The iVAX Toolkit: An in silico platform for epitope discovery with applications to human and animal vaccine design

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Abstract

Computational vaccine design, also known as computational vaccinology, encompasses epitope mapping, antigen selection and immunogen design using computational tools. In silico prediction of immune response to emerging infectious diseases and cancers can accelerate the design of novel and next generation vaccines. The iVAX toolkit is an integrated set of immunoinformatics algorithms that has been in development since 1998. It comprises a suite of immunoinformatics algorithms for triaging candidate antigens, selecting immunogenic and conserved T cell epitopes, eliminating regulatory T cell epitopes, and optimizing antigens for immunogenicity and protection against disease.

iVAX harnesses computing power, genomic data, and advanced immunoinformatics tools to identify T cell epitopes capable of inducing productive T cell immune mechanisms for the generation of safe and effective vaccines. Novel algorithms have been implemented to predict peptide binding to human, murine and swine class I and class II MHC alleles. Highly immunogenic peptides conserved across multiple strains of pathogen sequences are identified using the Conservatrix, EpiMatrix and EpiAssembler algorithms. Potential vaccine candidate epitopes can be aggregated into a string-of-beads design with the VaxCAD algorithm, simultaneously minimizing deleterious junctional epitopes that may be created in the linking process. Two newer tools, discovered and refined in the past few years, include JanusMatrix and iTEM. JanusMatrix is a specially tailored homology analysis tool that examines pathogen/host sequence similarity at the MHC:TCR interface for any given peptide, and predicts potentially cross-reactive epitopes, allowing candidate sequences with potential host cross-reactivity to be preferentially excluded from vaccine constructs. iTEM enables identification of epitopes unique for specific human subjects based on individual MHC allele expression. Combining iTEM and JanusMatrix results (J-iTEM) allows for selfness-adjusted individualized epitope prediction and is ideal for cancer mutanome processing and personalized cancer vaccine design.

The iVAX toolkit has been adapted to accelerate the development of targeted, safe and efficacious vaccines, which will address important global health and biodefense challenges for humans and livestock. Most recently, low-immunogenicity H7N9 influenza antigens with high human cross-conservation were engineered to include epitopes more highly cross-conserved with circulating influenza strains, resulting in a 5-fold increase in post-vaccination antibody titers compared to wild-type protein. Subsequently, the JanusMatrix tool was utilized to successfully identify cross-reactive epitopes between the MAGE A3 immunotherapeutic and human titin implicated in two fatalities among affinity-enhanced TCR cancer immunotherapy trial participants. The poor immunogenicity of unadjuvanted H7N9 vaccines illustrates the challenges associated with time-honored approaches to vaccine development, while modern cancer vaccine research has underscored the danger of auto-reactive vaccines and immunotherapeutics. We have applied immunoinformatics tools to develop safe and effective responses to these challenges. Academic and commercial collaborations are welcomed and encouraged.

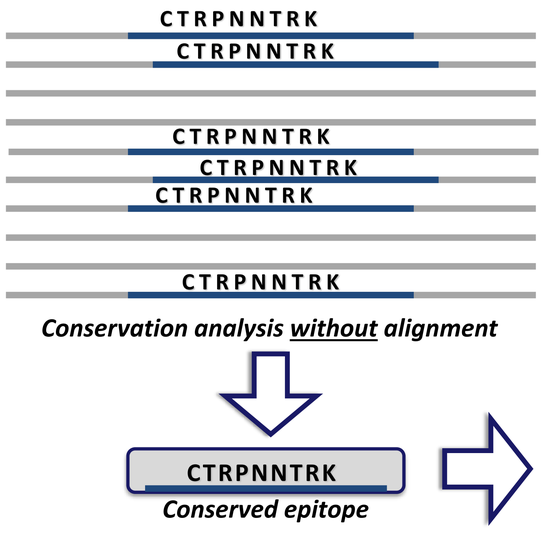
Online Access

Web Based Vaccine Design Process

CONSERVATRIX:

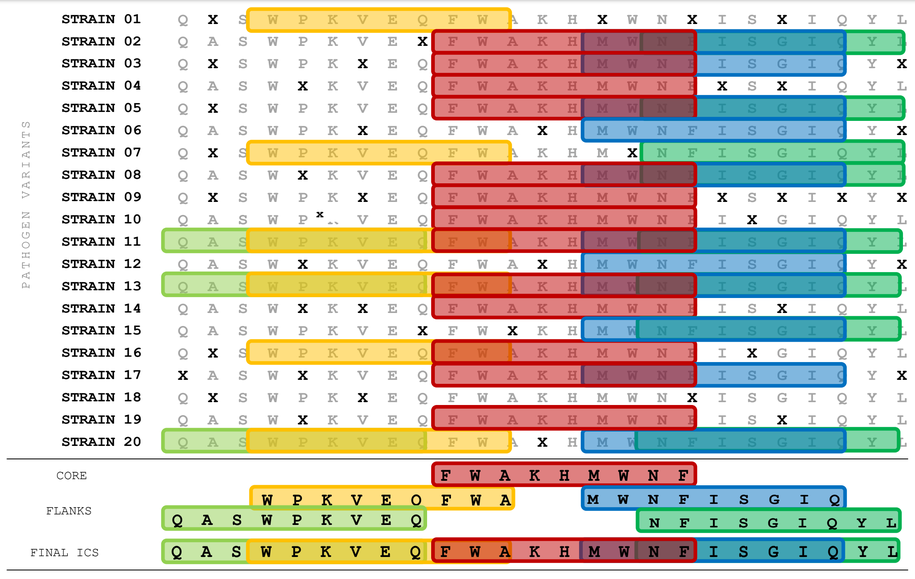
Conserved peptides scored for epitope content

CONSERVATION ANALYSIS



EPIASSEMBLER:

Assemble conserved, promiscuous epitopes into Immunogenic Consensus Sequences (ICS)



ICS peptides provide broad strain coverage, bind multiple HLA

HOMOLOGY ANALYSIS:

Triage candidate epitopes by evaluating their homology to host, microbiome, published seqs

Multiple Databases Available to Search:

HUMAN

- Swiss-Prot, manually annotated and reviewed.
- Updated monthly.

HUMAN MICROBIOME

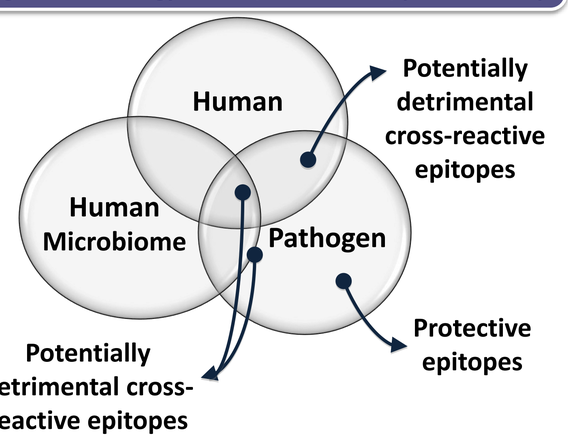
- Collected genomes from NIH Human Microbiome Project.

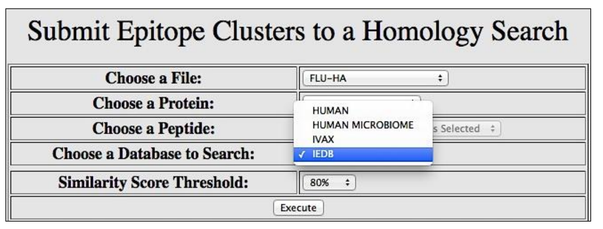
IEDB

- MHC Binding, T Cell Assays, and MHC Ligand Elution Assays.
- Updated biweekly.

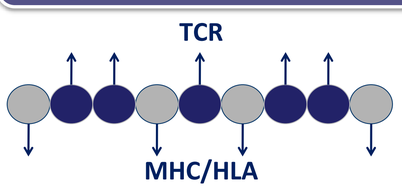
iVAX

- Search against custom files.





JANUSMATRIX: *Conserved, promiscuous sequences screened for TCR facing homology with human genome and/or microbiome epitopes in order to avoid autoimmunity and/or tolerogenic effect*

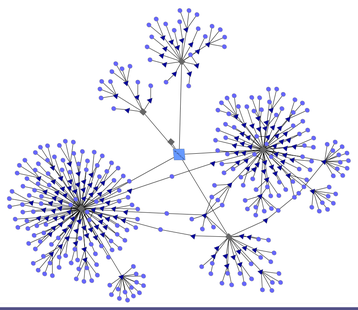


At the level of an HLA-bound peptide, certain amino acids are in contact with the HLA molecule itself while others are accessible to the T-cell receptor (TCR). If TCR-facing residues from a pathogenic epitope are conserved among multiple HLA-binding sequences from the human genome, the pathogenic epitope may activate T cells specific to these human proteins.

Protein ID	Protein Description	Start Position	Sequence	Cluster Score	Janus-Adjusted Score	Δ	# Janus Matches	DRB1* 0101	DRB1* 0301	DRB1* 0401	DRB1* 0701	DRB1* 0801	DRB1* 1101	DRB1* 1301	DRB1* 1501
CHICAGO-2013	hemagglutinin	102-123	VPDYASLRSLVASSGSLEFISNK	30.9	11.44	19.46									
		102	VPDYASLRS				0	-0.57	-0.7	0.12	-0.32	-0.06	-0.15	0.78	0.93
		103	PDYASLRSL				0	-0.56	0.1	-1.02	-0.2	-0.49	-0.14	-0.38	0.07
		104	DYASLRSLV				0	0.73	1.34	-0.33	1.33	0.05	0.73	0.88	0.74
		105	YASLRSLVA				7	2.56	1.32	2.71	2.3	1.89	2.62	1.67	2.25
sp|Q8NAA5|FA11A_HUMAN	Leucine-rich repeat-containing protein FAM211A	98	YASFRNLVD					1.14	-0.01	1.68	1.08	2.24	1.19	1.37	1.85
sp|O00555|CAC1A_HUMAN	Voltage-dependent P/Q-type calcium channel subunit...	597	WASLRNLVY					2.71	0.8	2.6	2.55	1.81	2.45	1.16	1.82
sp|Q13278|CAC1E_HUMAN	Voltage-dependent R-type calcium channel subunit a...	586	WASLRNLVY					2.71	0.8	2.6	2.55	1.81	2.45	1.16	1.82
sp|Q62523|RBM43_HUMAN	RNA-binding protein 43	203	LASFYRLVY					0.73	0.81	1.67	1.11	0.58	1.39	1.15	1.05
sp|P10253|LYAG_HUMAN	Lysosomal alpha-glucosidase	581	IASRSRLVK					0.65	0.26	1.44	1.46	0.32	0.71	1.92	0.92
sp|O94762|RECQ5_HUMAN	ATP-dependent DNA helicase Q5	294	KASRSRLVQ					0.69	0.38	1.92	0.5	-0.24	0.9	0.31	-0.23
sp|Q99497|PARK7_HUMAN	Protein DJ-1	1	MASRALIVI					0.97	1.49	0.41	1.63	1.29	0.78	2.07	1.04
		106	ASLRSLVAS				0	-0.5	-0.33	-0.86	-1.18	0.42	-0.25	0.74	0.13
		107	SLRSLVASS				0	1.11	1.48	1.57	0.51	0.66	1.54	1.34	0.1
		108	LRSLVASSG				10	2.78	2.04	2.55	1.63	2.7	2.46	2.12	2.44
sp|A6NFD8|HELT_HUMAN	Hairy and enhancer of split-related protein HELT	102	VRSLVLSSA					1.37	1.83	1.4	1.15	1.77	2.19	1.92	2.13
sp|Q7L2K0|CP059_HUMAN	Uncharacterized protein C16orf59	108	SRSIVTSSS					1.36	0.85	1.88	0.43	0.58	1.24	1.08	1.25
sp|Q32NB8|PGPS1_HUMAN	CDP-diacylglycerol--glycerol-3-phosphate 3-phospha...	524	LRSDVVRSA					1.39	1.89	1.92	1.35	0.85	1.28	1.3	1.1
sp|P38567|HYALP_HUMAN	Hyaluronidase PH-20	114	FRSFVKSSG					1.64	1.87	1.78	0.37	3.19	2.77	2.96	2.83
sp|Q92187|SIA8D_HUMAN	CMP-N-acetylneuraminate-poly-alpha-2,8-sialyltrans...	45	SRSLVNSSG					1.2	0.6	0.96	0.95	1.83	0.64	0.72	1.15
sp|O15111|IKKA_HUMAN	Inhibitor of nuclear factor kappa-B kinase subunit...	663	AASLVGSSL					2.05	0.7	1.11	1.66	0.68	0.73	0.81	1.49
sp|Q8WUI4|HDAC7_HUMAN	Histone deacetylase 7	76	KRSAVASSV					1.79	1.54	1.51	2.17	0.85	0.84	1.39	1.81
sp|O75916|RGS9_HUMAN	Regulator of G-protein signaling 9	237	MRSTVKSSV					1.23	1.63	0.57	1.53	1.51	1.18	2.31	2.04
sp|P42858|HD_HUMAN	Huntingtin	457	SRSTVSSSA					0.98	1.97	1.88	0.57	0.39	0.22	0.75	0.75
sp|Q8NAR2|MARH4_HUMAN	Probable E3 ubiquitin-protein ligase MARCH10	402	FRSFVNSSY					0.89	1.82	0.83	0.63	-0.4	0.42	1.35	0.8
		109	RSLVASSGS				0	0.75	0.94	0.9	0.66	0.09	0.68	1.03	1.47
		110	SLVASSGSL				2	1.09	1.25	0.56	1.87	0.47	0.1	0.13	1.78
sp|A6NMZ7|COA6_HUMAN	Collagen alpha-6(VI) chain	625	FLVDSSGSI					1.85	2.86	1.84	2.02	1.42	0.58	0.65	1.56
sp|P12111|CO6A3_HUMAN	Collagen alpha-3(VI) chain	1119	LLYDSAGSR					1.4	2.43	0.89	1.66	1.26	1.03	0.97	1.49
		111	LVASSGSLE				6	1.32	1.98	1.09	1.41	1.3	0.63	1.51	0.98
sp|P0C639|POTEJ_HUMAN	POTE ankyrin domain family member J	892	MVASSSSLE					1.16	2.33	1.26	1.82	1.54	0.86	1.85	1.37
sp|P49643|PRI2_HUMAN	DNA primase large subunit	166	IVASSSSLG					2.29	2.69	2.26	2.1	1.63	2.22	2.21	2.04
sp|P08172|ACM2_HUMAN	Muscarinic acetylcholine receptor M2	28	LVASSSLFY					0.65	1.74	0.6	1.14	0.6	0.94	1.16	1.87
sp|Q9NP58|DCP1A_HUMAN	mRNA-decapping enzyme 1A	445	KVASASRLS					1.39	1.9	1.6	0.94	1	0.94	1.75	1.9
sp|O75643|U520_HUMAN	U5 small nuclear ribonucleoprotein 200 kDa helicas...	1487	IVALSSRLS					2.92	2.23	2.94	2.3	2.47	2.71	2.31	2.81
sp|P49754|VPS41_HUMAN	Vacuolar protein sorting-associated protein 41 hom...	764	LVADSSLSL					0.87	2.73	1.2	0.88	1.17	0.59	1.5	1.35
		112	VASSSLEFF				0	0.77	1.66	1.38	1.66	0.36	1.06	1.45	1.2
		113	ASSSLEFFS				0	-1.13	0.2	-0.59	-1.53	-0.51	-0.27	-0.71	-0.29
		114	SSSSLEFSN				0	-0.65	-0.63	-1.67	-0.12	-1	-0.37	-1.01	0
		115	SSSLEFSNE				0	-1.28	-0.49	-1.2	-0.86	0	-0.96	-0.36	-1.28

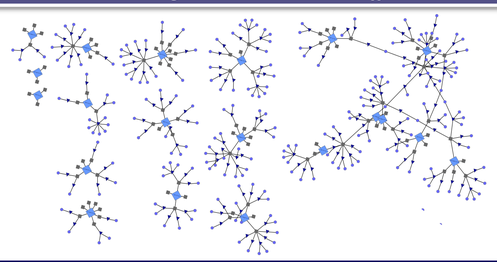
Differential responses may be expected depending on the particular autologous protein with which any given vaccine candidate epitope shares a TCR profile. A detailed printable report allows vaccine designers to review all possible cross-reactive relationships.

- Predicted 9-mer epitope from a source protein or peptide
- ▲ 9-mer from human proteome, 100% TCR face identical to source epitope
- Human protein where cross-reactive epitopes are present



Epitope Networks:

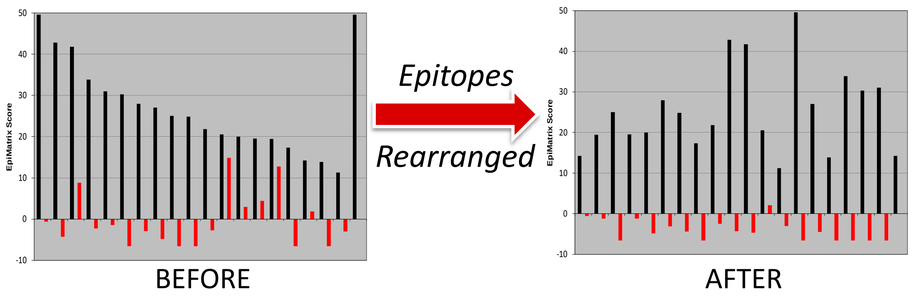
Visually distinguish T_{reg} (above) from T_{eff} epitopes (below)



# HOMOLOGY HITS	SEQUENCE	AA IDENTITIES	EPITOPE ID	T CELL	MHC BINDING
--	VPDYASLRSLVASSGTLKFIN	--	CVX-DATA-2	n/a	n/a
TOP 8 HOMOLOGY HITS	1 CTPYD----------E---EDFNWT	20/21	7431	POSITIVE	NONE
	1 **----------E---EGFNWTGVTQNGGSSAC	18/21	10850	POSITIVE	NONE
	1 CTPYD----------******	15/21	138761	POSITIVE	NONE
	1 YD----------*******	14/21	73603	POSITIVE	POSITIVE
	1 CTPYD----------*******	14/21	7430	POSITIVE	NONE
	1 ***----------E-**	15/21	73443	POSITIVE	POSITIVE
	1 *----------******	14/21	113677	POSITIVE	NONE
	1 PYD----------********	12/21	50080	POSITIVE	NONE
Total:	8				

After triage, generate string of beads in optimal epitope order

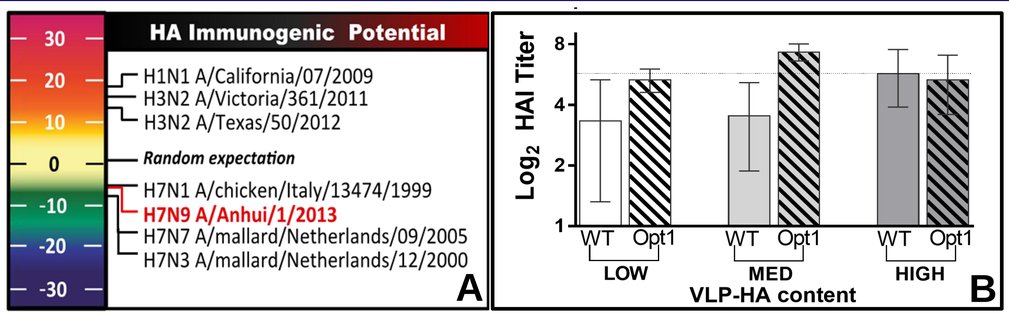
VAXCAD: *Organizes component epitope sequences in order to reduce potential nonsense immunogenicity at junctions between ICS*



Avian-origin influenza virus (H7N9): Shanghai, Feb 2013[1]

- Human-to-human transmission limited, but high mortality rate (27%)
- Immunoinformatics tools used to estimate immunogenic potential of H7N9 antigens

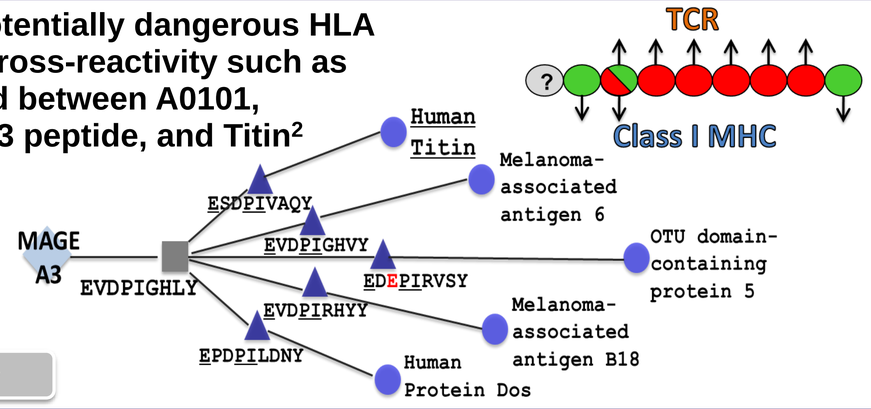
A) Found that HA proteins derived from human-derived H7N9 strains contain fewer T cell epitopes than most other circulating strains of influenza, explaining why previous H7 HA-containing vaccines have been poorly immunogenic.



B) Human epitope content depleted H7 HA seroconverted earlier and at a lower dose than low immunogenicity wild type antigen in VLP format.
(Study performed in collaboration with T Ross, UGA)

Infectious disease

Avoid potentially dangerous HLA Class I cross-reactivity such as observed between A0101, MAGE-A3 peptide, and Titin[2]



Oncology

Discussion and Conclusions

The recent swine-origin H1N1 influenza and avian-origin H7N9 outbreaks[1] have illustrated the difficulties associated with 'standard' approaches to vaccine development, while recent cancer vaccine research[2] has underscored the danger of auto-reactive vaccines and immunotherapeutics. These studies have provided an opportunity to apply immunoinformatics tools to guide the development of a safe and effective public health response. With proof of principle established in animal models for three out of five vaccines for which we currently have prototypes[3,4,5], the iVAX toolkit is poised to accelerate the development of targeted, efficacious vaccines which will address important global health and biodefense challenges. Collaborations are accepted and encouraged.

References / Acknowledgements

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For questions regarding immunogenicity screening and vaccine design, please contact: Katie Porter at 401-272-2123; or at info@epivax.com *www.epivax.com*