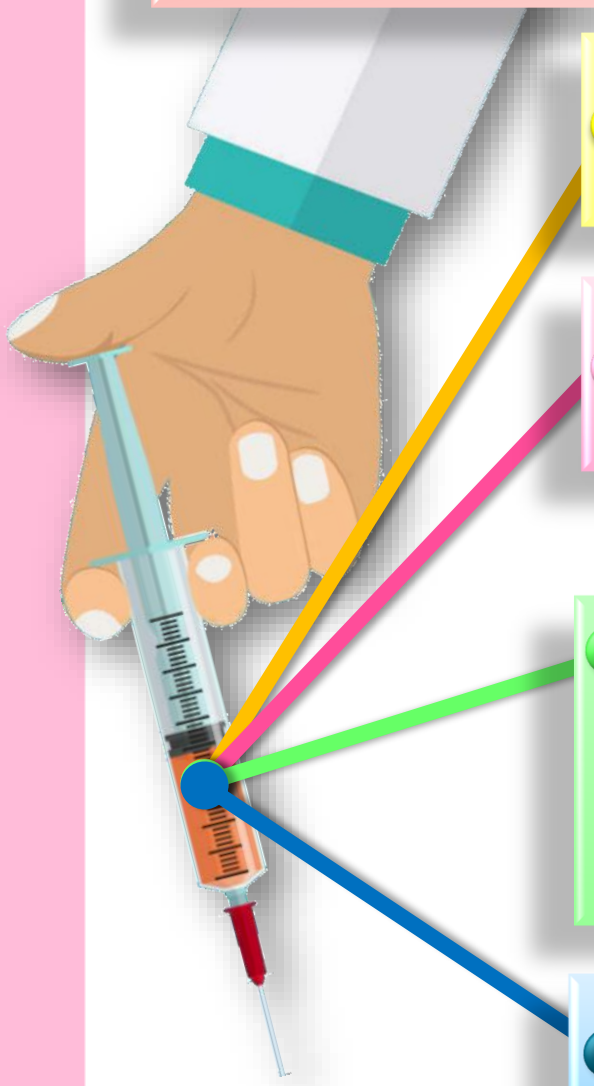
In the Name of God

In-silico design of a multi-epitope vaccine candidate

Presenter:

Dr. Samira Dodangeh

Classification of vaccines



Vaccination Using Extracts or Killed microorganism:

- sterile immunity cannot be achieved using killed vaccines in any of the infection models used

Vaccination Using Live, Attenuated microorganism :

- The use of such strains in immune compromised animals or humans should be restricted.

DNA vaccine:

- attractive due to inducing a **wide range of immune responses** including easy to produce, stable, very immunogenic, and possess the potential for long-lasting immunity.
- **Unfortunately**, It is likely to **occur autoimmune complications** in the recipient and the gene vaccine may be incorporated into the host cell genome complex.

Recombinant protein vaccine:

- Strong Humoral and Cellular responses, long-term memory and specific immunogenic responses and safe

Classification of vaccines

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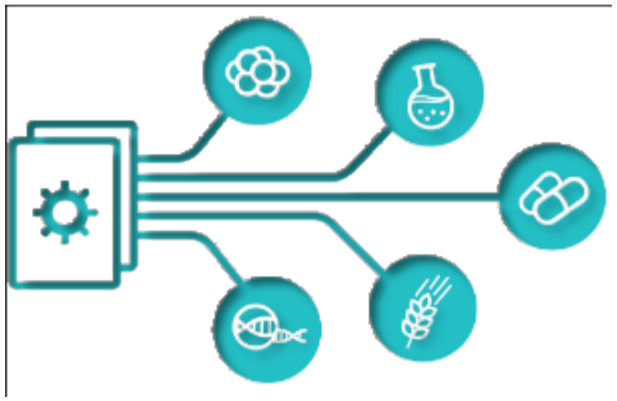
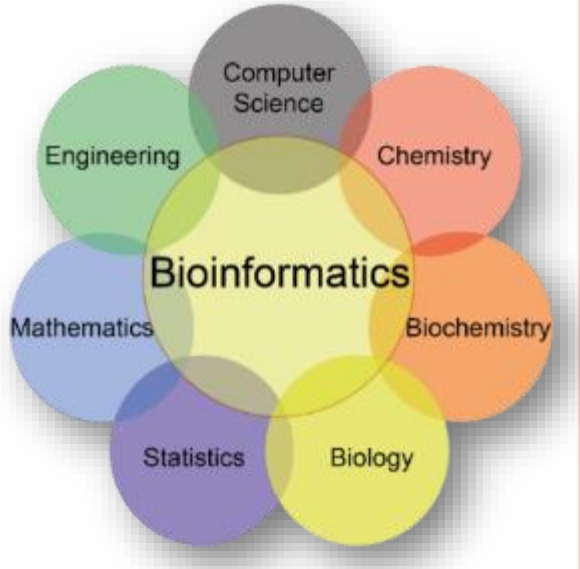
Epitope-Based Vaccines:

- The concept of epitope vaccines relies heavily on the prediction of **B cell and T cell** epitopes that can elicit **specific** and **Epitopes** or **antigenic** determinants are the minimal immunogenic part of any particular antigen.
- **Protective immune responses**.

- Immunization with **multi-epitope** vaccine expressing **T-cell** or/and **B-cell** epitopes against different pathogens showed significant increase in both cellular and humoral immunes responses, as well as **prolonged survival time.**

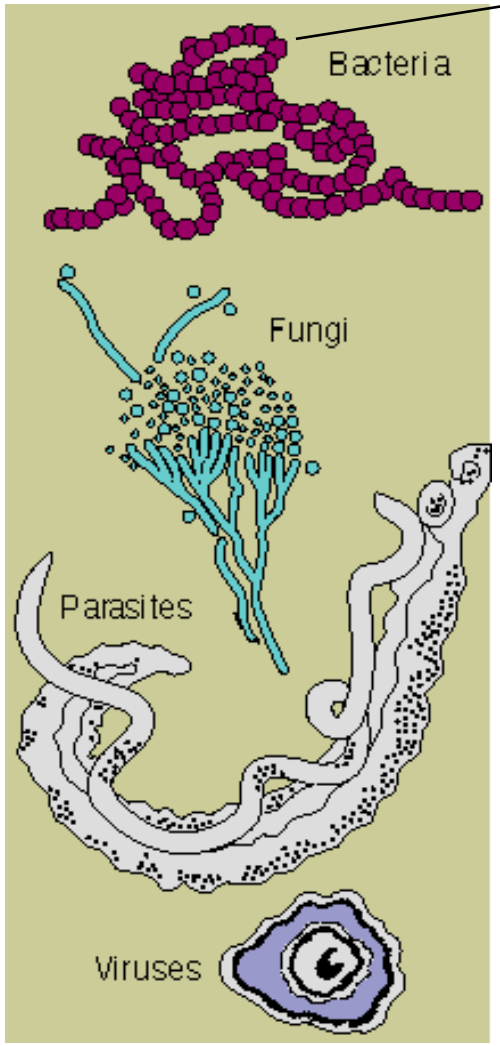
- As an interdisciplinary science, **bioinformatics** has been widely used to predict protein structures, functions, and epitopes.





- Prediction of epitopes plays a significant role in the **immunogenicity design of new vaccines**.

Disease Causing Agents



Host Immune Defense System
For Microbial (Pathogens) Attack

Non-Specific Defense
(Innate Immunity)

Specific Defense
Adaptive Immunity
Responsible for Generating
Memory B & T Cells

TOLL LIKE RECEPTORS
PATHOGEN ASSOCIATED
MOLECULAR PATTERNS

Humoral Immunity
(B-Lymphocytes)

Cell-Mediated Immunity
(T-Lymphocytes)

B-Cell Epitopes
Antibody Prod.
Neutralization

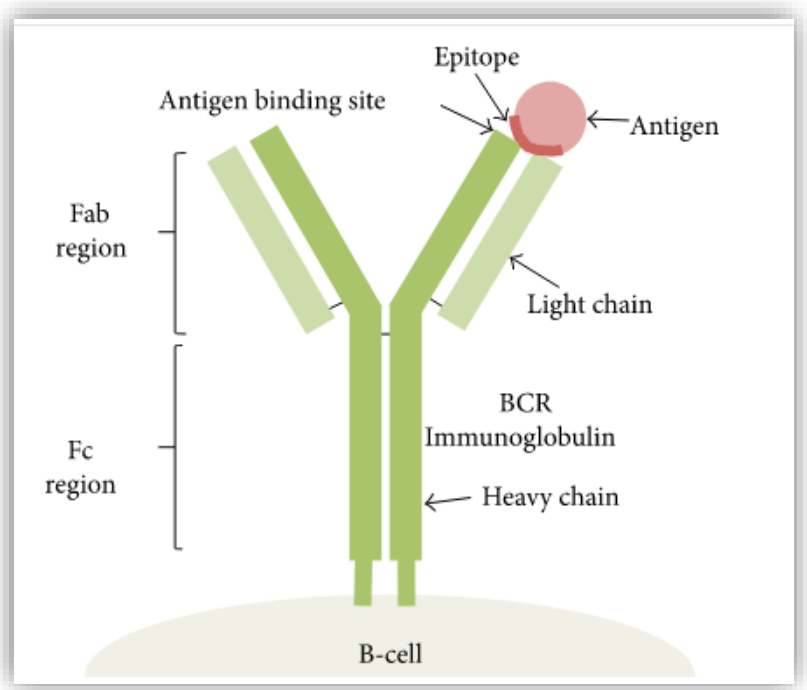
Endogenous Antigen Processing

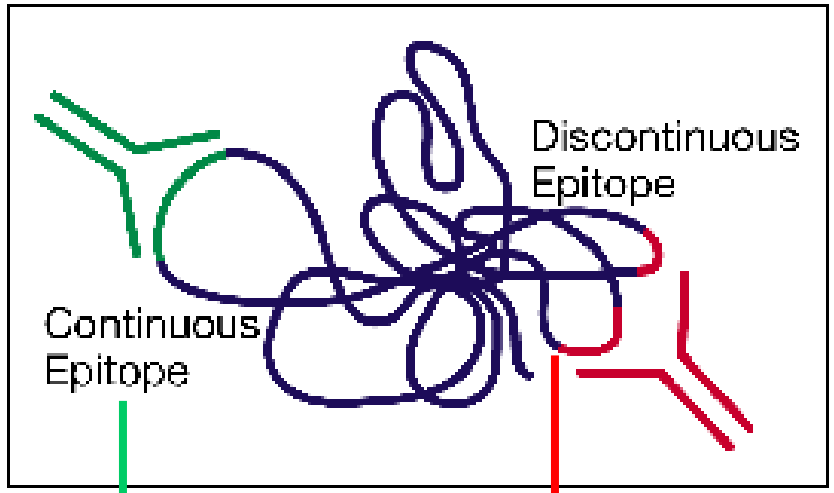
Exogenous Antigen Processing

CTL Epitopes
MHC I binders
TAP Binders
P. Cleavage Sites

T-Helper Epitopes
MHC II binders

B-cell epitopes recognition



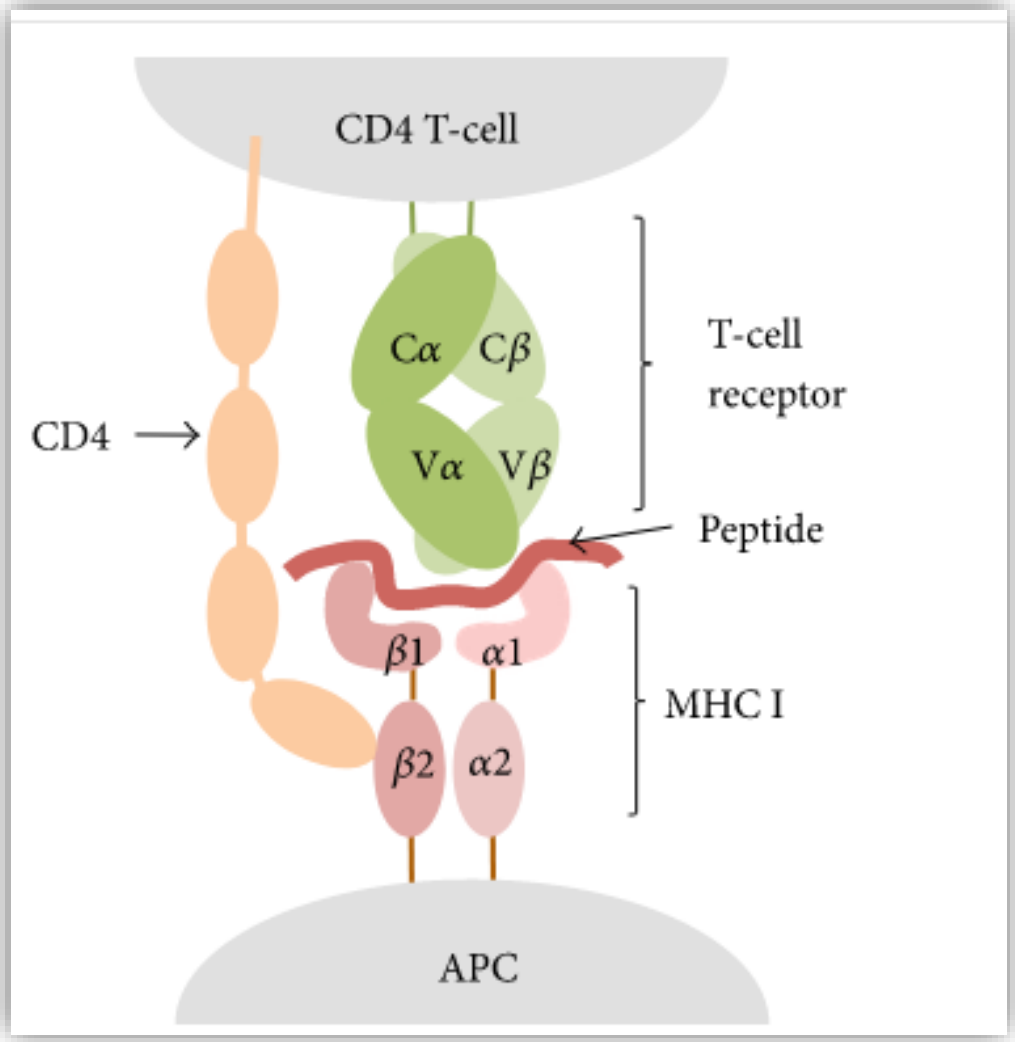


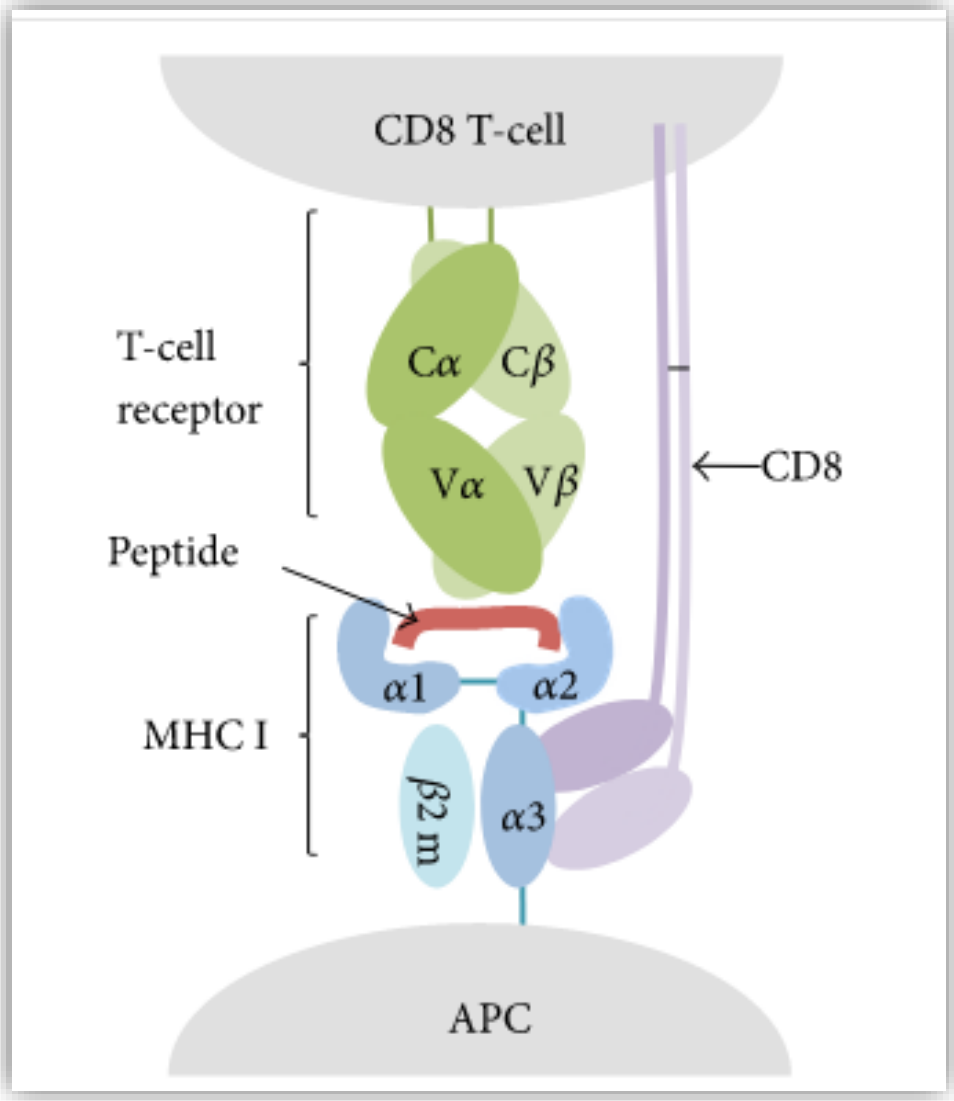
Sequential

Conformational

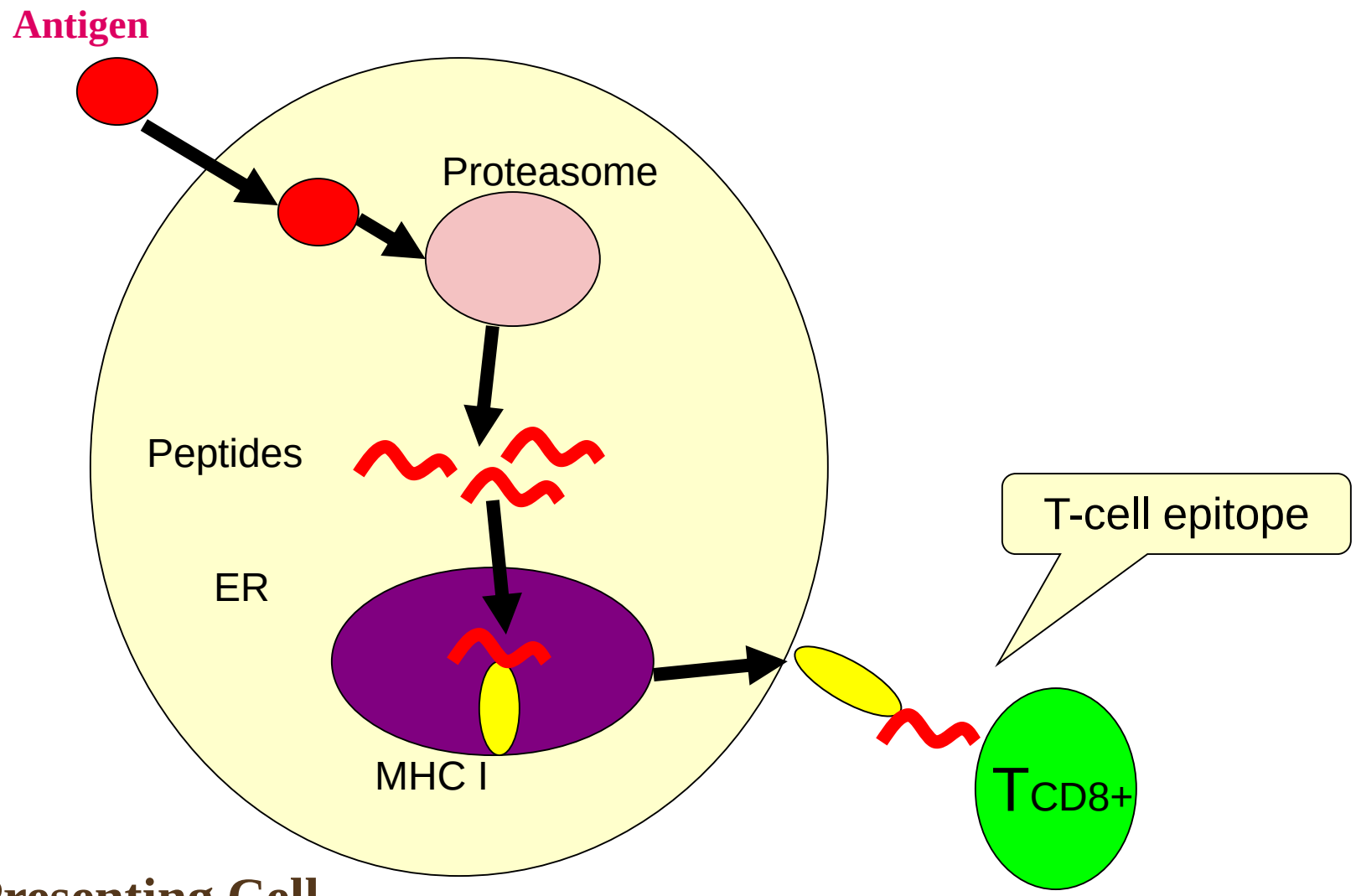
Ab-binding sites

T-cell epitopes recognition

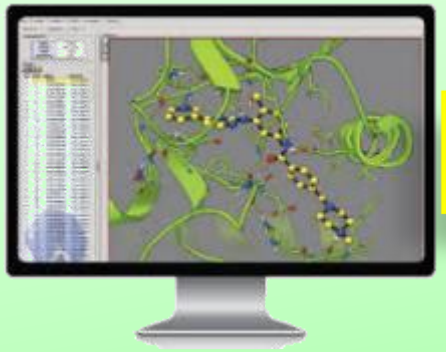




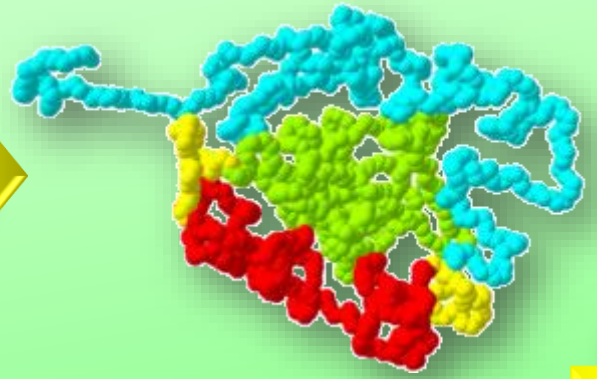
The MHC class I pathway



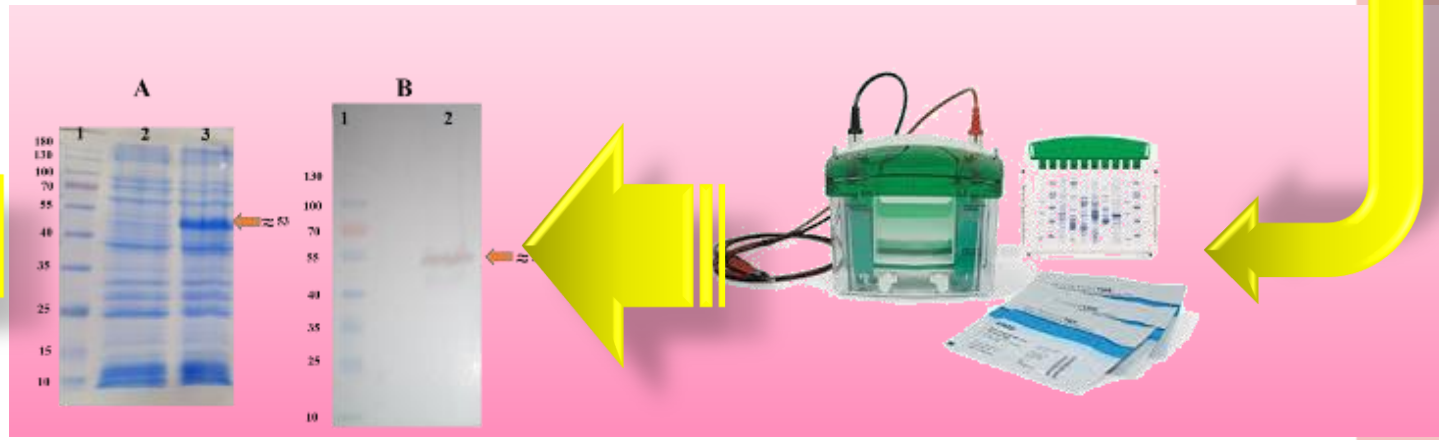
STEP 1 : *In Silico*



MGSSHHHHHHSSGLVPRGSHMQKSVQLGSFDKVVPSREVVSESLAPSFA
VTETHSSVQSPSKQETQLCAISSEGKPCRNRQNGYFIGASCPKSACCSKTMCGP
GGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGHRCDKNAECVENLHTDL
DAGGGVHCKCKDGFVGAEAAAKEAAAKAEDKHEGSWLEQEAAEDVTPLPG
SHTEAQTQSPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLR
RVGTFFRRDIPAAALRFFRRFRRVRAEAAAKEAAAKATALTEPPTLAYSPNRQI
CPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQT
FVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEGP
TTMTLVCGKDGVKVPQDNNQYCSGTTL

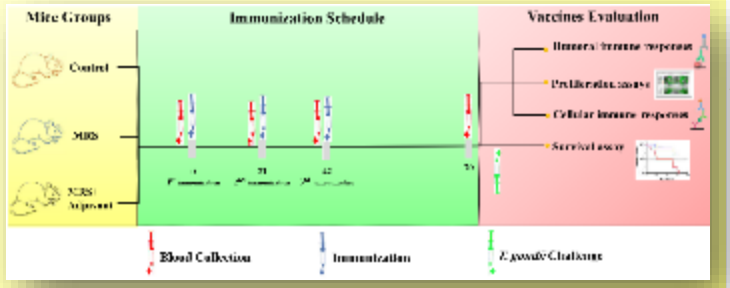


STEP 2 : Production & Purification



STEP 3 : Immunization

survey on immunogenicity in BALB /c mice



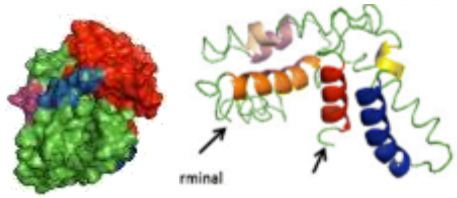
In silico

1 **UniProt and NCBI (Protein sequence)**

2 **Prediction of B-cell and T-cell epitopes**

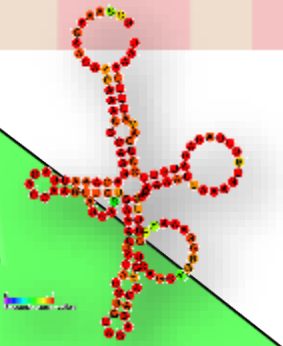
IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

3 **Tertiary structure prediction**



4 **Optimization of the chimeric gene**

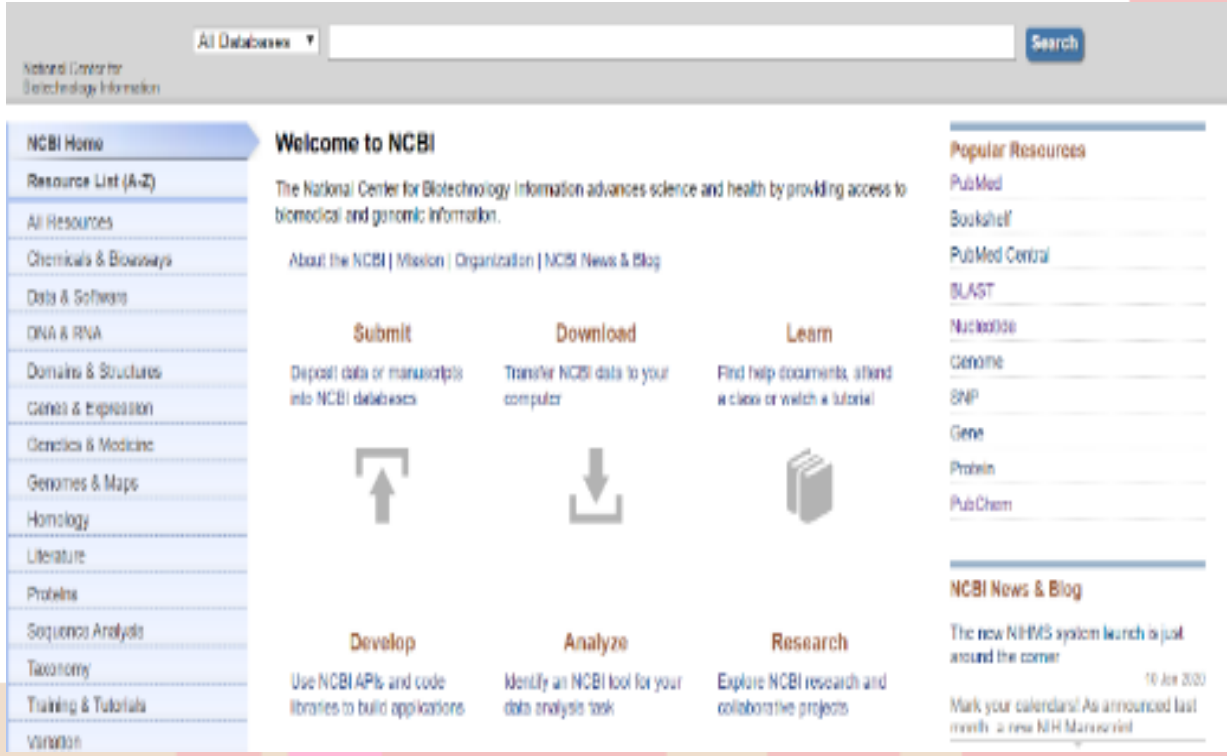
5 **mRNA structure prediction**



UniProt AND NCBI (Protein sequence)

https://www.uniprot.org/

https://www.ncbi.nlm.nih.gov/



UniProt (Protein sequence)

Similar proteins

90% Identity | 50% Identity

Protein	Similar proteins		Species	Score	Length	Source
Q86GT0	Major surface antigen p30		TOXGO	●●○○○	336	UniRef90_P13664
	SAG-related sequence SRS29B		TOXGG	●○○○○	336	
	SAG-related sequence SRS29B		Toxoplasma gondii FOU	●○○○○	336	
	SAG-related sequence SRS29B		Toxoplasma gondii CAST	●○○○○	336	
	SAG-related sequence SRS29B		Toxoplasma gondii TgCATBr9	●○○○○	336	
	+50					

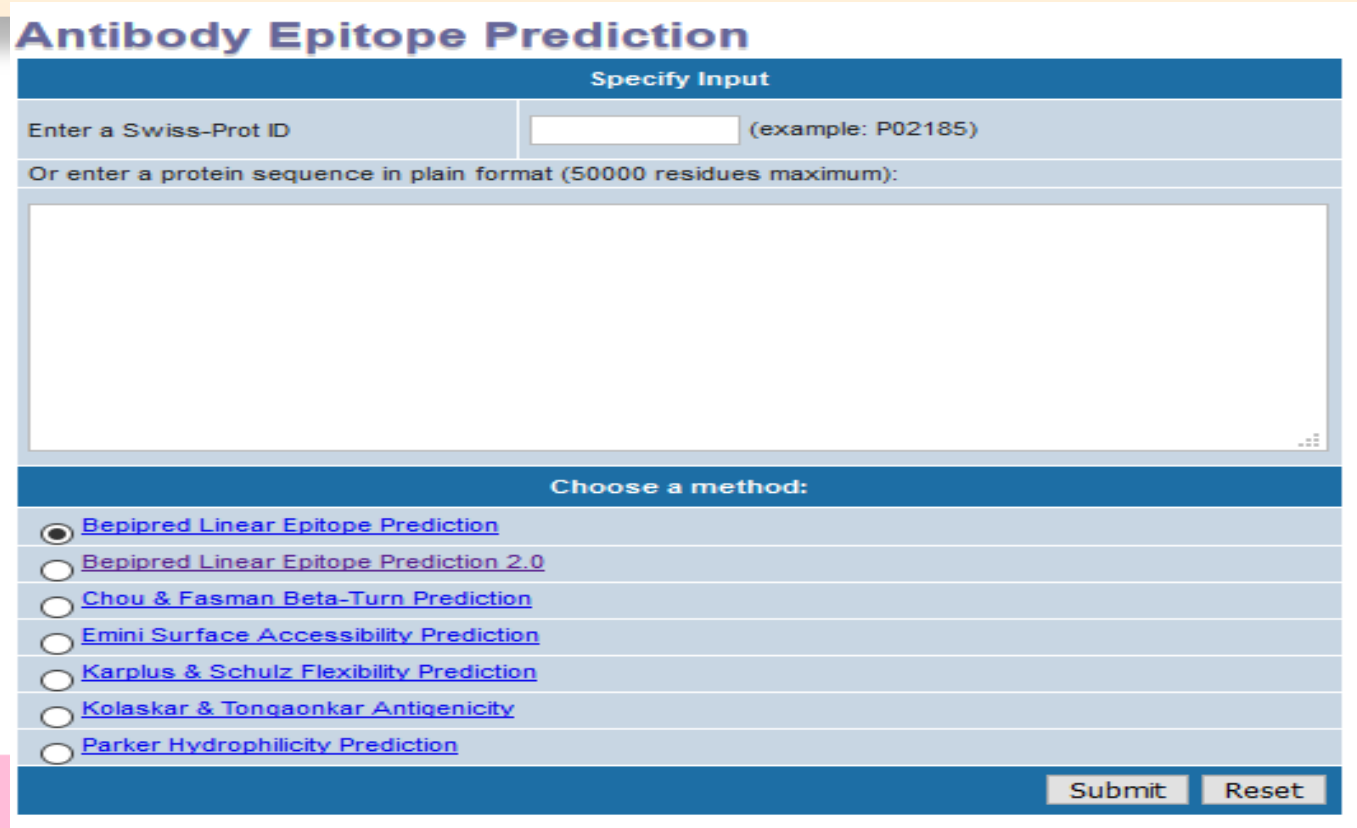
Full view

Name of Protein	Code of Protein	Amino acid length of Protein	Length of Signal Peptide
SAG1	C7E5T3	336	1-26

Prediction of B-cell Epitope

1 The B-cell epitopes of proteins were predicted using **IEDB** and **BCPREDS**, available at http://tools.iedb.org/bcell/ and http://ailab.ist.psu.edu/bcpreds/predict.html, respectively.

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

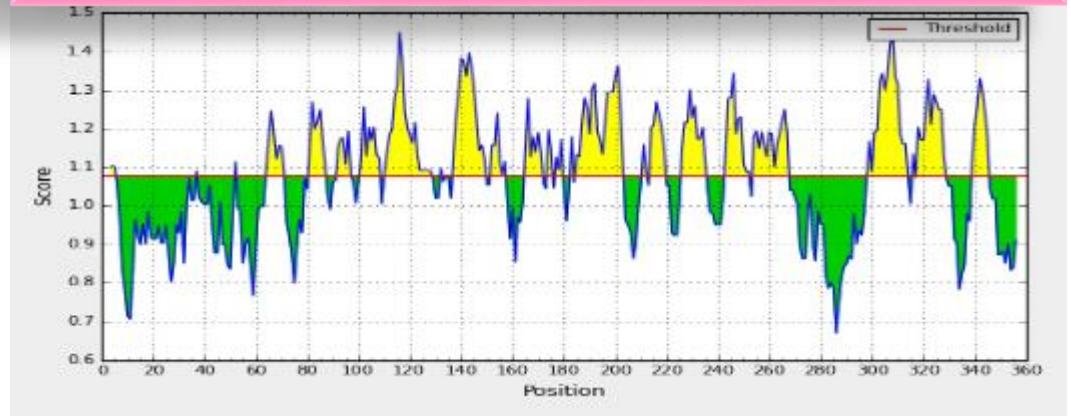


Prediction of B-cell Epitope

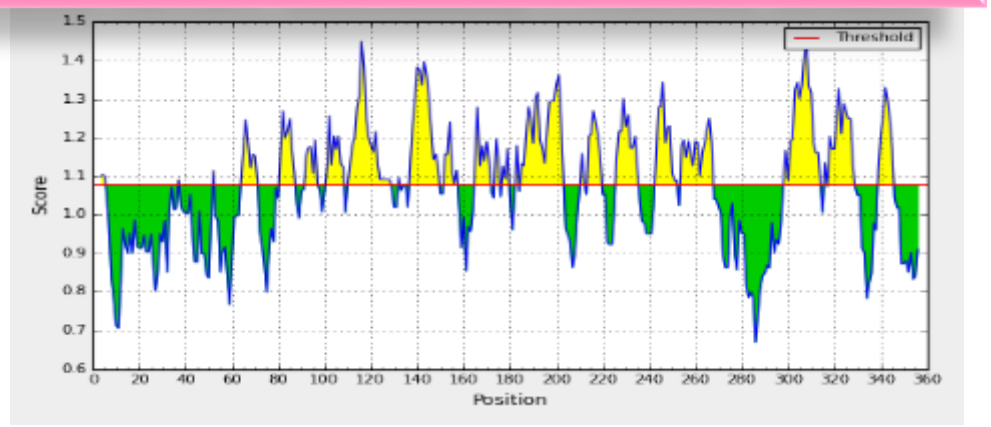
Tool	Web site	Reference
LEPS	http://leps.cs.ntou.edu.tw/	Wang et al. (*28*)
BayesB	http://www.immunopred.org/bayesb/	Wee et al. (*31*)
COBEpro	http://scratch.proteomics.ics.uci.edu	Sweredoski & Baldi (*30*)
BCPREDS/ FBCPRED	http://ailab.cs.iastate.edu/bcpreds	El-Manzalawy et al. (*27, 29*)
ABCpred	http://www.imtech.res.in/raghava/abcpred	Saha & Raghava (24)
BepiPred	http://www.cbs.dtu.dk/services/BepiPred	Larsen et al. (*25*)
Bcepred	http://www.imtech.res.in/raghava/bcepred	Saha & Raghava (*22*)
BEPITOPE/ PREDITOP	Standalone for Windows systems	Odorico & Pellequer (*20*)

Prediction of B-cell epitopes (SAG1)

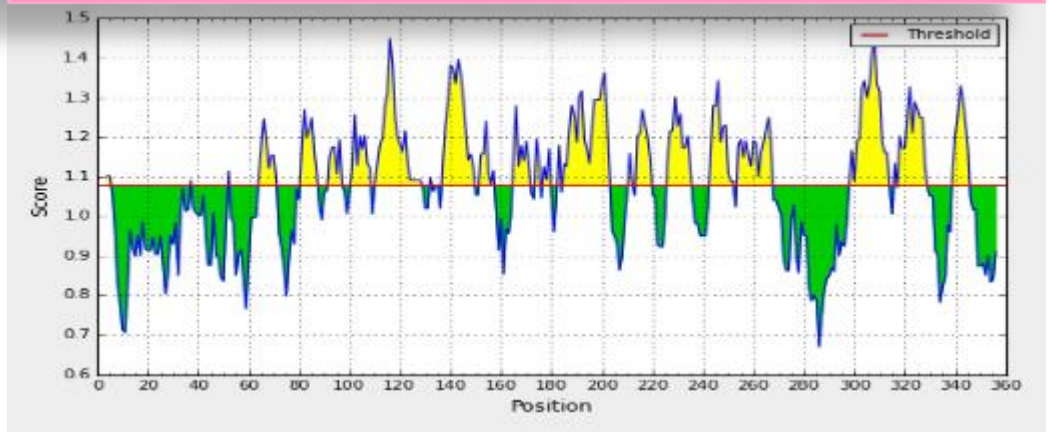
Bepipred Linear Epitope Prediction Results



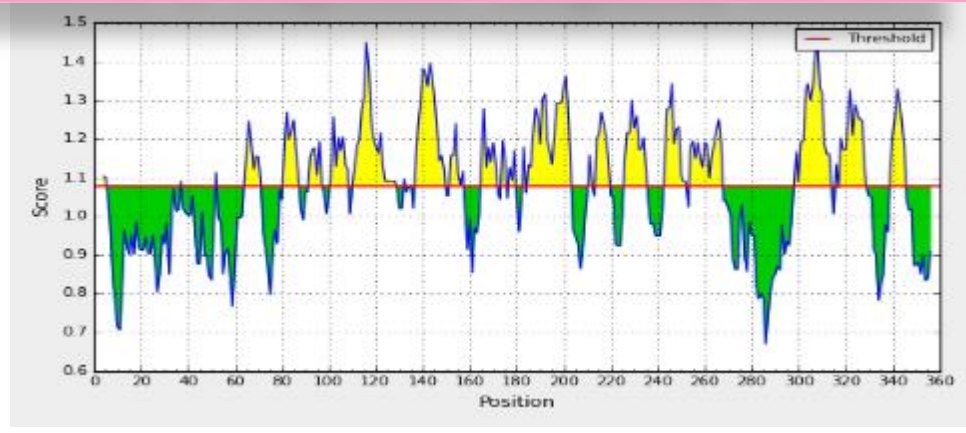
Emini Surface Accessibility Prediction Results



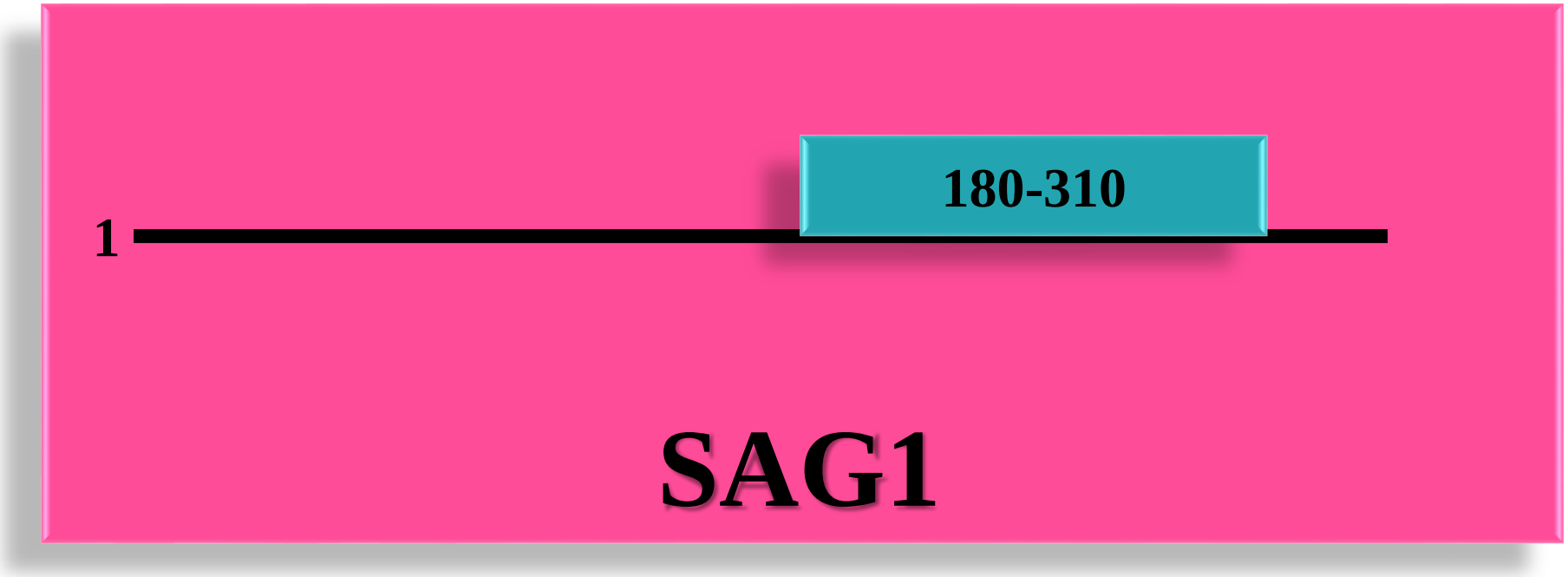
Karplus & Schulz Flexibility Prediction Results



Kolaskar & Tongaonkar Antigenicity Results



Prediction of B-cell epitopes (SAG1)



Prediction of T-cell Epitope

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

2

The **IEDB** online service (http://cvc.dfci.harvard.edu/balbc/) was employed to predict the affinity of peptides that bind to the **major histocompatibility complex (MHC) class I and class II molecules**.

http://cvc.dfci.harvard.edu/balbc/

PRED$^{BALB/c}$

Description | Help | Contact Us

For prediction of MHC binding peptides in a protein, select the MHC alleles of your interest and paste the protein sequence in the textbox.
Predict peptides binding to

- ○ All $H2^d$ alleles
- ○ $H2^d$ class I alleles
- ○ $H2^d$ class II alleles
- ◉ Single $H2^d$ allele [I-Ed ⌄]

ENTER YOUR PROTEIN SEQUENCES: (Please use input sequence of less than 2000 amio acids.)
Click here for the input data format

- ◉ Protein sequence ○ A list of peptide sequences

SEQUENCE NAME:

Submit Reset

For details of the system, please refer to the paper below:
Zhang,G.L., Srinivasan,K.N., Veeramani,A., August,J.T., Brusic,V. (2005) PRED$^{BALB/c}$: a system for prediction of peptide binding to the H2d molecules, a haplotype of the BALB/c mouse. Nucleic Acids Res. 33, W180 -W183.

Prediction of T-cell epitopes

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

MHCI												MHCII							
Prediction to H2-K^d				Prediction to H2-Dd				Prediction to H2-Ld				Prediction to I-Ed				Prediction to I-Ad			
Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction
1	335	RFIFPFDLV	9.7	1	59	RSPGGASPR	10	1	111	AQTQSPSAF	8.62	1	165	FFRRDIPAA	9.9	1	53	EAAVSVRSP	9.7
2	482	TFSFDAWAL	9.7	2	249	IGQPFRVES	10	2	70	HSPIEPVAF	8.44	2	177	FFRRFRRVR	9.9	2	153	TRILQYLRR	9.7
3	436	VFLTGFEHL	9.6	3	288	TGESFEVHV	10	3	17	GSSSCLIWL	7.9	3	309	KQMKQEVLR	9.9	3	291	SFEVHVPLS	9.7
4	369	LYPRMQTNL	9.6	4	369	LYPRMQTNL	10	4	186	QPVFPPDEF	7.8	4	404	LQVIRLLAG	9.9	4	390	THKSLVHHA	9.7
5	233	YMQSAADSL	9.58	5	536	RYPQENRLL	10	5	251	QPFRVESEL	7.8	5	332	VHLRFIFPF	9.8	5	464	LEARRATIS	9.7
6	29	FFVSALGHV	9.32	6	148	RPPLRTRIL	9.94	6	377	LQTLGEVLL	7.8	6	128	FWRRGVTGG	9.8	6	524	PQPVRALLA	9.7
7	484	SFDAWALGL	9.28	7	433	RGGVFLTGF	9.88	7	494	IYWIWCADL	7.8	7	423	FRAVDIVLD	9.8	7	19	SSCLIWLAA	9.6
8	68	HFHSPIEPV	9.24	8	522	NIPQPVRAL	9.86	8	523	IPQPVRALL	7.8	8	492	LVIYWIWCA	9.8	8	167	RRDIPAAAL	9.6

Prediction of T-cell epitopes (MIC3)

MHCI												MHCII							
Prediction to H2-K^d				Prediction to H2-D^d				Prediction to H2-L^d				Prediction to I-E^d				Prediction to I-A^d			
Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction
1	19	WMCTP AEAL	9.16	1	113	CGPGGC GEF	10	1	67	SPSKQE TQL	10	1	189	PCSKRG NAK	9.88	1	6	SALLHA LTF	9.7
2	133	LIYHPD KSY	9.08	2	217	GDGETL VNL	10	2	48	VSESLA PSF	7.5	2	266	GYSREV TSK	9.8	2	55	SFAVTE THS	9.7
3	127	IFCSSSL IY	8.3	3	345	PRYHA HTVT	10	3	27	LPIQKS VQL	7.5	3	329	LSEKM NIVF	9.8	3	72	ETQLCA ISS	9.7
4	126	WIFCSS SLI	8.04	4	82	GKPCRN RQL	9.9	4	104	KSACCS KTM	7.5	4	75	LCAISSE GK	9.7	4	280	VEGVEV TLA	9.7
5	204	CIVVDS VSY	7.32	5	341	SGYHPR YHA	9.7	5	300	SSCKCD NGY	7.5	5	172	CKCKD GFVG	9.62	5	294	EFGISAS SC	9.7
6	328	SLSEKM NIV	7.1	6	265	TGYSRE VTS	9.7	6	310	GSASAT SHH	7.5	6	332	KMNIVF KCP	9.6	6	132	SLIYHP DKS	9.6
7	34	QLQSFD KVV	6.98	7	21	CTPAEA LPI	9.6	7	329	LSEKM NIVF	7.5	7	287	LAEKCE KEF	9.5	7	124	SNWIFC SSS	9.6
8	175	KDGFVG TGL	6.18	8	198	CGPNGT CIV	9.5	8	339	CPSGYH PRY	7.5	8	37	SFDKVV PSR	9.5	8	130	SSSLIYH PD	9.6
9	44	SREVVS ESL	6.12	9	94	NGYFIG ASC	9.4	9	340	PSGYHP RYH	7.5	9	280	VEGVEV TLA	9.5	9	347	YHAHT VTCE	9.6
10	1	MRGGT SALL	6.1	10	306	NGYSGS ASA	9.4	10	342	GYHPRY HAH	7.5	10	282	GVEVTL AEK	9.4	10	24	AEALPI QKS	9.52
11	37	SFDKVV PSR	6.1	11	133	LIYHPD KSY	9.4	11	83	KPCRNR QLH	7.4	11	338	KCPSGY HPR	9.4	11	203	TCIVVD SVS	9.5

20- 180

MIC3

Prediction of T-cell epitopes (ROP8)

MHCI												MHCII							
Prediction to H2-K^d				Prediction to H2-Dd				Prediction to H2-Ld				Prediction to I-Ed				Prediction to I-Ad			
Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction
1	335	RFIFPF DLV	9.7	1	59	RSPGG ASPR	10	1	111	AQTQS PSAF	8.62	1	165	FFRRDI PAA	9.9	1	53	EAAVS VRSP	9.7
2	482	TFSFDA WAL	9.7	2	249	IGQPFR VES	10	2	70	HSPIEP VAF	8.44	2	177	FFRRFR RVR	9.9	2	153	TRILQY LRR	9.7
3	436	VFLTGF EHL	9.6	3	288	TGESFE VHV	10	3	17	GSSSCL IWL	7.9	3	309	KQMK QEVLR	9.9	3	291	SFEVH VPLS	9.7
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5	233	YMQSA ADSL	9.58	5	536	RYPQE NRLL	10	5	251	QPFRV ESEL	7.8	5	332	VHLRFI FPF	9.8	5	464	LEARR ATIS	9.7
6	29	FFVSAL GHV	9.32	6	148	RPPLRT RIL	9.94	6	377	LQTLGE VLL	7.8	6	128	FWRRG VTGG	9.8	6	524	PQPVR ALLA	9.7
7	484	SFDAW ALGL	9.28	7	433	RGGVF LTGF	9.88	7	494	IYWIW CADL	7.8	7	423	FRAVDI VLD	9.8	7	19	SSCLIW LAA	9.6
8	68	HFHSPI EPV	9.24	8	522	NIPQPV RAL	9.86	8	523	IPQPVR ALL	7.8	8	492	LVIYWI WCA	9.8	8	167	RRDIPA AAL	9.6

60- 185

ROP8

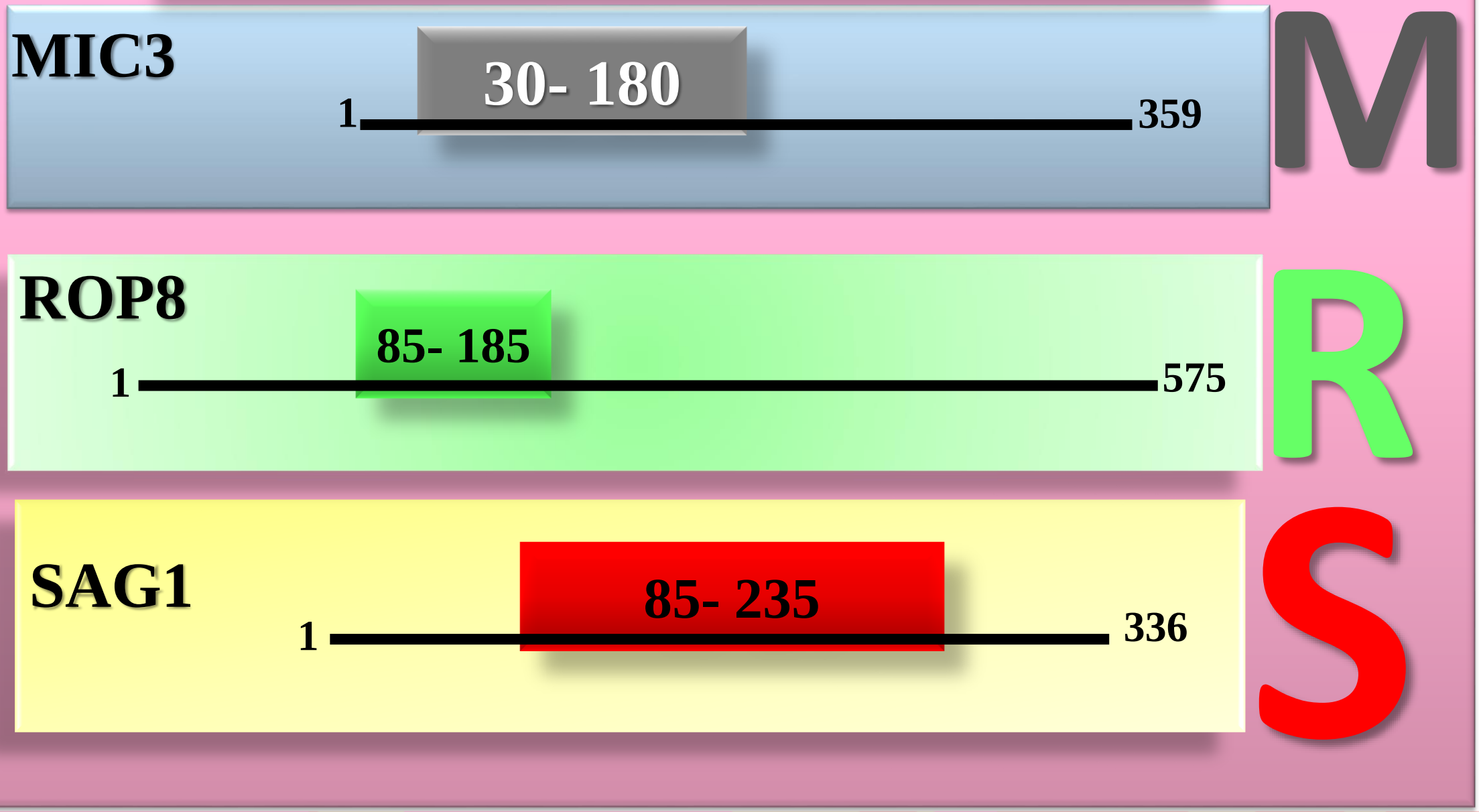
Prediction of T-cell epitopes (SAG1)

MHCII												MHCI							
Prediction to H2-K^d				Prediction to H2-L^d				Prediction to H2-D^d				Prediction to I-E^d				Prediction to I-A^d			
Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction	Rank	Position	Peptide	Prediction
1	320	IFAMVI GLI	9.8	1	315	ASHVSI FAM	8.8	1	259	ASSDKG ATL	10	1	73	PTENHF TLK	10	1	24	RRAVT AAVF	9.7
2	242	SFKDIL PKL	9.7	2	259	ASSDKG ATL	7.82	2	149	KFPVTT QTF	9.86	2	241	KSFKDI LPK	9.9	2	28	TAAVF AAPT	9.7
3	7	HFIISSG FL	9.7	3	38	MSFLRC GVM	7.8	3	198	LGPVKL SAE	9.8	3	22	AVRRA VTAA	9.8	3	112	SKAVTL SSL	9.7
4	273	AFPAES KSV	9.6	4	227	NQYCS GTTL	7.8	4	312	AGTAS HVSI	9.8	4	142	KLTVPI EKF	9.6	4	140	GIKLTV PIE	9.7
5	45	VMASD PPLV	9.58	5	72	TPTENH FTL	7.64	5	206	EGPTTM TLV	9.7	5	221	KVPQD NNQY	9.6	5	169	SCMVT VTVQ	9.7
6	79	TLKCPK TAL	9.5	6	203	LSAEGP TTM	7.5	6	139	AGIKLT VPI	9.6	6	110	CTSKA VTLS	9.6	6	171	MVTVT VQAR	9.7
7	37	LMSFLR CGV	9.18	7	11	SSGFLT SMF	7.5	7	190	CSYGA DSTL	9.5	7	271	KEAFPA ESK	9.6	7	173	TVTVQ ARAS	9.7
8	135	SLDTAG IKL	8.82	8	190	CSYGA DSTL	7.5	8	93	LAYSPN RQI	9.44	8	157	FVVGCI KGD	9.5	8	167	AQSCM VTVT	9.6
9	212	TLVCG KDGV	8.3	9	195	DSTLGP VKL	7.5	9	227	NQYCS GTTL	9.44	9	171	MVTVT VQAR	9.5	9	96	SPNRQI CPA	9.6

105- 225

SAG1

Prediction of B-cell AND T-cell epitopes



Effect of linker length and residues on the structure and stability of a fusion protein with vaccine application

Flexible

Rigid

Clevable

لینکرهای proline-rich نسبتا rigid هستند، بنابراین stability و biological activity در recombinant fusion pro دارند.

Fusion peptide design

Three immunodominant peptide domains associated with the **B-cell** and **T-cell** immunity types from proteins **SAG1, MIC3**, **ROP8** and with different segment arrangements were joined together via a **helix-forming linker** containing the

$A(EAAAK)_2A$ motif to produce various constructs.

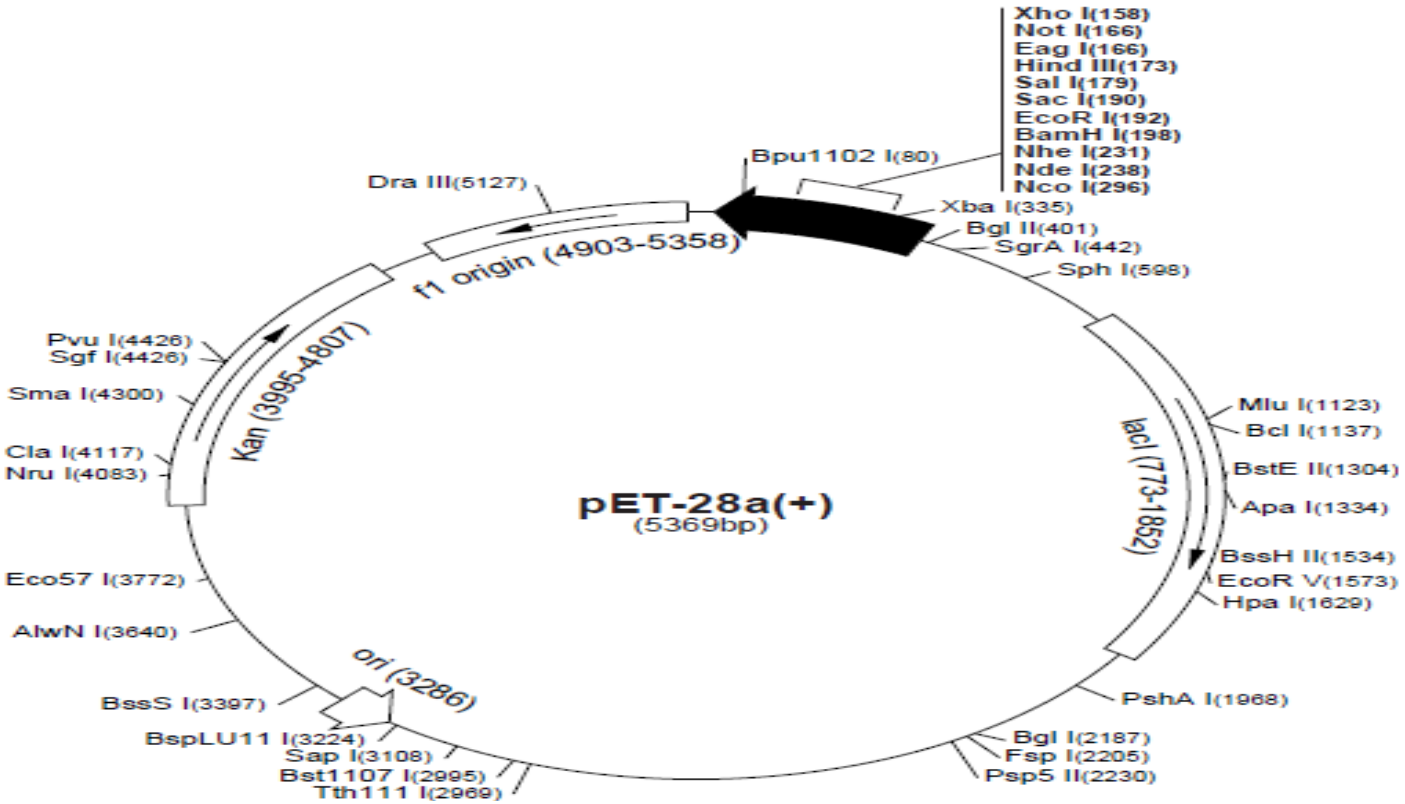
A(EAAAK)nA]m (n=2–4, m=1 or 2)
n= 2, m= 1

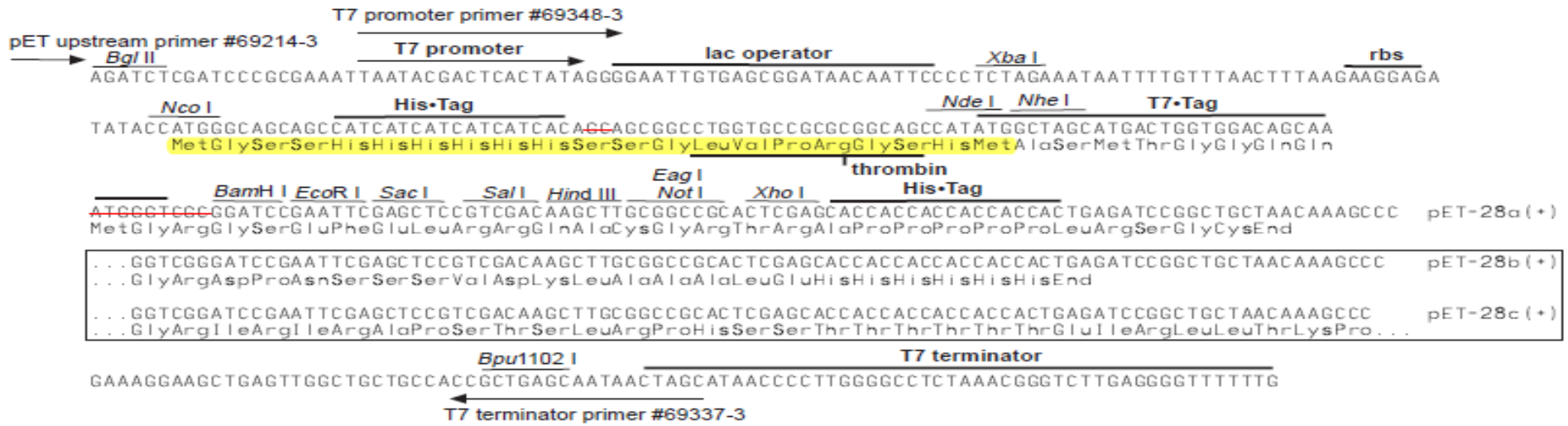
Linker

pET-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.





pET-28a-c(+) cloning/expression region

MIC3, ROP8, SAG1

1

MGSSHHHHHHSSGLVPRGSHMEDKHEGSWLEQEAAEDVTPLPGSHTEAQTQSPSAFRRLTRRLRFWRRG
VTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRRFRRVRAEAAAKEAAAKATALTEPPTL
AYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQTFVVGCIKGDDAQ
SCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEGPTTMTLVCGKDGVKVPQDNNQYCSGTTLAEAA
AKEAAAKAQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCAISSEGKPCRNRQLHTDN
GYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGHRCDKNAECVENLDAG
GGVHCKCKDGFVG

2

MGSSHHHHHHSSGLVPRGSHMEDKHEGSWLEQEAAEDVTPLPGSHTEAQTQSPSAFRRLTRRLRFWRRG
VTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRRFRRVRAEAAAKEAAAKAQKSVQLGS
FDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCAISSEGKPCRNRQLHTDNGYFIGASCPKSACCSKT
MCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGHRCDKNAECVENLDAGGGVHCKCKDGFVGAE
AAAKEAAAKATALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEK
FPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEGPTTMTLVCGKDGV
KVPQDNNQYCSGTTL

3

MGSSHHHHHHSSGLVPRGSHMTALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSA
SLDTAGIKLTVPIEKFPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEG
PTTMTLVCGKDGVKVPQDNNQYCSGTTLAEAAAKEAAAKAQKSVQLGSFDKVVPSREVVSESLAPSFAVTET
HSSVQSPSKQETQLCAISSEGKPCRNRQLHTDNGYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYH
PDKSYGGDCSCEKQGHRCDKNAECVENLDAGGGVHCKCKDGFVGAEAAAAKEAAAKAEDKHEGSWLEQEA
AEDVTPLPGSHTEAQTQSPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIP
AAALRFFRRFRRVR

4

MGSSHHHHHHSSGLVPRGSHMTALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSA
SLDTAGIKLTVPIEKFPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEG
PTTMTLVCGKDGVKVPQDNNQYCSGTTLAEAAAKEAAAKAEDKHEGSWLEQEAAEDVTPLPGSHTEAQTQS
PSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRRFRRVRAEAA
AKEAAAKAQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCAISSEGKPCRNRQLHTDN
GYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGHRCDKNAECVENLDAG
GGVHCKCKDGFVG

5

MGSSHHHHHHSSGLVPRGSHMQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCAIS
SEGKPCRNRQLHTDNGYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGH
RCDKNAECVENLDAGGGVHCKCKDGFVGAEAAAKEAAAKATALTEPPTLAYSPNRQICPAGTTSSCTSKAVT
LSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVAR
CSYGADSTLGPVKLSAEGPTTMTLVCGKDGVKVPQDNNQYCSGTTLAEAAAKEAAAKAEDKHEGSWLEQE
AAEDVTPLPGSHTEAQTQSPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDI
PAAALRFFRRFRRVR

6

MGSSHHHHHHSSGLVPRGSHMQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCAIS
SEGKPCRNRQLHTDNGYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGH
RCDKNAECVENLDAGGGVHCKCKDGFVGAEAAAKEAAAKAEDKHEGSWLEQEAAEDVTPLPGSHTEAQTQ
SPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRRFRRVRAEA
AAKEAAAKATALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEKF
PVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEGPTTMTLVCGKDGVK
VPQDNNQYCSGTTL

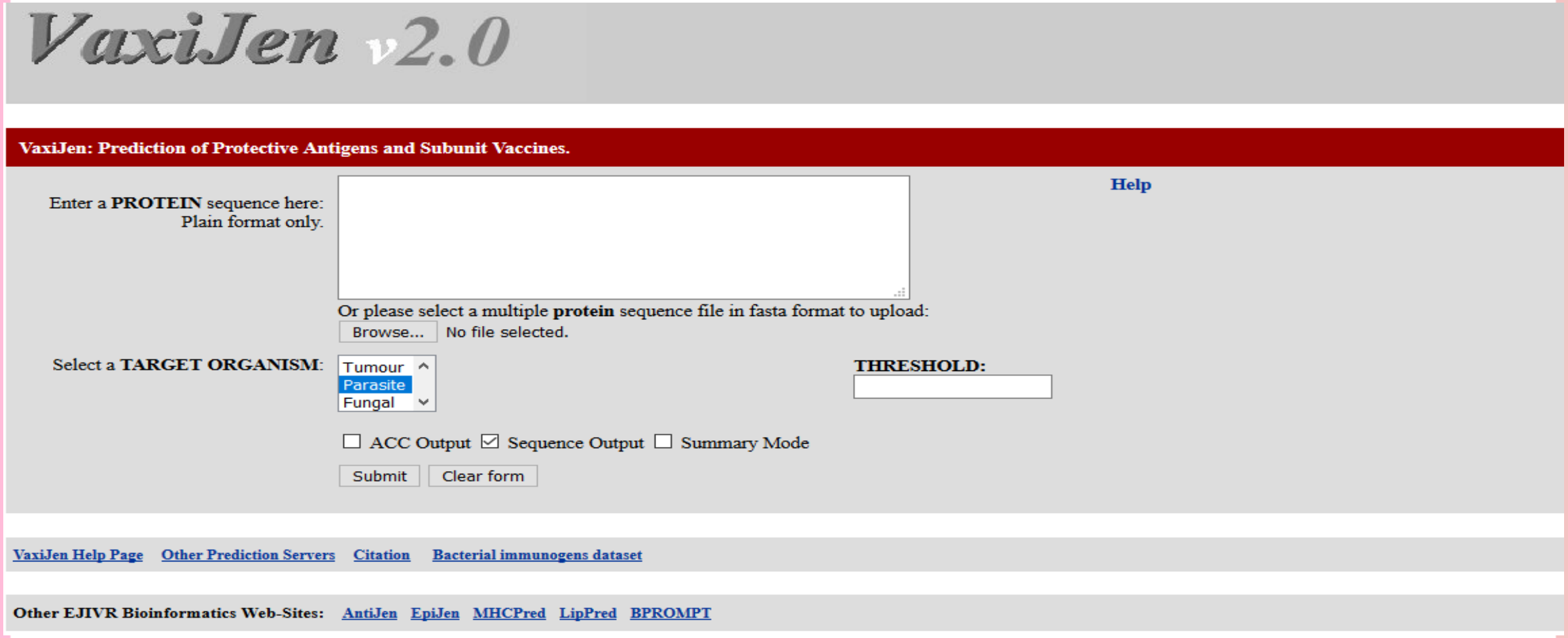
Antigenicity and Allergenicity evaluation

- Antigenicity analysis of different constructs was conducted using **VaxiJen v.2.0**, which is freely available online at http://www.jenner.ac.uk/VaxiJen and can perform antigenicity prediction solely based on the **physicochemical properties** of protective antigens regardless of the sequence length and alignment. **Threshold scores of more than 0.5 were assumed to indicate probable antigens.**

- The web server **AlgPred** (http://www.imtech.res.in/raghava/algpred/) was used to predict the allergenicity of the sequences.

The several structures were selected

VaxiJen is the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment.



1

MGSSHHHHHHSSGLVPRGSHMQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCA
ISSEGKPCRNRQLHTDNGYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGH
RCDKNAECVENLDAGGGVHCKCKDGFVGAEAAAKEAAAKATALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLS
SLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQTFVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYG
ADSTLGPVKLSAEGPTTMTLVCGKDGVKVPQDNNQYCSGTTLAEAAAKEAAAKAEDKHEGSWLEQEAAEDVT
PLPGSHTEAQTQSPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFF
RRFRRVR

Probable Antigenicity: **0.7962**

2

MGSSHHHHHHSSGLVPRGSHMQKSVQLGSFDKVVPSREVVSESLAPSFAVTETHSSVQSPSKQETQLCA
ISSEGKPCRNRQLHTDNGYFIGASCPKSACCSKTMCGPGGCGEFCSSNWIFCSSSLIYHPDKSYGGDCSCEKQGH
RCDKNAECVENLDAGGGVHCKCKDGFVGAEAAAKEAAAKAEDKHEGSWLEQEAAEDVTPLPGSHTEAQTQS
PSAFRRLTRRLRFWRRGVTGGSDAGEEAPQTPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRRFRRVRAEAAAK
EAAAKATALTEPPTLAYSPNRQICPAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLDTAGIKLTVPIEKFPVTTQT
FVVGCIKGDDAQSCMVTVTVQARASSVVNNVARCSYGADSTLGPVKLSAEGPTTMTLVCGKDGVKVPQDNNQ
YCSGTTL

Probable Antigenicity: **0.7983**

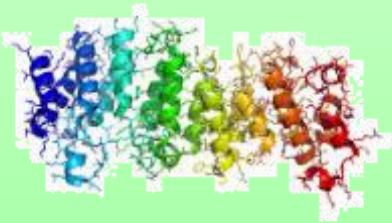
Tertiary structure prediction

I-TASSER
Protein Structure & Function Predictions

❖ **3D structures** of the designed sequences were predicted by online service:

I-TASSER service (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) .

❖ **I-TASSER** was used to determine the **confidence score (C-score).**



❖ The structural models was analyzed in Molegra Molecular Viewer.

Online Services
- I-TASSER
- QUARK
- LOMETS
- COACH
- COFACTOR
- MetaGO
- MUSTER
- SEGMER
- FG-MD
- ModRefiner
- REMO
- SPRING
- COTH
- BSpred
- SVMSEQ
- ANGLOR
- BSP-SLIM
- SAXSTER
- ThreaDom
- ThreaDomEx
- EvoDesign
- GPCR-I-TASSER
- BindProf
- BindProfX
- ResQ
- IonCom
- STRUM
- TM-score
- TM-align
- MM-align
- NW-align
- LS-align
- EDTSurf

I-TASSER

Protein Structure & Function Predictions

(The server completed predictions for 437777 proteins submitted by 104627 users from 140 countries)
(The template library was updated on 2018/12/17)

I-TASSER (Iterative Threading ASSEmbly Refinement) is a hierarchical approach to protein structure and function prediction. It first identifies structural templates from the PDB by multiple threading approach LOMETS, with full-length atomic models constructed by iterative template-based fragment assembly simulations. Function insights of the target are then derived by threading the 3D models through protein function database BioLiP. I-TASSER (as 'Zhang-Server') was ranked as the No 1 server for protein structure prediction in recent community-wide CASP7, CASP8, CASP9, CASP10, CASP11, CASP12, and CASP13 experiments. It was also ranked as the best for function prediction in CASP9. The server is in active development with the goal to provide the most accurate structural and function predictions using state-of-the-art algorithms. Please report problems and questions at I-TASSER message board and our developers will study and answer the questions accordingly. (>> More about the server ...)

[Queue] [Forum] [Download] [Search] [Registration] [Statistics] [Remove] [Potential] [Decoys] [News] [Annotation] [About] [FAQ]

I-TASSER On-line Server (View an example of I-TASSER output):

Copy and paste your sequence below ([10, 1500] residues in FASTA format). Click here for a sample input:

Or upload the sequence from your local computer:

Browse... No file selected.

Email: (mandatory, where results will be sent to)

Password: (mandatory, please click here if you do not have a password)

ID: (optional, your given name of the protein)

▶ **Option I**: Assign additional restraints & templates to guide I-TASSER modeling.

▶ **Option II**: Exclude some templates from I-TASSER template library.

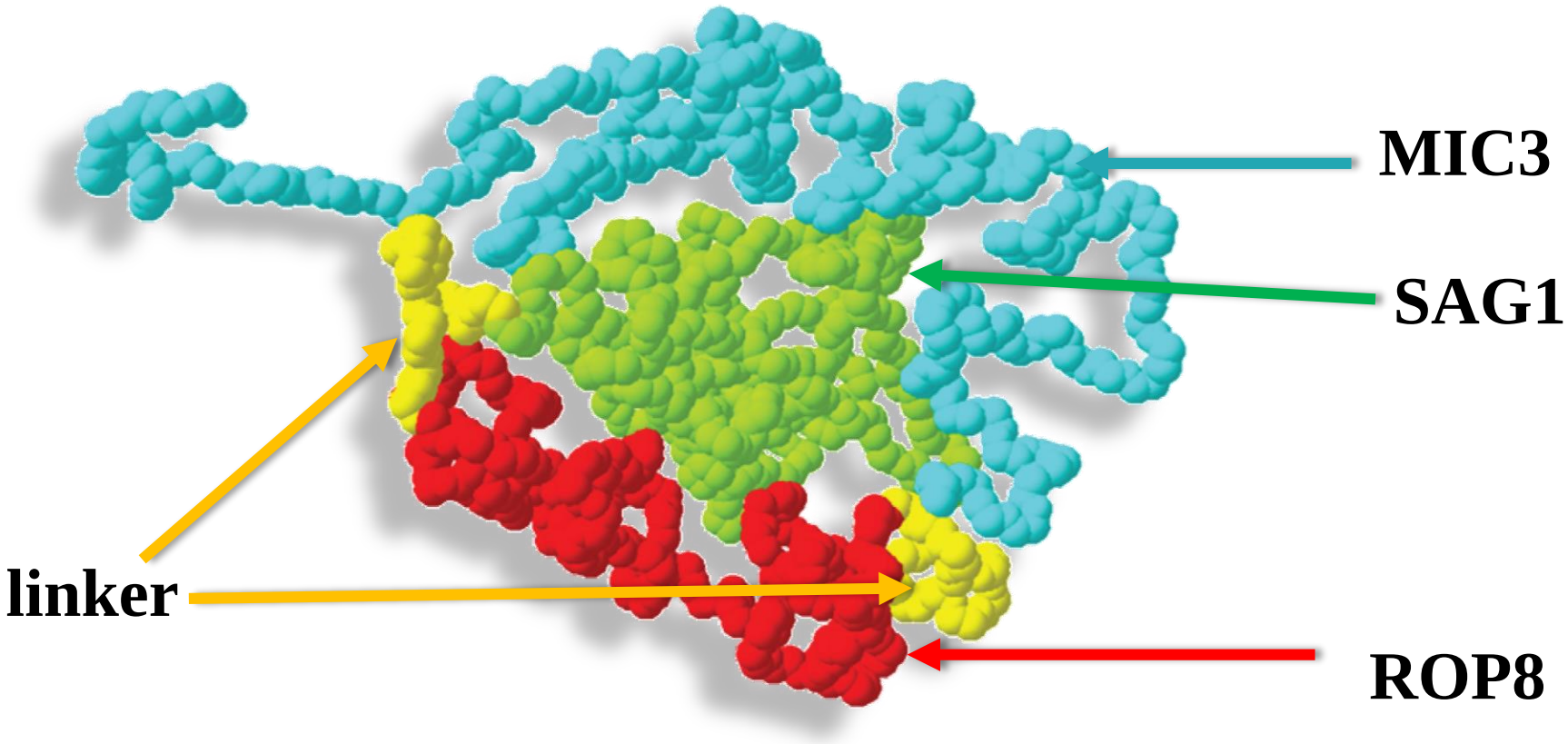
▶ **Option III**: Specify secondary structure for specific residues.

☑ Keep my results public (uncheck this box if you want to keep your job private. A key will be assigned for you to access the results)

Run I-TASSER | Clear form

(Please submit a new job only after your old job is completed)

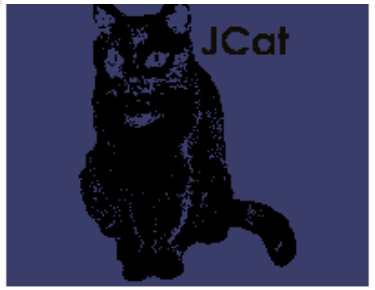
Tertiary structure prediction



Optimization of the chimeric gene

The **Jcat tool** (http://www.prodoric.de/JCat) was employed for reverse translation and codon optimization to determine the optimized DNA sequence of the recombinant protein for cloning and expression in *Escherichia coli* K12.

http://www.jcat.de/



JAVA Codon Adaptation Tool

Bioinformatic tools from our team:

PRODORIC Release 2

JVirGel

PrediSi

JCat was published in NAR (Nucleic Acids Research).

Codon-Adaptation

1. Type/paste sequences below:

```
AQTQSPSAFRRLTRRLRFWRRGVTGGSDAGEEAPQ
TPRPPLRTRILQYLRRVGTFFRRDIPAAALRFFRR
FRRVRAEAAAKEAAAKATALTEPPTLAYSPNRQIC
PAGTTSSCTSKAVTLSSLIPEAEDSWWTGDSASLD
TAGIKLIVPIEKFPVTTQTFVVGCIKGDDAQSCMV
TVTVQARASSVVNNVARCSYGADSTLGPVKLSAEG
PTIMTLVCGKDGVKVPQDNNQYCSGTTL
```

Standard genetic code is used for the input sequence. Click here to change!

2. Specify the pasted Sequence:

- (●) DNA/RNA Sequence
- ( ) Protein Sequence

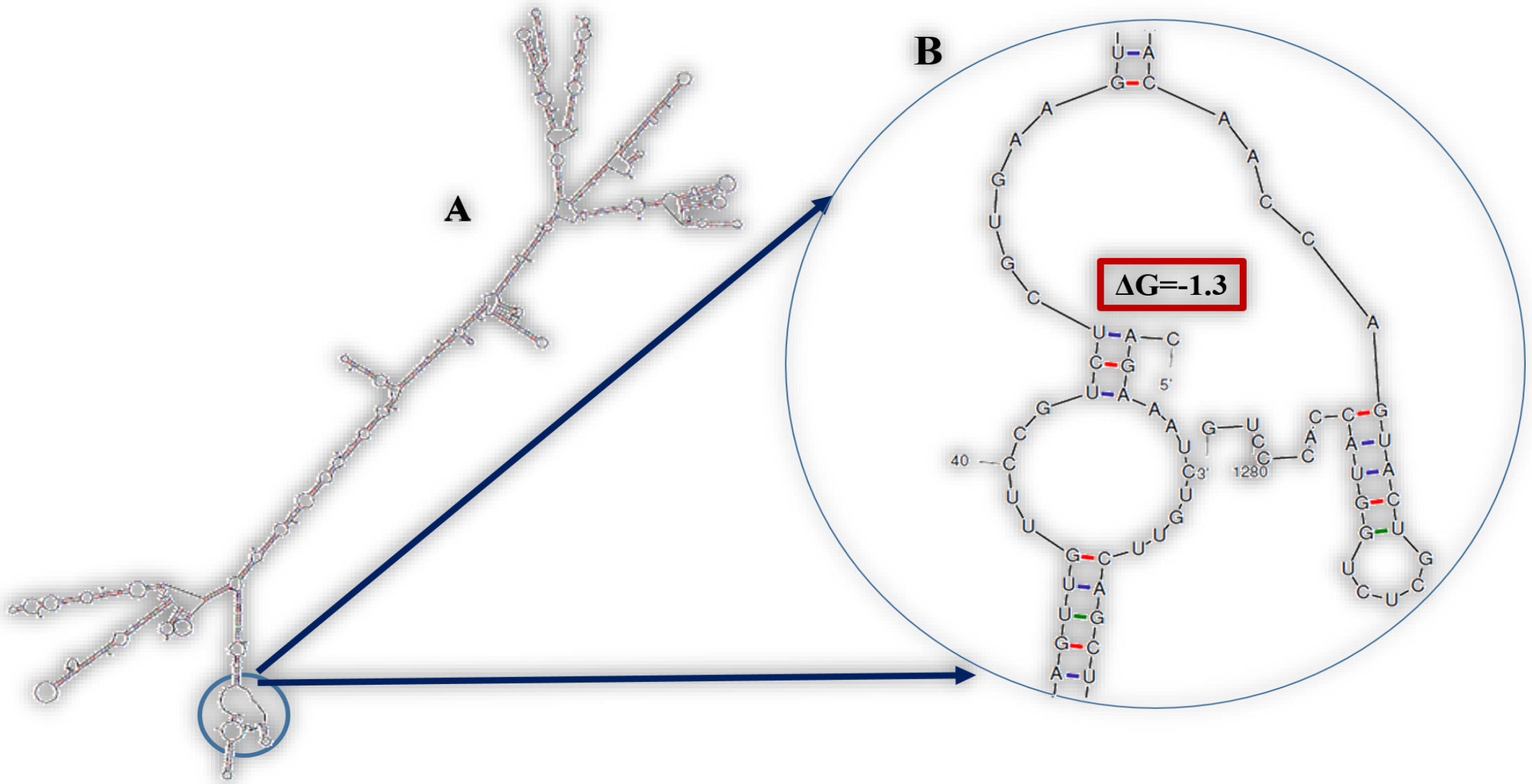
3. Select organism:

Escherichia coli (strain K12)

mRNA structure prediction

The **RNA secondary structure** was predicted with the mfold tool (http://unafold.rna.albany.edu/?q=mfold) to determine the free energy associated with the 5′ end in the mRNA of the chimeric gene.

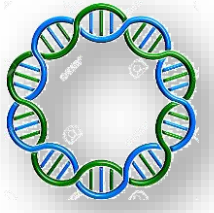
mRNA structure prediction

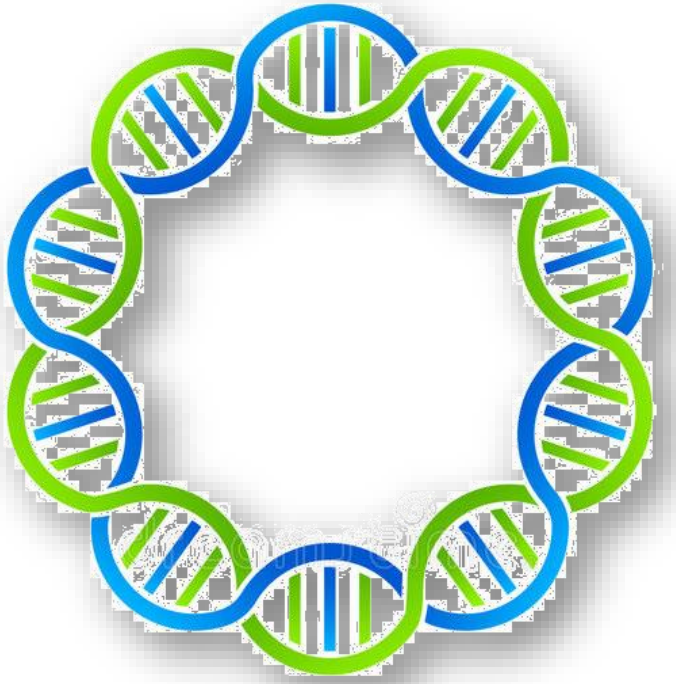


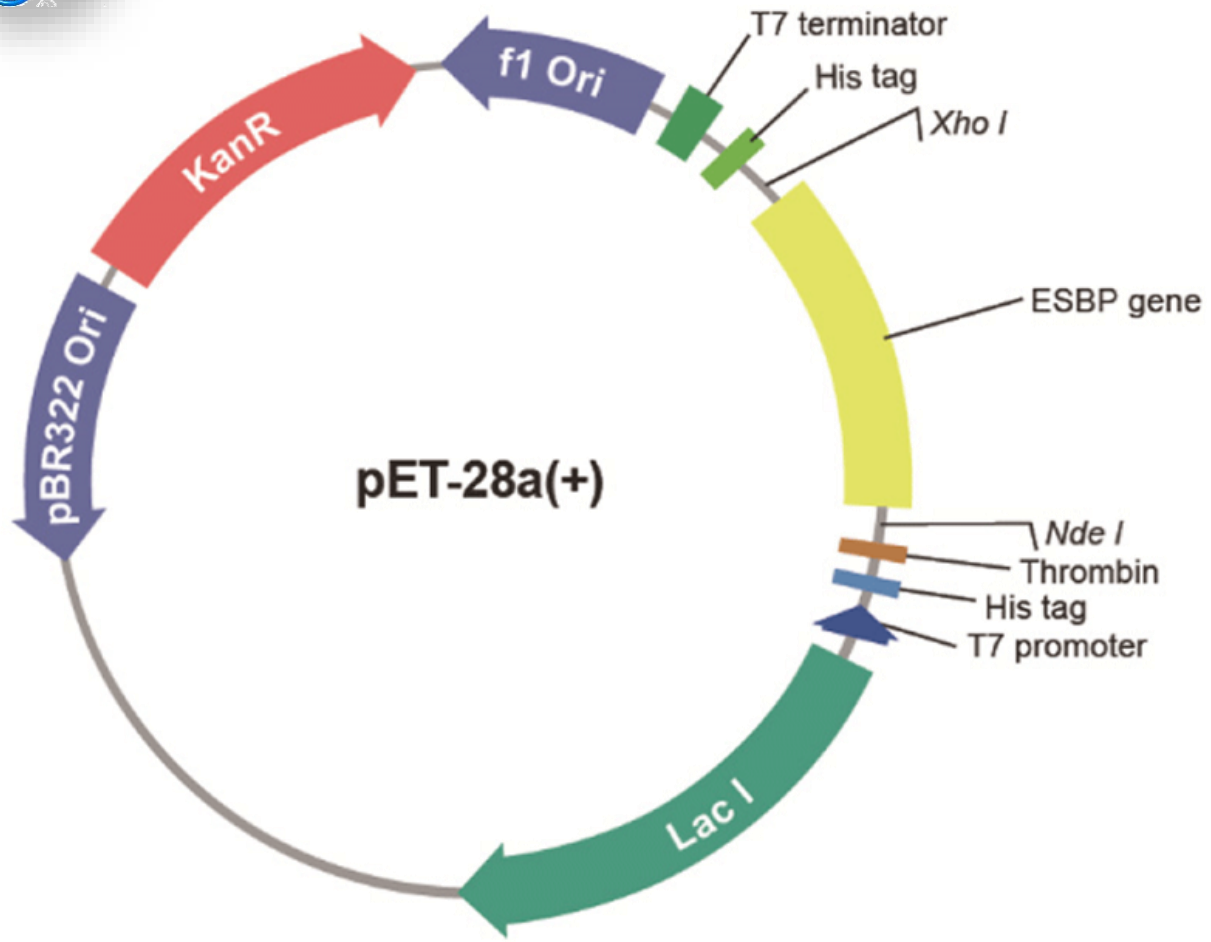
mRNA structure prediction

Stack	-2.90	External closing pair is C 115-G 127
Helix	-5.20	3 base pairs.
Hairpin loop	3.10	Closing pair is C 116-G 126
Stack	-2.30	External closing pair is G 91-C 109
Stack	-0.90	External closing pair is A 92-U 108
Helix	-3.20	3 base pairs.
Hairpin loop	3.90	Closing pair is A 93-U 107
Stack	-1.70	External closing pair is A 2-U 45
Stack	-2.30	External closing pair is G 3-C 44
Helix	-4.00	3 base pairs.
Interior loop	2.70	External closing pair is A 4-U 43
Stack	-1.80	External closing pair is C 13-G 37
Stack	-0.50	External closing pair is A 14-U 36
Stack	-2.10	External closing pair is G 15-U 35
Stack	-1.70	External closing pair is C 16-G 34
Helix	-6.10	5 base pairs.
Interior loop	0.00	External closing pair is U 17-A 33
Stack	-2.10	External closing pair is G 20-C 30
Stack	-1.10	External closing pair is U 21-A 29
Helix	-3.20	3 base pairs.
Hairpin loop	3.50	Closing pair is U 22-G 28

pET-28a Plasmid







- **Numerous studies** identified potential **epitope-based antigens** that could effectively induce high and protective immunity against diverse pathogens.
- The approach has been used to **develop and evaluate vaccines** to various infectious agents, such as Influenza **Virus, Human Immunodeficiency Virus, Epstein-Barr Virus, and hepatitis B virus.**

Potential advantages of using this epitope-based Vaccination Strategy

- It has the ability to **optimize the epitope structure** to **increase potency in eliciting** strong immunity;
- It provides the opportunity to **focus** and **generate specific immune responses** to known immunodominant epitopes;
- It shows **chemical stability**.

Bioinformatic tools remain the vital option for analyzing immunogenic epitopes with high **antigenicity** and **immunogenicity**.


Thanks For your Attention