



Reverse Vaccinology for lactococcosis (Bioinformatics workflow)



The PRIMA programme has been 100% funded by the European Union under the Horizon Europe programme (2021-2027) for the first time. This is the first time that the European Union has funded a research and innovation programme in the Mediterranean area.



Vaccinology in the era of genomics

The concept of reverse vaccinology

- Disease prevention is the most effective approach for health
- Development of vaccines has proved a milestone in prevention of diseases

SUPER TROUT



The PRIMA program is a joint effort of the European Union and the Mediterranean countries.

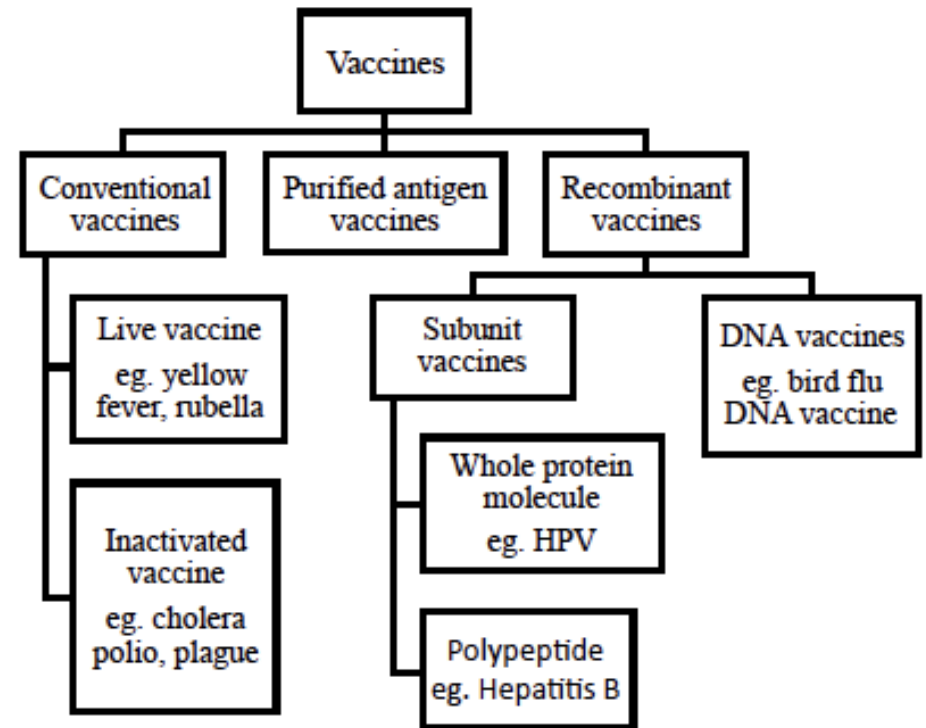
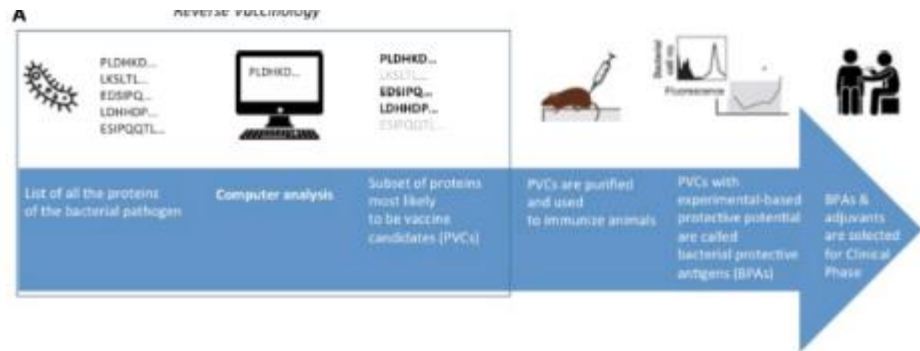


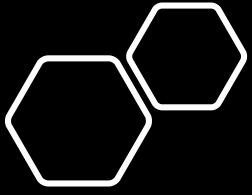
Figure 1: Classification of vaccines.



- Reverse Vaccinology (RV) is a genome-based approach developed for the first time in early 1990's by Rappuoli to identify protein vaccine candidates against meningococcus

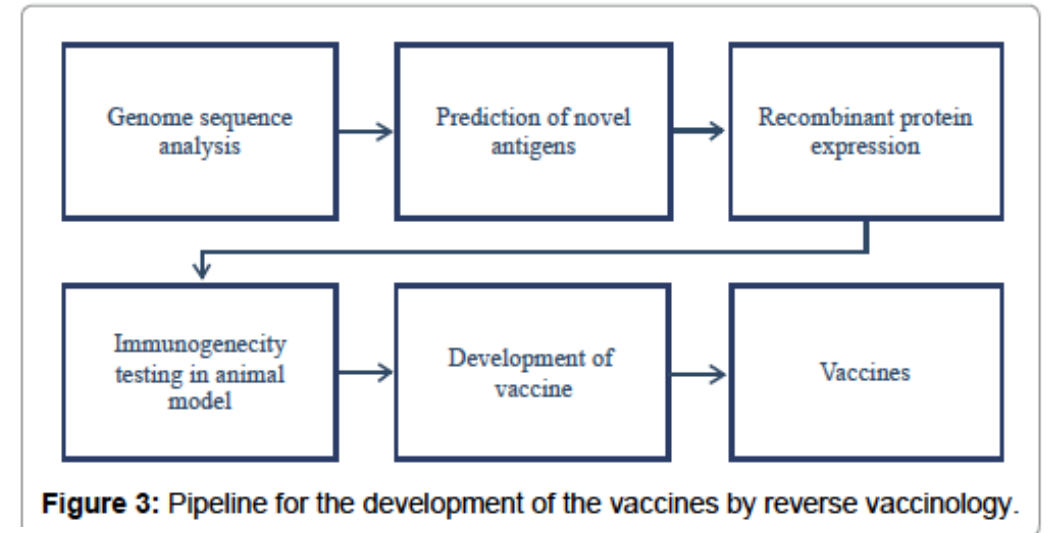
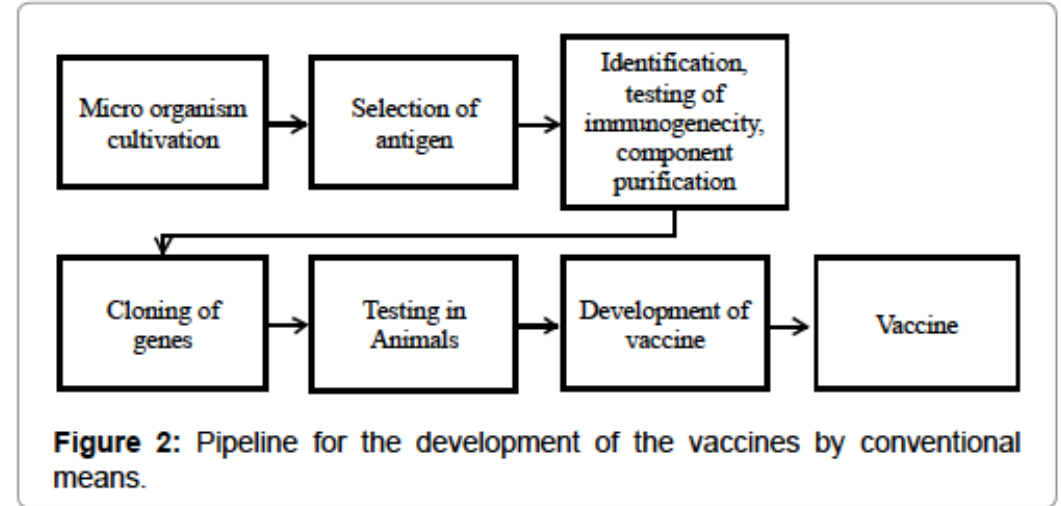


- RV approach adopts computerized screening of protein sequences from the pathogen as the first step of the process, to select a subset of promising antigens, as potential vaccine candidates
- offers two main advantages compared to traditional vaccine development approaches: (i) identification of candidate antigens without the need to grow the pathogen (ii) identification of any antigen independently by its purified quantity to be suitable for vaccine testing.



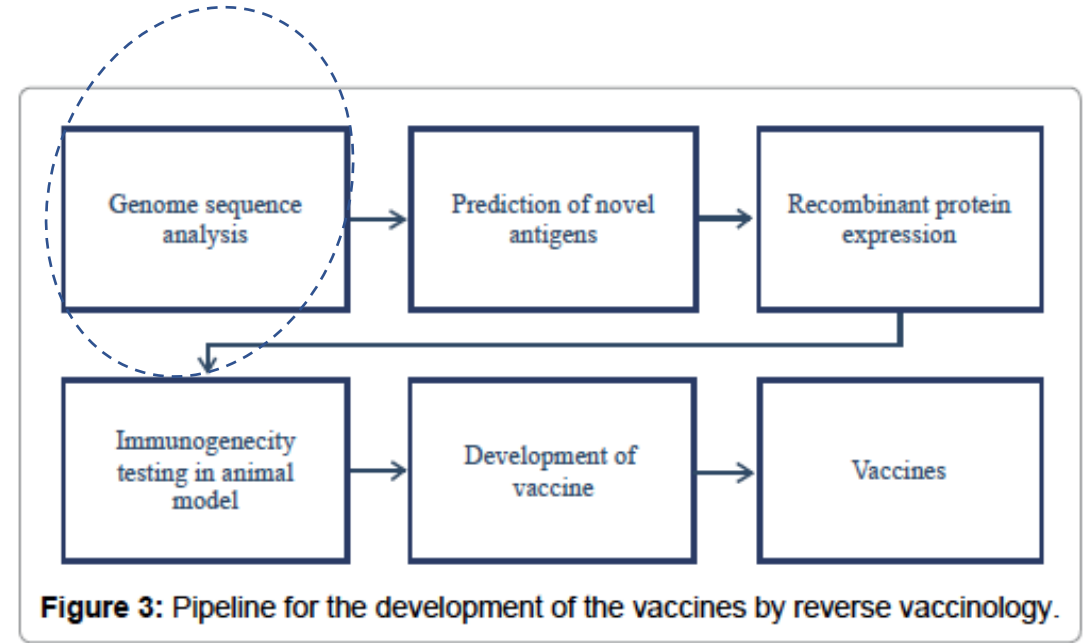
The use of genomic information with aid of computer for the preparation of vaccines without culturing microorganism is known as reverse vaccinology (RV).

- RV explores the protein coding sequences that can be used as a potential target for vaccine preparation





- The genome sequences provide at once all protein antigens that the pathogen can express at any time. What follows:
 - 1. Genome sequences
 - 2. Computer analysis
 - 3. Prediction of epitope/ antigen
 - 4. construction of candidate vaccine





Role of Epitope Prediction in Reverse Vaccinology

The original idea behind reverse vaccinology:

To **screen in silico** the entire genome of a pathogen to **identify epitopes** in proteins which are suitable vaccine targets, for example, proteins that are predicted to be surface exposed and are well conserved between strains .

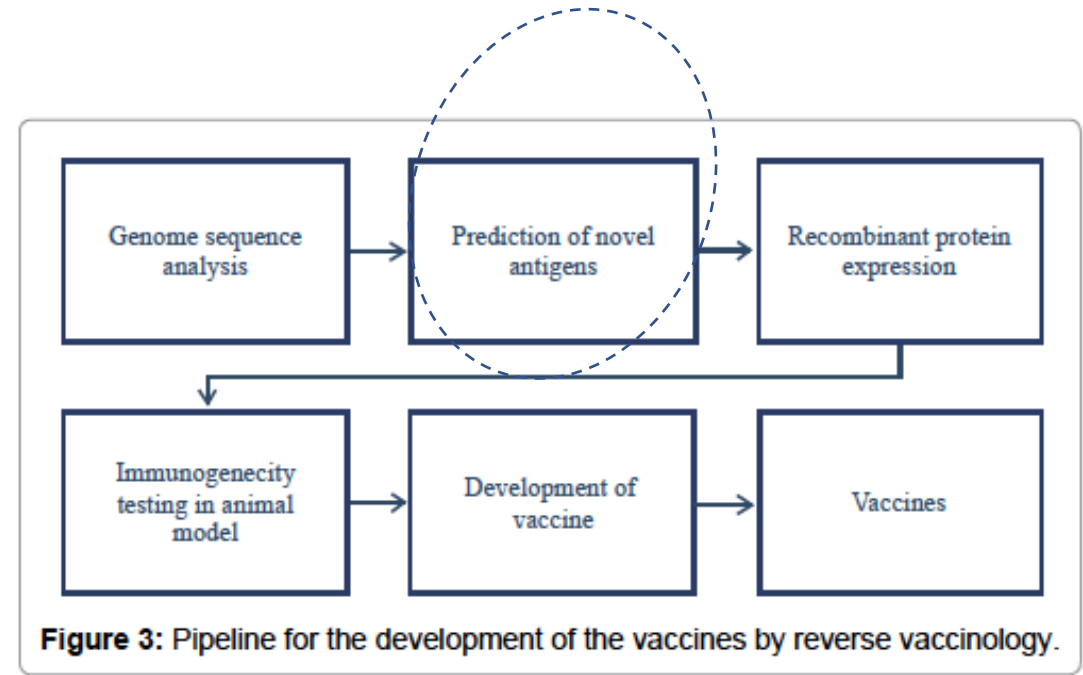


Figure 3: Pipeline for the development of the vaccines by reverse vaccinology.



- **Role of Epitope Prediction in Reverse Vaccinology**

How can we screen **in silico** for new epitopes?

- Improved sequencing technologies have greatly increased the number of genomes available and reverse vaccinology has evolved to include the analysis of several genomes within a species (**pan-genomic analysis**)
- Between closely related species-(**comparative-genomic analysis**)
- or between pathogenic and commensal members of the same species (**comparative/subtractive genomics**)

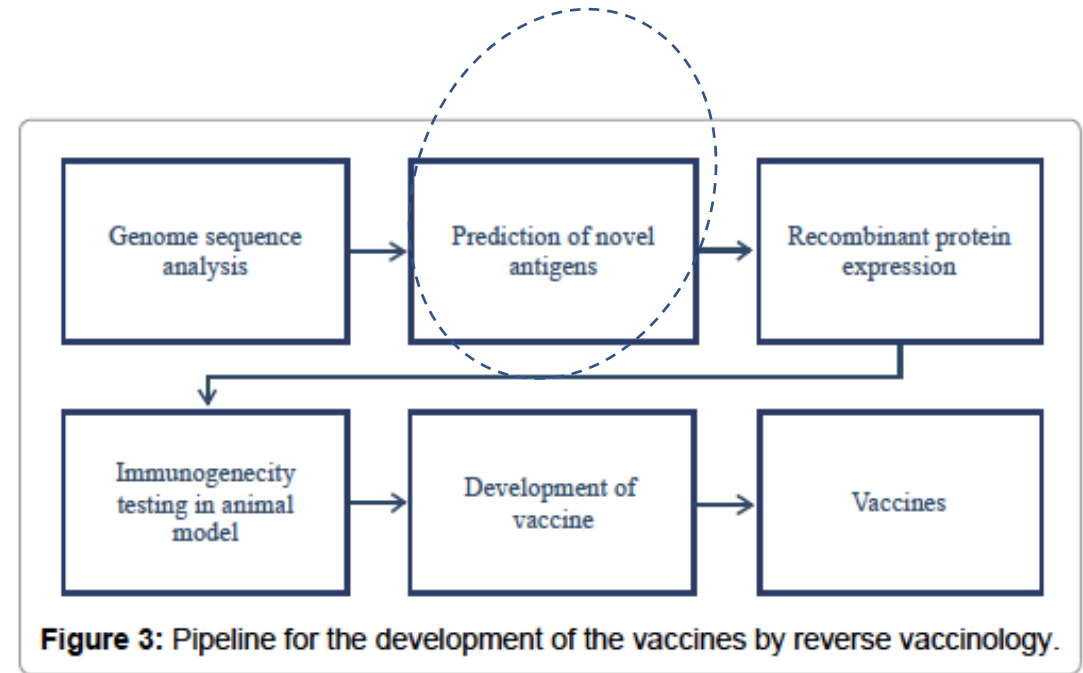


Figure 3: Pipeline for the development of the vaccines by reverse vaccinology.

Role of Epitope Prediction in Reverse Vaccinology

- The identification of antigens to induce immunogenic responses is crucial for the development of an effective vaccine. An epitope is an antigenic determinant that plays an important role in immunity of an organism.
- What kind of epitopes we try to identify?
- **T-Cell Epitope Mapping and Prediction.** T cell recognizes the antigenic peptides only when they are presented by MHC I or II, with the help of the CD4 and CD8 molecules. The target is to predict epitopes against a panel of MHC class I and/or class II alleles.
- **B-Cell Epitope Mapping and Prediction**

The B-cell epitopes are defined by a specific surface region of an antigenic protein and may be divided into two different types of epitopes: linear epitopes and conformational epitopes.

The linear epitopes are short peptides while conformational epitopes composed of amino acid folded in 3- dimensional protein structure

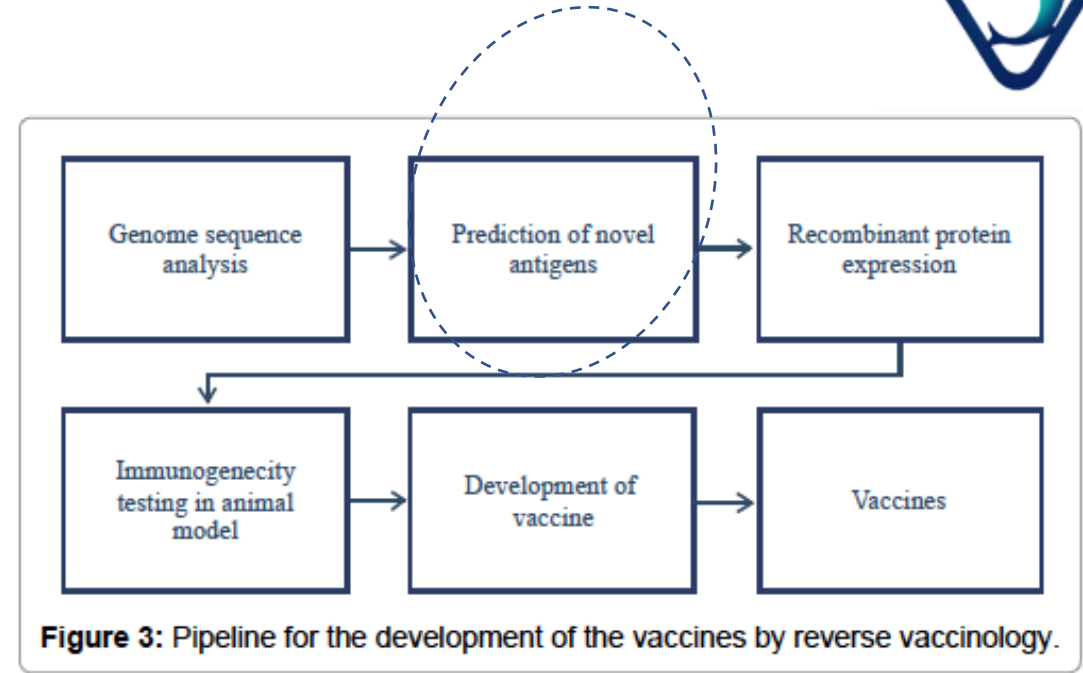


Figure 3: Pipeline for the development of the vaccines by reverse vaccinology.



Reverse Vaccinology for lactococcosis (Bioinformatics workflow)

A case study:

In this work, we are introducing an integrated framework that combines immuno-informatics approaches, bioinformatics tools, and supervised machine learning-based tools for vaccine construction against lactococcosis in supertrouts

To develop a recombinant subunit-protein vaccine against lactococcosis to be administered by immersion.



Objectives

Immunogenic protein identification

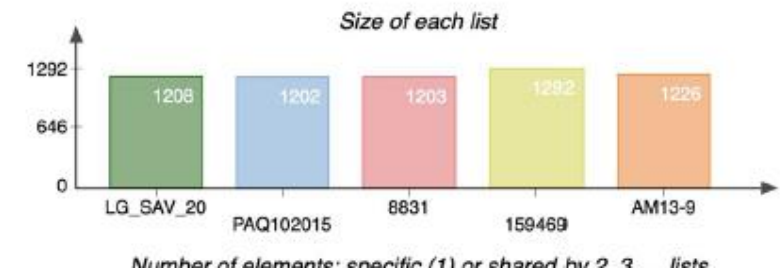
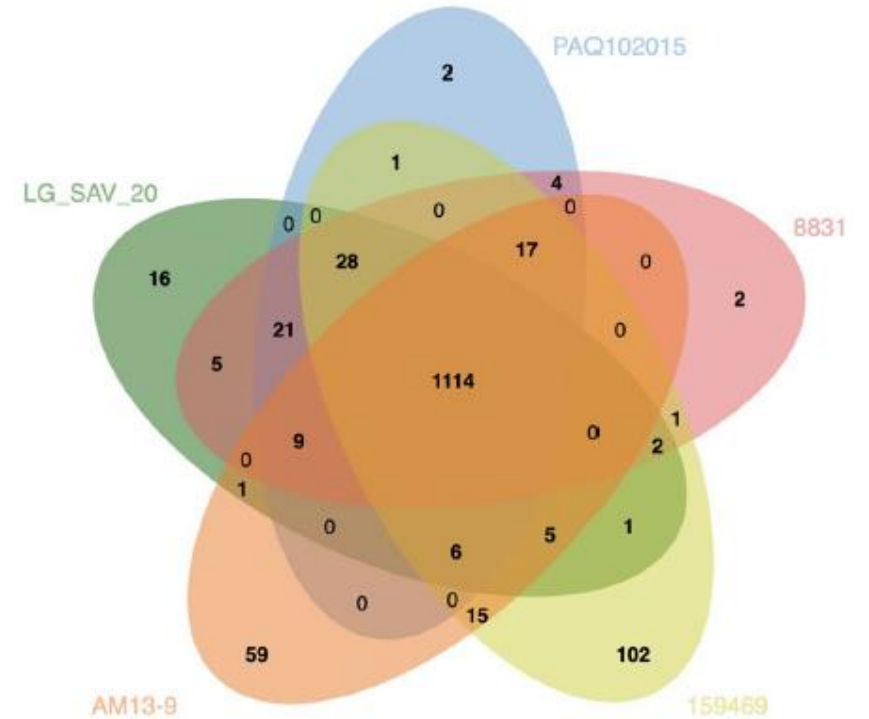
recombinant-protein production

experimental trial for vaccine efficacy



What we know for lactococcosis in rainbow trout

- *Lactococcus garvieae*, a highly diverse species, is considered to be the causative agent of the disease
- However, genomic information provides new insights into the role of novel species: *Lactococcus petauri* as an etiological agent of lactococcosis



Number of elements: specific (1) or shared by 2, 3, ... lists



First steps towards polypeptide vaccine construction

1. Protein selection and identification of highly conserved amino-acid loci
 2. Prediction of Cytotoxic T Lymphocytes epitopes (CTL)
 3. Prediction of Helper T Lymphocytes epitopes (HTL)
 4. Prediction of B cell linear epitopes
 5. Comparative analysis and selection of the best-fitted epitopes
-



Lactococcus petauri strains from Rainbow Trout

In an early approach, we retrieved the core genome data from 6 complete genome sequences of *L. petauri* strains isolated from diseased fish and employed the first steps of a reverse vaccinology analysis for the prediction of potential vaccine candidates. More sequences of *L. garvieae* and *L. petauri* isolated from lactococcosis cases from Greece, Italy, Spain, Turkey will be included in the study.

1. Five *L. petauri* strains isolated and analyzed at VRI in Greece (LG-SAV-20, LG1, LG3, LG5, LG6)
2. One *L. petauri* strain isolated at Faculty of Marine Sciences in Turkey (D375?)



we performed a detailed analysis of the genomic dataset of *L. garvieae* and *L. petauri* to shortlist 7 proteins as possible vaccine antigen candidates using properties such as mucus binding ability, surface-exposed nature, fibrogen-binding

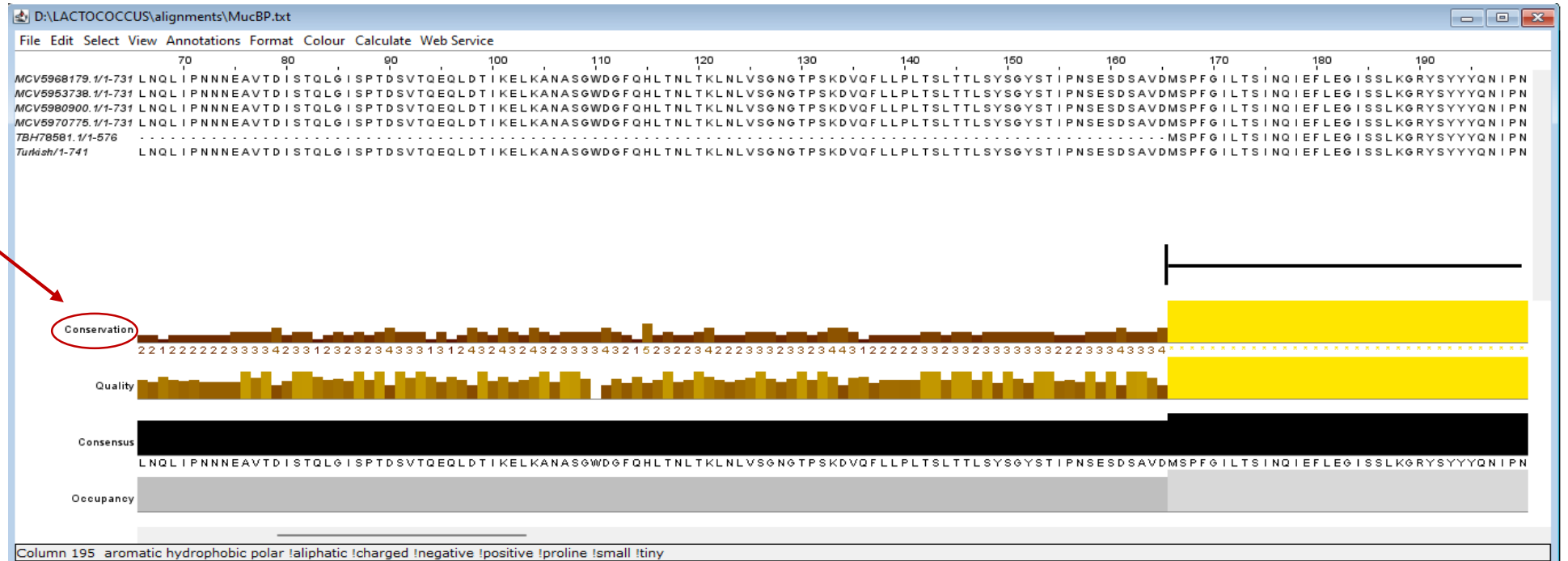
Target Proteins:

1. PsaA, metal ABC transporter substrate-binding protein Cytoplasmic Membrane
2. Muc, mucus binding protein (MucBP domain)
3. Fibronectin/Fibrinogen-binding protein
4. LPXTG cell wall anchor domain-containing protein I (107 AA)
5. LPXTG cell wall anchor domain-containing protein II (225 AA)
6. LPXTG cell wall anchor domain-containing protein III (924 AA)
7. LPXTG cell wall anchor domain-containing protein IV (988 AA)

Multiple alignment of proteins

```

D:\LACTOCOCCUS\proteins from given strains\PsaA.fasta - Notepad++
File Edit Search View Encoding Language Settings Tools Macro Run Plugins Window ?
PsaA.fasta LPXTG 924.fasta LPXTG 976.fasta JAOYNZ01.1.fsa_aa new 1 Fbp.fasta
1 >MCV5968045.1 metal ABC transporter substrate-binding protein [Lactococcus petauri]
2 MNRKQLSLITILASLLFLTACSPKKEESKASDILKVVITYSIIADIAENIGKDHVDVYSMVPRGTDPHQ
3 YDPKPNDTQAVEKADLVFYNGLNLETGKGWFDKLIKNSRKEDSTFMVSGVTPIHLSKSGKSEEDPHAW
4 LNIQNGIITYAQNIKELSKKDPQNKEDYQKNLKVYTDKQLDTEAKAKIATIPQEDRILVTSEGAFKYF
5 SKQYGLTAEYIWEINTDNQGTPAQLNRINTIVKDKNKALFVETSVSPKTMESVSRQTGVKIYSKIIFDTS
6 LADEGQKGDYYDMLQWNIHITDGLSGK
7
8 >MCV5952953.1 metal ABC transporter substrate-binding protein [Lactococcus petauri]
9 MNRKQLSLITILASLLFLTACSPKKEESKASDILKVVITYSIIADIAENIGKDHVDVYSMVPRGTDPHQ
10 YDPKPNDTQAVEKADLVFYNGLNLETGKGWFDKLIKNSRKEDSTFMVSGVTPIHLSKSGKSEEDPHAW
11 LNIQNGIITYAQNIKELSKKDPQNKEDYQKNLKVYTDKQLDTEAKAKIATIPQEDRILVTSEGAFKYF
12 SKQYGLTAEYIWEINTDNQGTPAQLNRINTIVKDKNKALFVETSVSPKTMESVSRQTGVKIYSKIIFDTS
13 LADEGQKGDYYDMLQWNIHITDGLSGK
14
15 >MCV5980937.1 metal ABC transporter substrate-binding protein [Lactococcus petauri]
16 MNRKQLSLITILASLLFLTACSPKKEESKASDILKVVITYSIIADIAENIGKDHVDVYSMVPRGTDPHQ
17 YDPKPNDTQAVEKADLVFYNGLNLETGKGWFDKLIKNSRKEDSTFMVSGVTPIHLSKSGKSEEDPHAW
18 LNIQNGIITYAQNIKELSKKDPQNKEDYQKNLKVYTDKQLDTEAKAKIATIPQEDRILVTSEGAFKYF
19 SKQYGLTAEYIWEINTDNQGTPAQLNRINTIVKDKNKALFVETSVSPKTMESVSRQTGVKIYSKIIFDTS
20 LADEGQKGDYYDMLQWNIHITDGLSGK
21
  
```



Identification of conservative regions

The screenshot shows a sequence viewer with a protein sequence: `.VDMSPFGILTSINQIEFLEGISSLKGRYSYYYQNIPN`. A red bar highlights residues 170-190. A context menu is open over the sequence, with the following options: Selection, Sequence Details, Show annotations, Hide annotations, Add reference annotations, Edit, Output to Textbox..., Create Sequence Feature..., Create Group, and Edit New Group. The 'Output to Textbox...' option is selected, and a sub-menu is open showing the following output formats: Fasta, PFAM, Stockholm, PIR, BLC, AMSA, JSON, PileUp, MSF, Clustal, and PHYLIP.

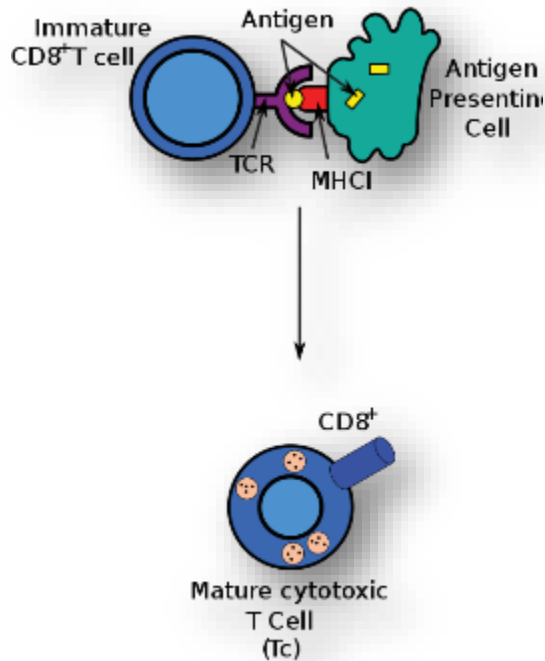
The screenshot shows a text editor window with the following menu: File, Edit, Search, View, Encoding, Language, Settings, Tools, Macro, Run, Plugins, Window. The window title is 'MUC conserved.fasta'. The sequence is as follows:

```
1 >muc 156-731
2 MSPFGILTSINQIEFLEGISSLKGRYSYYYQNIPNPFIGKGERVMPETDFAPNIFEYESAIDTFKTKVED
3 GREQIVSAGFAYTPFGDPLTSVSEGITYKFLRVSFSPYVYGNLVTSLPEIDYDVNSSVSESQFLADINAKF
4 SPSGAVPGVSLSSNFSETVDLTKPGSYKVMLNTILPDTIDSRAKAAPVEVTVNVKNTSIKAEDVTVRYVDED
5 GKPIPNVSAQTI SGNVGDSDYDATTDVYKLSIDGYTLDESKLPANGKGSLSDKAQTVTYVYKQTKDQSTVIVH
6 DSELIVGDTWEPEDNFDSDATDYDGNVFPFSHITVDGSVDTSKVGTYKITYSRILPSFFSAENQGEYSAVATI
7 TVKDAQPVKGGDVTAKYIDTDGNKISDDIVKTVGSVGETYKTEQKAIDGYTFKEVQGNMSGQFTDQAQTVTVV
8 YTKNEIPNITGTVLVKYVDTDGNKISEDIVKSGTVGEGYSTEKKAIEGYTFKEVQGNITGQFTEQVQTVTVV
9 YTKNRVNSEPKPENKQSSNDKNNNQGTISSTQHGLPETGENERMTMMSIILGLILLALGAVVWIFRFKLNK
10
```

T-cell epitope prediction

The objective of T-cell epitope prediction is to identify short peptide sequences within an antigen that can act as a stimulant of CD4+ or CD8+ T-cells. More specifically:

1. **Selection of cytotoxic T lymphocytes (CTL) epitopes.** It predicts the MHC-class I binding peptide sequences
2. **Selection of helper T cells (HTL) epitopes**



1. Selection of cytotoxic T lymphocytes (CTL) epitopes, there is no selection of fish MHC I alleles to perform the analysis and this is a major limitation

But there is a solution :

NetMHCpan 4.1 & IPD-MHC Database, is a tool for predicting the binding capacity of protein regions that we submit. What interests us about this particular tool and differentiates it from the rest, is that there is an option to submit our own MHC I sequence and have the analysis done based on it.

1. Salmonids

Welcome to the IPD-MHC FISH database, a specialist database for the Major Histocompatibility Complex (MHC) in fish. The database includes at present two salmonid species: Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Atlantic salmon and rainbow trout possess a single classical MHC class I locus, UBA, encoded by closely linked DAA (IIA) and DAB (IIB) loci. Included in the database are binding domain-coding sequences from published studies and from the EMBL and NCBI databases.

For those readers who would like to know more about MHC sequences in salmonids, please see the [Salmonids](#) page.

Alleles Species Align

List all Salmonids alleles > Species belonging to the Salmonids group > Multi locus inter- and intra- species alignment >

2. Salmonids species

Below all organisms belonging to the *Salmonids* group are listed. The official designations are assigned by the Comparative MHC Nomenclature Committee as established by the International Society for Animal Genetics (ISAG) who are affiliated to the International Union of Immunological Societies (IUIS) - Veterinary Immunology Committee (VIC). Organism four-letters codes are written in parentheses.

If you are working on MHC nomenclature and your species is not represented, please [contact](#) the Comparative MHC Nomenclature Committee.

Species	Common Name	Class I	Class II
<i>Oncorhynchus mykiss</i> (Onmy)	Rainbow Trout	UBA	DAA, DAB
<i>Salmo salar</i> (Sasa)	Atlantic Salmon	UBA	DAA, DAB

3.

Accession	locus	species
FISH08144	*01:01:01	Onmy
FISH08145	*01:01:02	Onmy
FISH08146	Onmy-UBA*01:01:03	Onmy
FISH08147	Onmy-UBA*01:02	Onmy
FISH08148	Onmy-UBA*01:03	Onmy
FISH08149	Onmy-UBA*01:04	Onmy
FISH08150	Onmy-UBA*02:01	Onmy

Evaluation of Antigenic, Allergenic, Immunogenicity, and Toxicity of CTL epitopes

VaxiJen v2.0 Antigenicity Threshold: 0.4

VaxiJen: Prediction of Protective Antigens and Subunit Vaccines.

Enter a PROTEIN sequence here:
Plain format only.

Or please select a multiple protein sequence file in fasta format to upload:
Επιλογή αρχείου Δεν επιλέχθηκε κανένα αρχείο.

Select a TARGET ORGANISM: Bacteria
Virus
Tumour

THRESHOLD:

ACC Output Sequence Output Summary Mode

Class I Immunogenicity

Specify sequence(s) *

Enter peptide sequence(s)

Or select file containing sequence(s) Δεν επιλέχθηκε κανένα αρχείο.

Choose which positions to mask

Default (1st, 2nd, and C-terminus amino acids)
 Custom

Specify which positions to mask
 (Comma separated numbers)

Peptide lengths must be equal when using custom masking.

**Immunogenicity
Immunogenic > 0**

ToxinPred
Designing and prediction of toxic peptides

Home Design Peptide Batch Submission Protein Scanning Motif Scan Motif List QMSol Helpfiles

Virtual Scanning of Toxic Peptides

This tool allows the users to identify highly toxic or non-toxic peptides from large number peptides submitted by a user. It predict their acidity along with all the important physico-chemical properties like hydrophobicity, charge pi etc. of peptides submitted by users. To generate mutants of specific peptide, user need to click on the peptide. For more information click [Help](#).

Toxicity
E-value cut-off: 10

Type or paste peptide sequence(s) in single letter code (in FASTA format):

OR Submit sequence file: Δεν επιλέχθηκε κανένα αρχείο

Either select SVM method: SVM (Svm-Pro) based SVM (Svm-Pro) + Motif based SVM (T-EMBL) based SVM (T-EMBL) + Motif based

Peptide	Q1	Q2	Hydrophobicity	Charge	PI	Hydrophobicity	Charge	PI	Hydrophobicity	Charge	PI
1. PAA											
2. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
3. APTDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
4. AAL											
5. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
6. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
7. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
8. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
9. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
10. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
11. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
12. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
13. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
14. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
15. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
16. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
17. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
18. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
19. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
20. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
21. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
22. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
23. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
24. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
25. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
26. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
27. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
28. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
29. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
30. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
31. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
32. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
33. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
34. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
35. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
36. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
37. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
38. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
39. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000
40. AGLDLEHT	126	151.4 of 96				0.9012	0.4341	1.0614	1.0000	0.0000	0.0000

T-cell epitope prediction

2. Selection of helper T cells (HTL) epitopes. As in the case of CTL epitopes, antigen presentation is required, but they take part in the MHC Class II process.

The problem: There is no tool that can do anything like what we did with MHC I. The only choices are human, bovine, or mouse available MHC II. Therefore, we chose to perform the analysis based on mouse histocompatibility molecules.

NetMHCIIpan version 3.2					NetMHCIIpan version 3.2												
Input in 5x FAKVA format.					Input in 5x FAKVA format.												
Peptide length 15					Peptide length 15												
Threshold for Strong binding peptides (%Rank) 25					Threshold for Strong binding peptides (%Rank) 25												
Threshold for weak binding peptides (%Rank) 104					Threshold for weak binding peptides (%Rank) 104												
Allele: H-2-IAb					Allele: H-2-IAb												
Seq	Allele	Peptide	Identity	Pos	Seq	Allele	Peptide	Identity	Pos	Core	Core Rel	log50k(aff)	Affinity (nM)	%Rank	Exp	Bind	BindingLevel
141	H-2-IAb	MAEFGGAVGVRL	100	3	141	H-2-IAb	MAEFGGAVGVRL	100	3	FGGGAVG	0.770	0.025	57.91	0.03	NA	<88	Core_Histogram
140	H-2-IAb	IMKRFKRVAVTVR	100	4	140	H-2-IAb	IMKRFKRVAVTVR	100	4	FKRVAVTVR	0.760	0.018	62.63	0.03	NA	<88	Core_Histogram
142	H-2-IAb	AFEDDGVAVDGLS	100	2	142	H-2-IAb	AFEDDGVAVDGLS	100	2	FGDGVAVD	0.750	0.015	64.37	0.04	NA	<82	Core_Histogram
139	H-2-IAb	DKNMFVGGVAVTV	100	5	139	H-2-IAb	DKNMFVGGVAVTV	100	5	FGGGVAVG	0.749	0.018	71.73	0.05	NA	<82	Core_Histogram
143	H-2-IAb	KFSSGAVFVGLSS	100	1	143	H-2-IAb	KFSSGAVFVGLSS	100	1	FGGGVAVG	0.735	0.018	86.46	0.07	NA	<82	Core_Histogram
132	H-2-IAb	ADTKAFVGGVAVS	100	6	132	H-2-IAb	ADTKAFVGGVAVS	100	6	FGGGVAVG	0.750	0.040	132.31	0.20	NA	<68	Core_Histogram
133	H-2-IAb	TVKIFYKRLVGGFR	100	3	133	H-2-IAb	TVKIFYKRLVGGFR	100	3	IFYKRLVGG	0.695	0.020	217.63	0.40	NA	<88	Core_Histogram
130	H-2-IAb	GFYKIFYKRLVGGFR	100	4	130	H-2-IAb	GFYKIFYKRLVGGFR	100	4	IFYKRLVGG	0.665	0.049	226.08	0.40	NA	<88	Core_Histogram
134	H-2-IAb	YKLYSLLDGFVSS	100	2	134	H-2-IAb	YKLYSLLDGFVSS	100	2	IFYKRLVGG	0.675	0.091	248.38	0.70	NA	<82	Core_Histogram
144	H-2-IAb	FGDGVAVFVGLSS	100	0	144	H-2-IAb	FGDGVAVFVGLSS	100	0	FGGGVAVG	0.500	0.480	278.50	0.80	NA	<82	Core_Histogram
131	H-2-IAb	VGVYKIFYKRLVGGFR	100	5	131	H-2-IAb	VGVYKIFYKRLVGGFR	100	5	IFYKRLVGG	0.645	0.477	281.03	0.90	NA	<88	Core_Histogram
135	H-2-IAb	KIFYKRLVGGFR	100	1	135	H-2-IAb	KIFYKRLVGGFR	100	1	IFYKRLVGG	0.655	0.456	361.59	1.10	NA	<88	Core_Histogram
76	H-2-IAb	YKLYSLLDGFVSS	100	3	76	H-2-IAb	YKLYSLLDGFVSS	100	3	YKLYSLLD	0.695	0.440	382.51	1.40	NA	<88	Core_Histogram
25	H-2-IAb	KFYKIFYKRLVGGFR	100	6	25	H-2-IAb	KFYKIFYKRLVGGFR	100	6	YKLYSLLD	0.690	0.437	641.72	1.70	NA	<85	Core_Histogram
14	H-2-IAb	KFYKIFYKRLVGGFR	100	5	14	H-2-IAb	KFYKIFYKRLVGGFR	100	5	YKLYSLLD	0.660	0.434	654.98	1.90	NA	<82	Core_Histogram
300	H-2-IAb	KFYKIFYKRLVGGFR	100	6	300	H-2-IAb	KFYKIFYKRLVGGFR	100	6	IFYKRLVGG	0.550	0.431	670.94	1.90	NA	<82	Core_Histogram
140	H-2-IAb	KFGYKIFYKRLVGGFR	100	5	140	H-2-IAb	KFGYKIFYKRLVGGFR	100	5	YKLYSLLD	0.440	0.425	501.13	1.20	NA	<88	Core_Histogram
77	H-2-IAb	KFYKIFYKRLVGGFR	100	2	77	H-2-IAb	KFYKIFYKRLVGGFR	100	2	YKLYSLLD	0.640	0.424	501.26	1.20	NA	<88	Core_Histogram
851	H-2-IAb	KFYKIFYKRLVGGFR	100	3	851	H-2-IAb	KFYKIFYKRLVGGFR	100	3	YKLYSLLD	0.455	0.416	556.97	1.50	NA	<88	Core_Histogram
850	H-2-IAb	KFGYKIFYKRLVGGFR	100	4	850	H-2-IAb	KFGYKIFYKRLVGGFR	100	4	YKLYSLLD	0.445	0.416	558.90	1.50	NA	<82	Core_Histogram
98	H-2-IAb	KFYKIFYKRLVGGFR	100	5	98	H-2-IAb	KFYKIFYKRLVGGFR	100	5	IFYKRLVGG	0.460	0.414	589.75	1.50	NA	<82	Core_Histogram
99	H-2-IAb	KFYKIFYKRLVGGFR	100	4	99	H-2-IAb	KFYKIFYKRLVGGFR	100	4	IFYKRLVGG	0.505	0.412	578.35	1.50	NA	<82	Core_Histogram
100	H-2-IAb	KFYKIFYKRLVGGFR	100	3	100	H-2-IAb	KFYKIFYKRLVGGFR	100	3	IFYKRLVGG	0.525	0.403	639.00	1.00	NA	<88	Core_Histogram
75	H-2-IAb	KFYKIFYKRLVGGFR	100	1	75	H-2-IAb	KFYKIFYKRLVGGFR	100	1	YKLYSLLD	0.610	0.398	674.22	1.00	NA	<88	Core_Histogram
866	H-2-IAb	KFYKIFYKRLVGGFR	100	0	866	H-2-IAb	KFYKIFYKRLVGGFR	100	0	IFYKRLVGG	0.415	0.396	688.62	1.50	NA	<85	Core_Histogram
845	H-2-IAb	KFYKIFYKRLVGGFR	100	8	845	H-2-IAb	KFYKIFYKRLVGGFR	100	8	YKLYSLLD	0.375	0.395	701.64	1.50	NA	<82	Core_Histogram
97	H-2-IAb	KFYKIFYKRLVGGFR	100	6	97	H-2-IAb	KFYKIFYKRLVGGFR	100	6	IFYKRLVGG	0.415	0.393	711.87	1.50	NA	<82	Core_Histogram
102	H-2-IAb	KFYKIFYKRLVGGFR	100	5	102	H-2-IAb	KFYKIFYKRLVGGFR	100	5	KFAAVVET	0.350	0.391	726.04	1.50	NA	<82	Core_Histogram
103	H-2-IAb	KFYKIFYKRLVGGFR	100	2	103	H-2-IAb	KFYKIFYKRLVGGFR	100	2	KFAAVVET	0.505	0.387	755.22	1.50	NA	<82	Core_Histogram
104	H-2-IAb	KFYKIFYKRLVGGFR	100	4	104	H-2-IAb	KFYKIFYKRLVGGFR	100	4	KFAAVVET	0.365	0.386	770.05	4.00	NA	<88	Core_Histogram
28	H-2-IAb	KFYKIFYKRLVGGFR	100	6	28	H-2-IAb	KFYKIFYKRLVGGFR	100	6	YKLYSLLD	0.675	0.379	831.59	4.00	NA	<85	Core_Histogram
104	H-2-IAb	KFYKIFYKRLVGGFR	100	3	104	H-2-IAb	KFYKIFYKRLVGGFR	100	3	KFAAVVET	0.470	0.379	841.98	4.00	NA	<82	Core_Histogram
137	H-2-IAb	KFYKIFYKRLVGGFR	100	5	137	H-2-IAb	KFYKIFYKRLVGGFR	100	5	KFAAVVET	0.370	0.377	841.94	4.00	NA	<82	Core_Histogram
101	H-2-IAb	KFYKIFYKRLVGGFR	100	2	101	H-2-IAb	KFYKIFYKRLVGGFR	100	2	KFAAVVET	0.525	0.374	874.05	4.50	NA	<88	Core_Histogram

NetMHCIIpan 4.0 Server

SUBMISSION

How to use mouse cursor over the symbol for a short description of the options

INPUT TYPE:

Please enter a couple sentences or several keywords for [PubMed](#) search from this field below:

... or upload a file to [NCBI](#) server directly from your local disk

Enable support for multiple amino acids (aa)

... or load your sequence files

Enable support for aa

PEPTIDE LENGTH (specify variable length as a comma separated list):

Use context encoding:

SELECT SPECIES (LOC)

Mouse (1.7)

Select file(s) (max. 10 per submission)



Additional properties of HTL epitopes (interferon- γ inducing ability, prediction of toxicity, prediction of pro-inflammatory cytokine production)



PIP-EL: a new ensemble learning method for improved pro-inflammatory inducing peptide predictions

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Welcome to the Home Page of PIP-EL

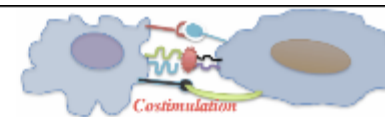
PIP-EL is web based prediction server for pro-inflammatory inducing peptides. Technically, PIP-EL was the fusion of 10 independent RF models, where each of the five different compositions including amino acid, dipeptide, composition-transition-distribution, physicochemical properties and amino acid index contained 10 RF models. For a given peptide, PIP-EL predicts its class and probability values.

Enter the protein sequences in FASTA format ([Example](#))

File: Διεύθυνση αρχείου

IFNepitope

A server for predicting and designing interferon-gamma inducing epitopes



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Epitope prediction

This tool has been developed to predict the IFNepitope, where users are allowed to paste or upload file with multiple peptide sequences and each sequence would be the predicted according to the model selected. For more help please visit [link](#).

Name of your job (Optional)

Email address (Optional)

Please paste/peptide amino acid sequence of peptides in FASTA format. Use example sequences.

OR Upload file containing peptide sequences. Επιλογή αρχείου Διεύθυνση αρχείου
How: Please upload file containing peptide sequence in fasta format.

Please select approach for predicting IFN-gamma epitopes: Motif based SVM based Motif and SVM hybrid

Select the Model for prediction: IFN-gamma versus Non-IFN-gamma IFN-gamma versus other cytokine IFN-gamma versus random

B-Cell epitope prediction

B-cell epitopes are two types, linear (continuous) and conformational (discontinuous).

In the case of vaccine design, only the linear epitopes are considered

IEDB Analysis Resource

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Antibody Epitope Prediction

Specify Input

Enter a Swiss-Prot ID (example: P02105)

Or enter a protein sequence in plain format (50000 residues maximum, 250 residues for Bepred 2.0)

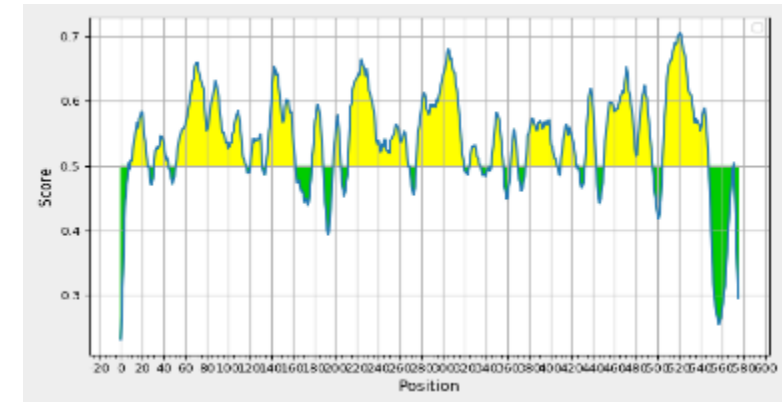
Choose a method:

- Bepred Linear Epitope Prediction 2.0
- Bepred Linear Epitope Prediction
- Chou & Fasman Beta-Turn Prediction
- Emini Surface Accessibility Prediction
- Kaplus & Schulz Flexibility Prediction
- Kolaskar & Toppoonaik Antigenicity
- Parker Hydrophobicity Prediction

Submit Reset

Predicted peptides:

No.	Start	End	Peptide	Length
1	0	0	T	1
2	10	25	INQIEFLEGISLKGK	16
3	32	45	NIPNPFIGIKGERV	14
4	53	117	APNIFEYESAIDTFKTKVEDGREQIVSAGFAYTPFGDPLTSVSEGITYKFLRVSFSPYVYGNLVI	65
5	122	132	EIDYDVISSVS	11
6	137	163	LADINAKFSPSGAVPGVLSLNFSETV	27
7	179	189	LPDTIDSRKA	11
8	199	205	KNTSIKA	7
9	213	268	VOEDGKPIPNVSAQTI5GNVGD5YDATTDVYKLSIDGYTLDESKLPANGKGLSDK	56
10	277	320	KQTKDQSTVTVHDSSELIVGDTWEPEDMFDSATDYDGNVPPFSHI	44
11	326	336	VDTSKVGTYKI	11
12	347	357	SAENQGEYSAV	11
13	365	370	AQPVKG	6
14	378	407	IDTDGNKISDDIVKTVGSVGETYKTEQKAID	30
15	411	425	FKEVQGNVSGQFTDQ	15
16	427	427	Q	1
17	434	443	TKNEIPNITG	10
18	451	496	DTDGNKISEDIVKSGTVGEGYSTKKAIEGYTFKVEVQGNITGQFTE	46
19	506	548	TKNRWSEPKPENKQSSNDKNNMQGTISSTQHGLPETGENERM	43
20	572	572	K	1





Initial results and further developments

1. Analysis completed for MHC-I alleles of rainbow trout
2. CTL epitopes were examined for their antigenicity, immunogenicity, allergenicity and toxicity.
3. For HTL epitopes there is no available reference MHC II alleles for rainbow trout.
4. Analysis completed for B-cell epitopes

Further steps are needed:

Analyses of the physicochemical properties of the peptide, 3D modelling, molecular docking, immuno-response simulations, etc.

More *L. petauri* strains and *L. garvieae* isolates are needed...

The workflow ends in the construction of a multi-epitope vaccine

The vaccine construct will be designed with the inclusion of high scored T-cell and B-cell epitopes predicted from various epitope prediction tools and exhibiting high immunogenicity, non-toxicity, non-allergenic and strong binding affinity to a maximum number of HLA alleles.



Reverse Vaccinology for lactococcosis (Bioinformatics workflow)

In conclusion:

Despite significant advancements in vaccinology, computational proteomics, machine learning, and reverse vaccinology, finding vaccine candidates, producing them in the laboratory, and confirming their efficacy in animal models remains a complicated undertaking.



Objectives

Immunogenic protein identification

recombinant-protein production

experimental trial for vaccine efficacy