36	0.75	1

EpiAssembler

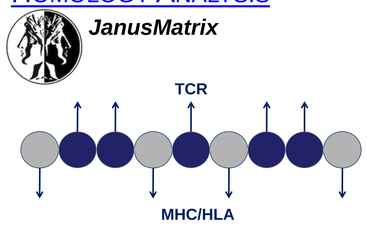
EpiAssembler identifies sets of overlapping, conserved, and immunogenic epitopes and assembles them into extended Immunogenic Consensus Sequences (ICS). A *highly conserved, putatively promiscuous 9-mer* is chosen as the core. Additional epitopes that overlap with the natural n- and c-terminal flanking regions are identified and integrated into the core. Processing of these sequences allows for presentation of the highly conserved peptides in the context of more than one MHC. Users may select construction criteria and view reports summarizing the immunogenicity and conservation of each ICS and each 9-mer within its input protein file.

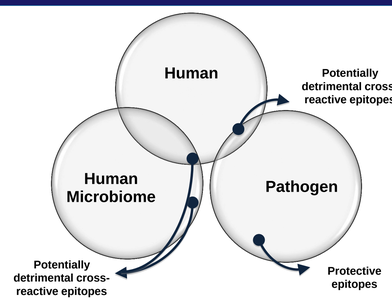
EpiAssembler – construction of an Immunogenic Consensus Sequence



Homology Analysis

JanusMatrix





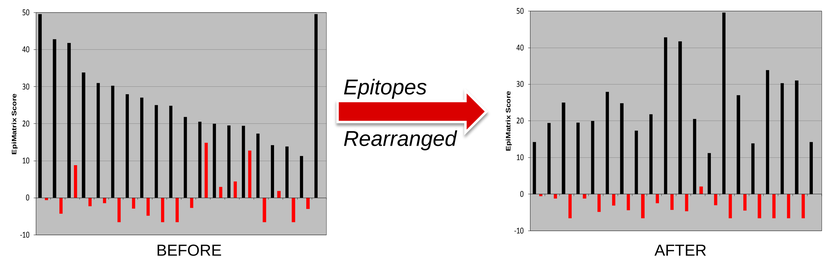
JanusMatrix evaluates homology specifically at the T cell receptor (TCR) interface. Pathogenic epitopes which may differ at the MHC binding interface but present the same residues to the TCR are identified and set aside.

JanusMatrix limits the possibility of cross-reactivity and autoimmunity in epitope-based vaccines. Epitopes derived from the pathogen that are homologous to components of the human genome can be set aside while the remaining "foreign" epitopes can be safely included in vaccine formulations.

Vaccine Design

VaccineCAD

When formed into a "string of beads" and joined with appropriate expression, targeting, and processing signals, T cell epitope clusters and immunogenic consensus sequences can also be used directly as vaccine components. In a default order, juxtaposed pathogen-derived epitopes, shown left as vertical black bars whose height is relative to their predicted immunogenicity, may create unintended, non-specific epitopes at the junctions, shown left as red vertical bars. Vaccine-CAD (Computer Assisted Design) reiteratively modifies the order of the epitopes until an optimal pseudoprotein sequence with minimzed junctional immunogenicity is created. If, after reordering, any junction still surpasses the threshold for immunogenicity, an immunologically inert spacer (Class II) or breaker (Class I) sequence is inserted to completely interrupt the remaining junctional epitope. Users may choose to optimize for supertype Class I or Class II alleles, or for a specific allele of interest.



Conclusions / Future Directions

We have achieved proof of principle in animal models for three out of five vaccines for which we currently have prototypes. A therapeutically administered vaccine construct engineered against *H. pylori* using the iVAX system resulted in a significant reduction in gastric colonization compared to unvaccinated controls.[1] Similarly, VennVax, a DNA vaccine expressing conserved epitopes from seven Smallpox genomes, conferred 100% survival to HLA-transgenic mice lethally challenged with *Vaccinia*.[2] TulyVax protected 57% of immunized mice against a lethal challenge with *F. tularensis*.[3] Two more vaccines are in the process of validation.

We are applying the approach to influenza, tuberculosis and HIV, along with biodefense projects. Additional collaborations in the field of neglected tropical diseases are under development. We believe these tools are of great utility for development of safer, more targeted vaccines.

References / Acknowledgements

[1] Moss SF, Moise L, Lee DS, Kim W, Zhang S, Lee J, Rogers AB, Martin W, De Groot AS. HelicoVax: epitope-based therapeutic Helicobacter pylori vaccination in a mouse model. Vaccine. 2011 Mar 3; 29(11):2085-91.

[2] Moise L, Buller RM, Schriewer J, Lee J, Frey SE, Weiner DB, Martin W, De Groot AS. VennVax, a DNA-prime, peptide-boost multi-T-cell epitope poxvirus vaccine, induces protective immunity against vaccinia infection by T cell response alone. Vaccine. 2011 Jan 10; 29(3):501-11.

[3] Gregory SH, Mott S, Phung J, Lee J, Moise L, McMurry JA, Martin W, De Groot AS. Epitope-based vaccination against pneumonic tularemia. Vaccine. 2009 Aug 27; 27(39):5299-306.

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For questions regarding immunogenicity prediction services and deimmunization options, please contact: Anthony Marcello at 401-272-2123, ext. 149; or at amarcello@epivax.com www.epivax.com