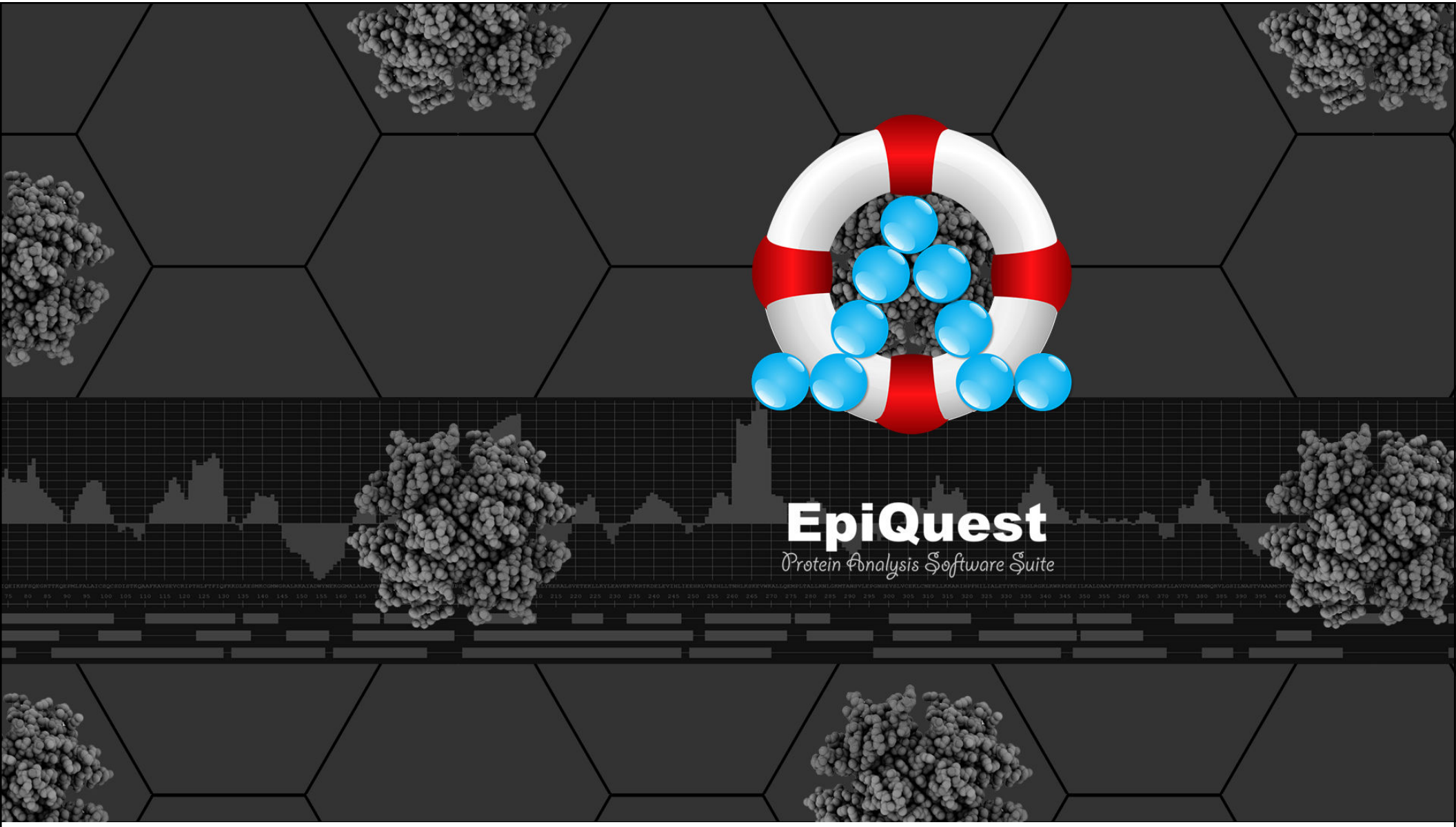

EpiQuest 2015

User's manual v 2014.12.01

How to operate EpiQuest programs and to interpret the results

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OS and Browser

You can work using a desktop PC with Windows (Vista, 7, 8/8.1), MacOS, Google OS. You can also work using a tablet with Android OS 4 and later. However, as your access to the server is mediated by internet browser, we strongly recommend using **Google Chrome** (download here).

Tests were performed using Internet Explorer (9 and later) and Safari (current versions), however, modifications introduced in these browsers by their developers cannot always be traced and taken into account.

Registration

To use the Demo version or full program you need to register. Please, follow this link, and you will open you the Server login screen. Press the Registration link:

At the registration screen, you will be asked to fill a form. Please accurately put your registration e-mail, as it is used to identify your account. After you confirm the data, you will get an e-mail confirming the registration.
You may now log in.

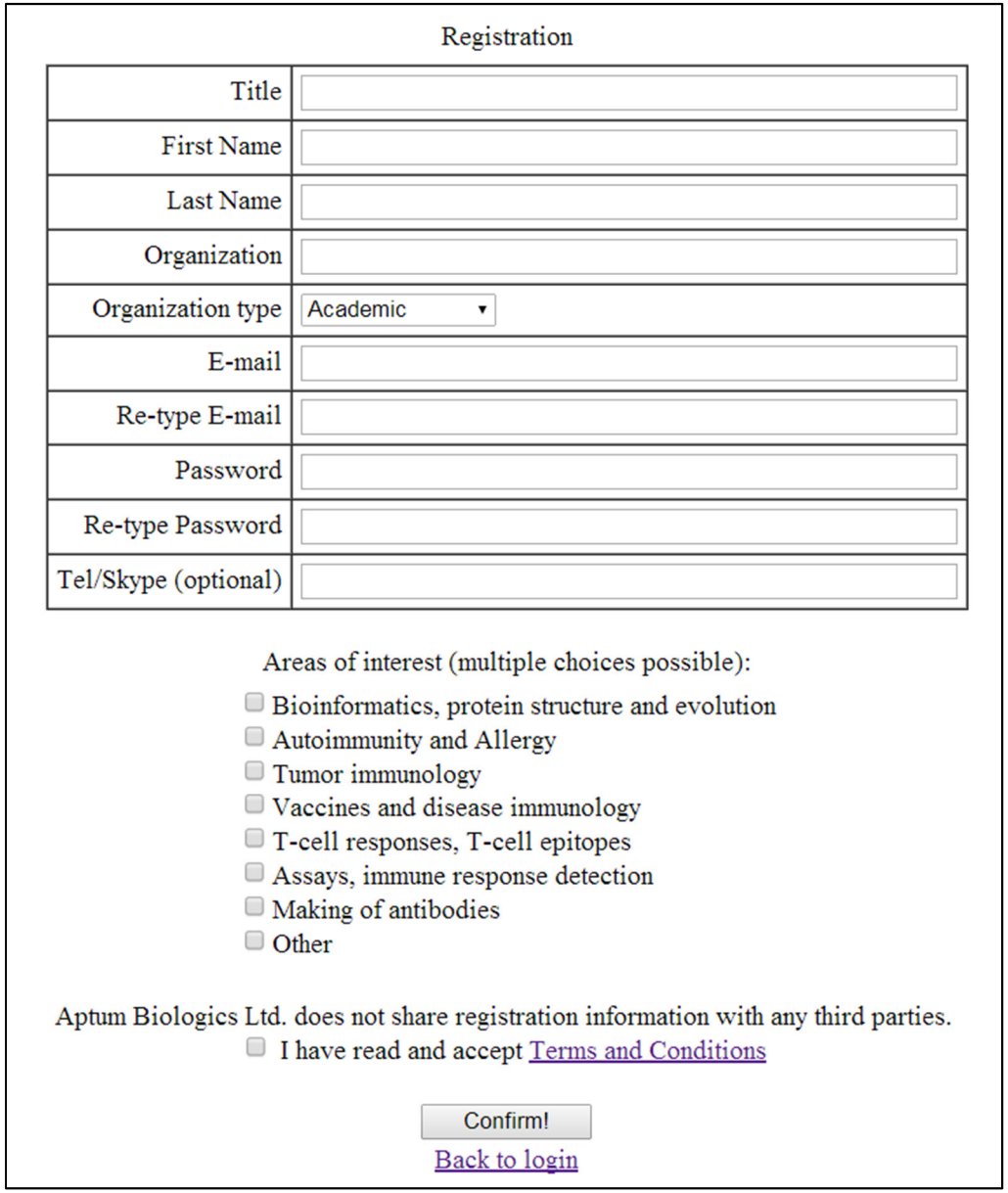


Table of content...

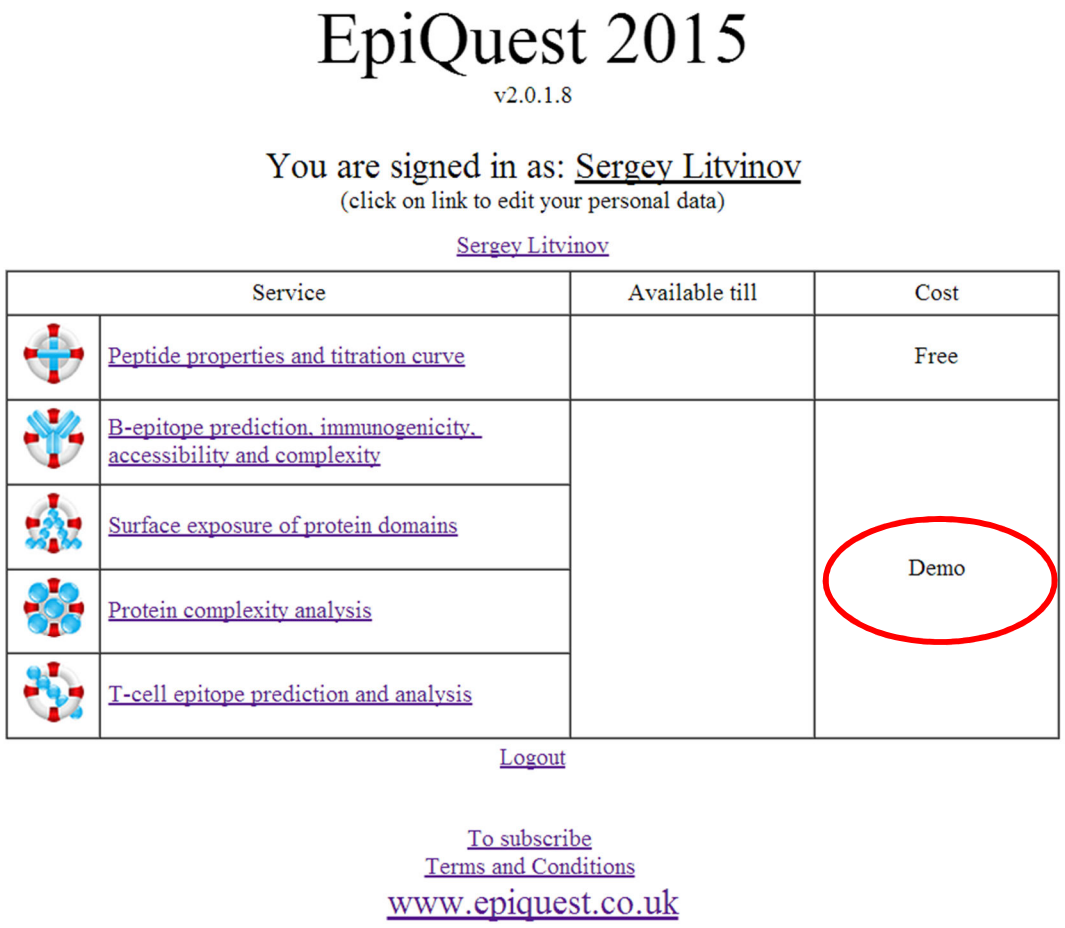
After the login

After you have logged into your profile on the server, you are met with a table of available programs.

At the top of the table your registration name is shown,

In Charge is a free program, so you can always access it.

EpiQuest sequence analysis programs are accessible by subscription. If you have no subscription, the word "Demo" in the right column will indicate that you have access to demo mode only. If you have a subscription, In the column "available till" you will see the date your current subscription will end.



After the login, you will be identified by the name you provided. The status (*demo and free versions* or *subscription* will be identified in the right section of the table.

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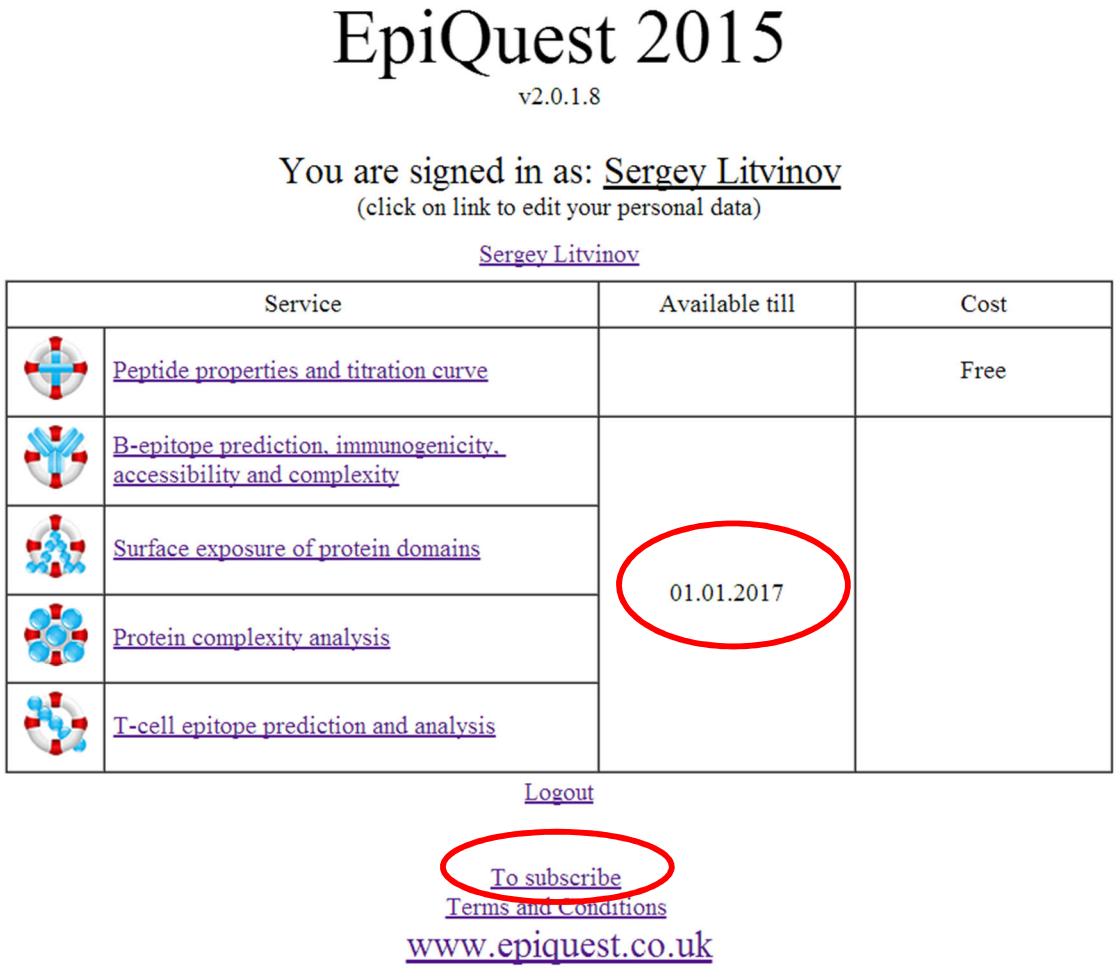
Full & Demo Mode

Demo mode allows you to use the full version of all the programs, but with a limited number or demo sequences. To analyse your own sequences you are required to use paid subscription, which is comparable with a price of one commercial antibody, and is for the duration of one year (for academic scientists).
Customers from companies are advised to inquire for a quotation.

To subscribe, please follow the link. The subscription may be paid for online using the secure PayPal gateway (Credit cards, PayPal, etc.) or you may ask for an invoice to be paid by your University the routine way. The subscription is activated within hours of the payment (maximum 24) upon verification of your academic status.



If you are subscribed, in the column “Available Till” the date your subscription ends will appear.

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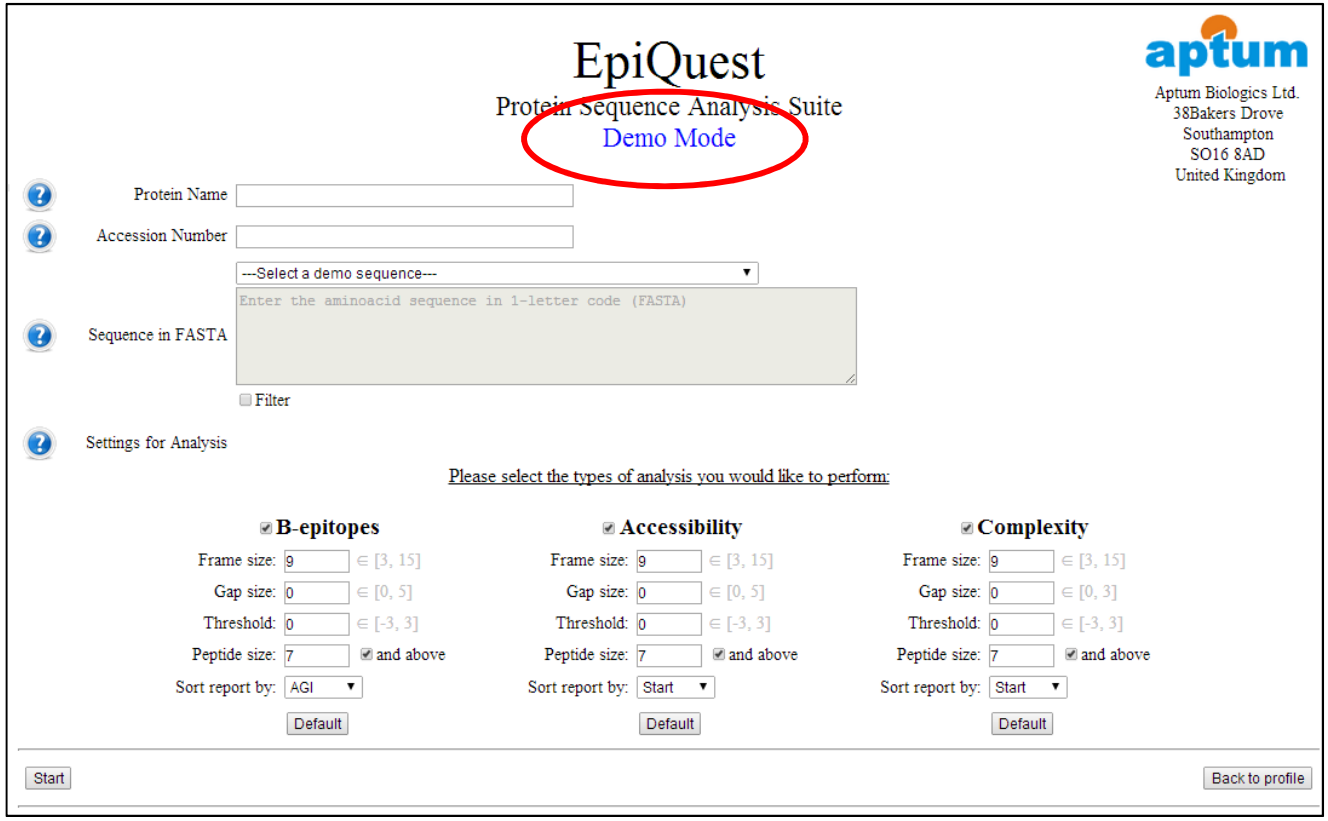
Demo Mode



When you are logged into the server in Demo mode, this will be indicated in the sequence entry screen. In Full Mode (when subscribed,) this indicator will be absent.

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How to evaluate B-cell epitopes using EpiQuest B, A & C

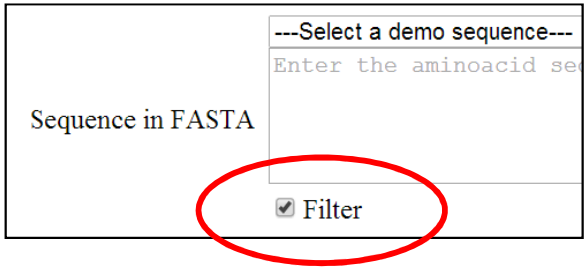
EpiQuest programs B (B-cell immunogenicity), A (surface accessibility) and C (complexity) join in one application to profile the protein's sequence of all three parameters, as all three are required to choose an optimal epitope. You will be able to save both ***graphical*** and ***numerical*** results of analysis for all three parameters.

To start the program, please click on ***"B-epitope prediction…"*** link.

Service		Available till	Cost
	Peptide properties and titration curve		Free
	B-epitope prediction, immunogenicity, accessibility and complexity		Demo
	Surface exposure of protein domains		
	Protein complexity analysis		
	T-cell epitope prediction and analysis		

Logout

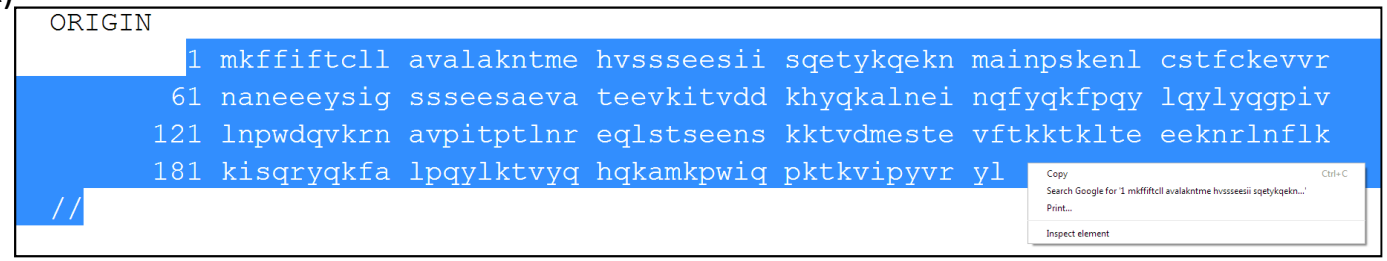
Protein sequence: Code and Filtering

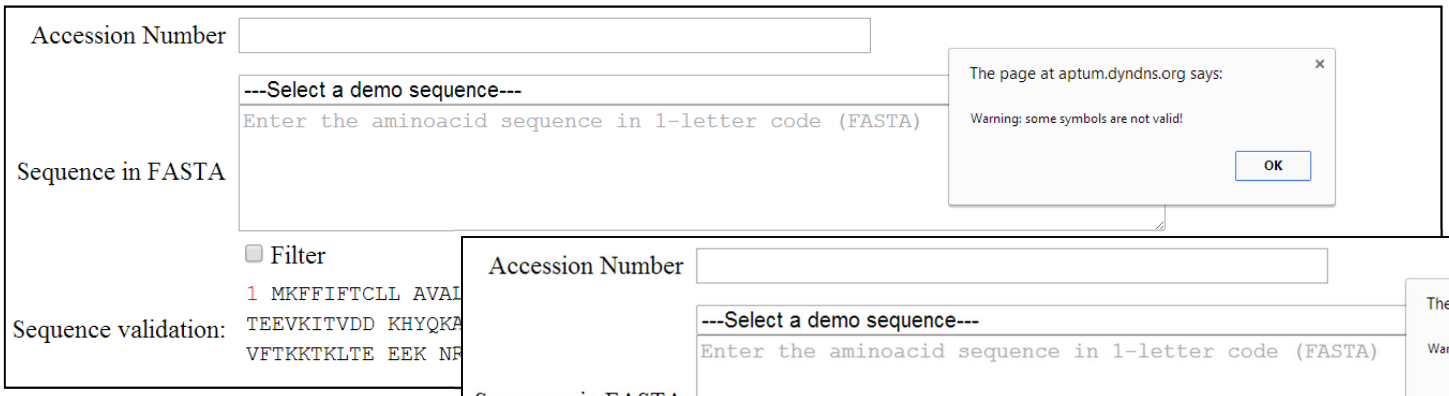


You may simply paste the sequence in ***one letter amino acid code*** (txt). The sequence can be taken from any FASTA file of the protein sequence. The program would not recognize three letter code.

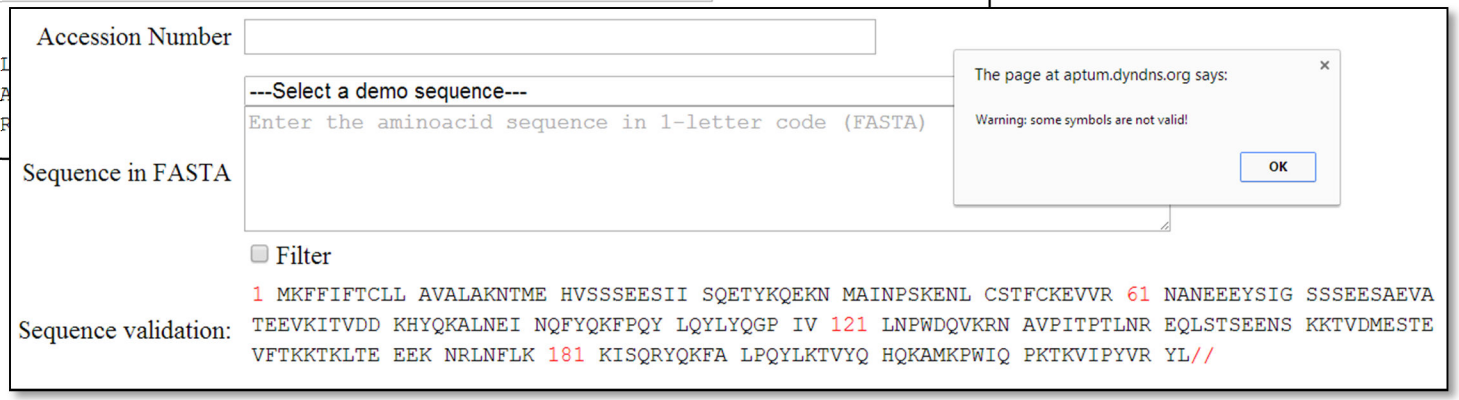
The sequence may be in Upper or Lower case letters. If the sequence contains ***any other symbols*** except for 20 AA codes i.e. gaps, numbers or extra letters, they will be eliminated from the sequence (click the ***Filter*** field below the sequence entry box)

If the Filter is not activated, you will be warned by validation service.





i.e. if you copy a protein sequence from NCBI record (as shown), the program will identify the non AA codes



If the Filter is activated, all non amino acid characters will be automatically filtered out of the sequence.

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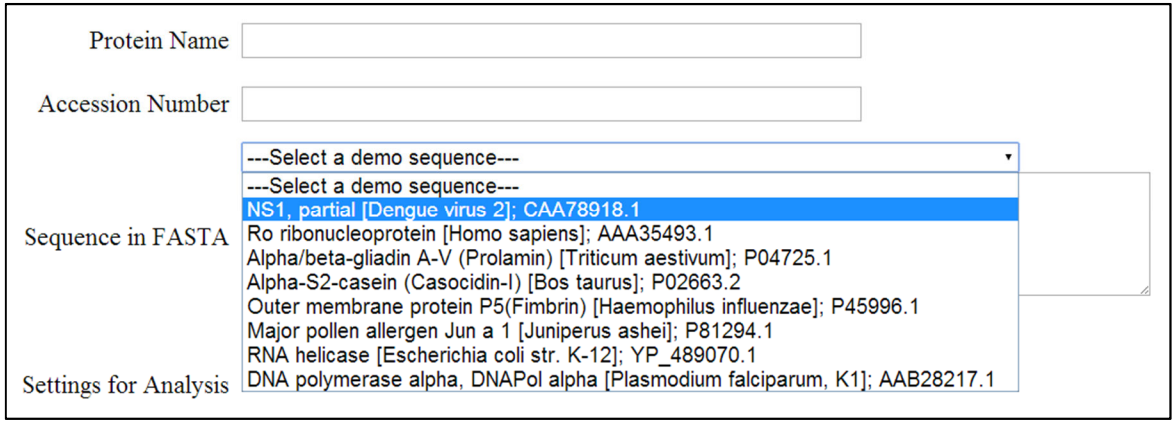
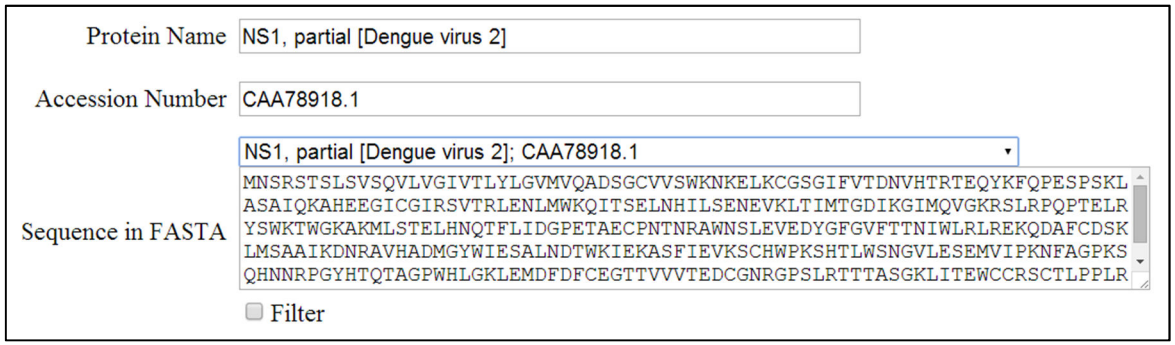
Choosing or Entering the Sequence

In Demo mode you cannot paste your own sequence, but can choose one of the several examples presented in drop-down list (---*Select a demo sequence*---).

In full mode you can simply paste the sequence in one letter code into the sequence window. We advise you to also enter a Protein name and Accession number sections, as you may later find the results being confusing if you do not record which isoform, splice variant, post-processed product etc. was used for the analysis.





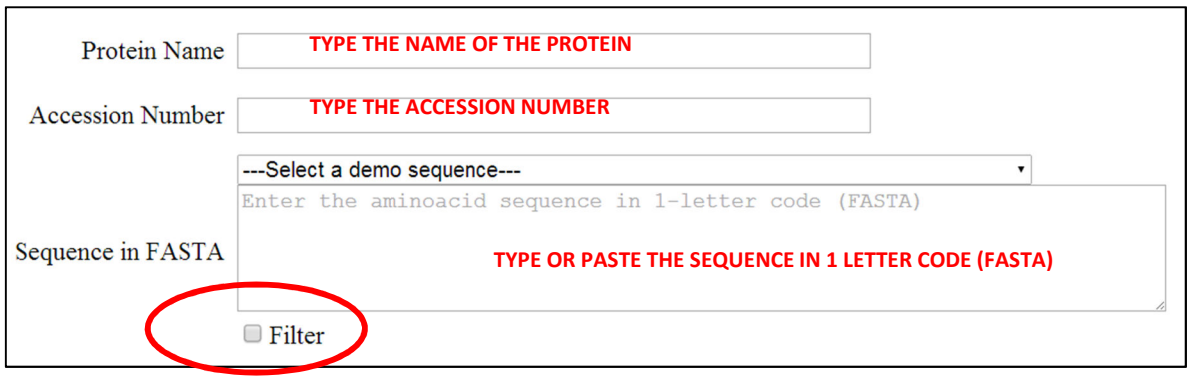


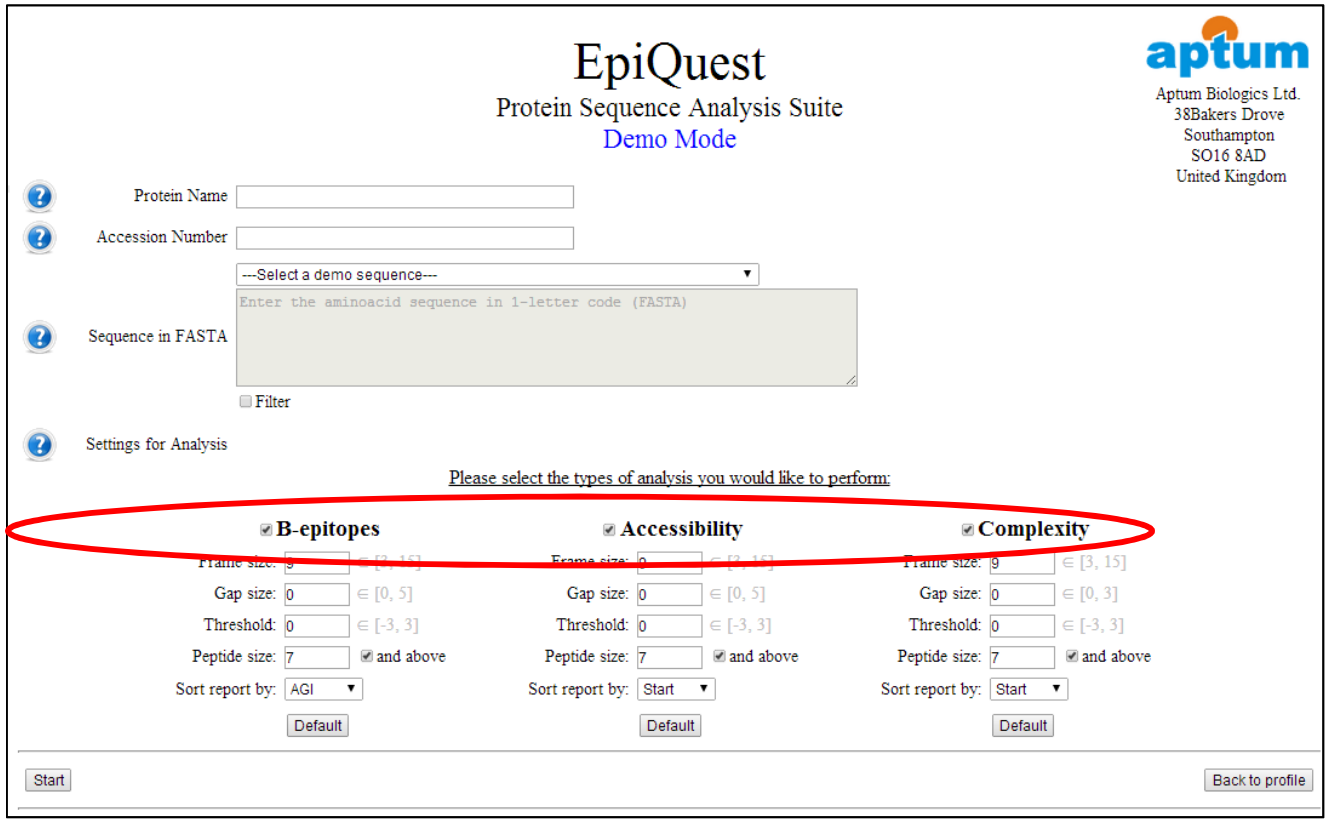
Table of content...

Select the programs to use

You may choose the analysis of all or some of the protein's parameters by clicking (or unclicking) the respective:

☑ B-epitopes	☑ Accessibility	☑ Complexity

When choosing an epitope for immunization (or selecting a determinant for assay), we advise to run all three algorithms: these allow you to define epitopes' immunogenicity, accessibility of it in a folded molecule of origin, and complexity (uniqueness for a particular protein)



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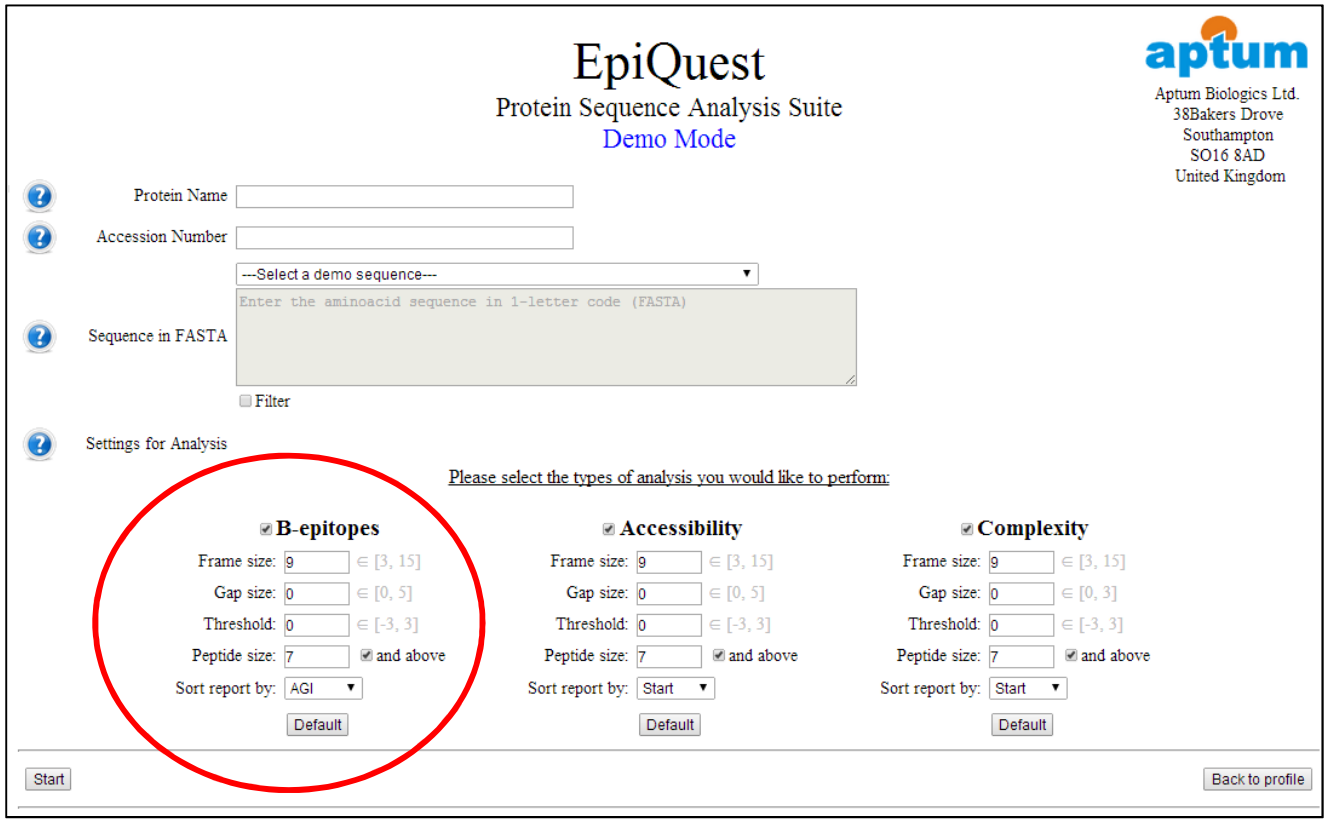
Changing the Analysis Settings



Parameters of the analysis may be modified according to the particular specifics of the required output. However, unless you are an advanced user, we strongly recommend using the ***Default settings***; these settings are optimized for simultaneous analysis of the parameters suitable for majority of sequences and tasks. If you, however, would like to make adjustments, please consider the guidelines that follow.

Parameters can be changed by typing the required number into respective window. The frames of parameter variability indicated in brackets by the window. By clicking the Default button you reset them to the original settings.

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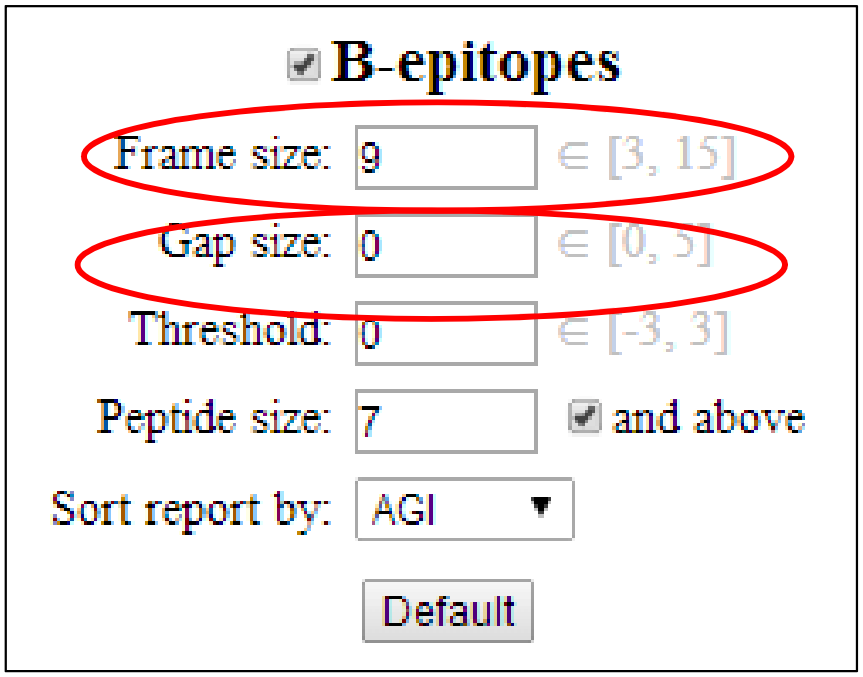
Settings for B-epitopes

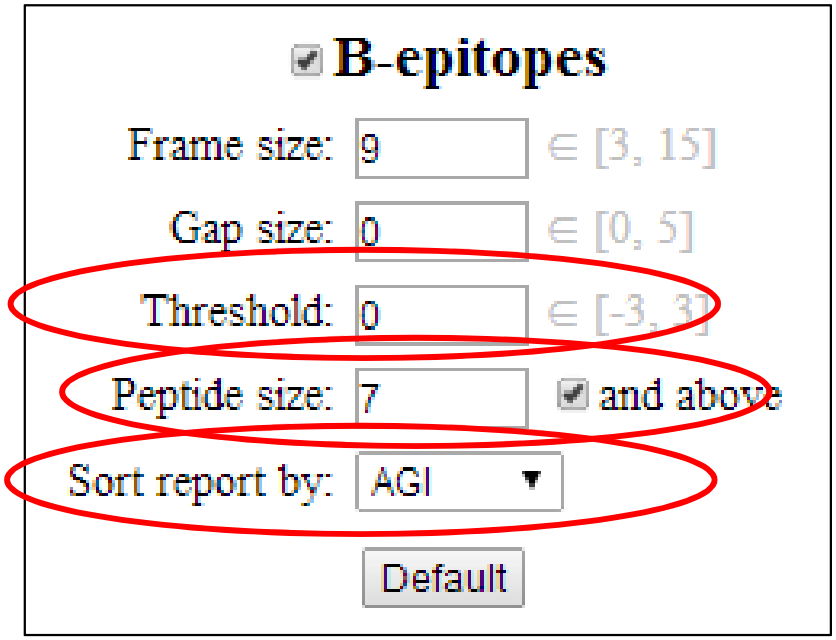


Frame size: by default it is 9, this is the setting when the predicted immunogenicity (AGI index) is in closest correlation to the actual ability of epitope eliciting antibody response. It gives you a most appropriate and corresponding to the actual immunogenicity profile of the protein. If you are interested in short areas of strong immunogenicity (usually they are the peak of the epitope), you may want to lower the Frame to 6, or even to 3.

Gap Size: If you would like to find a larger area of the protein, which probably contains several closely located epitopes, and want to do this within the program, you may increase the Gap up to 5. Do not increase the Gap when you want to predict a continuous epitope.

Table of content...

Settings for B-epitopes



Threshold: by default it is set at 0, and for most tasks you should keep it this way. When working with highly immunogenic proteins, and want to more clearly discriminate the strongest epitopes, raise the Threshold to +1. So far we have not met the task when a higher Threshold would be required. When analysing low immunogenic areas, and want to see the relative immunogenicity, possible epitopes etc. in domains of low immunogenicity, you may want to lower Threshold to -1 or even -2. So far, we have encountered the proteins where -2 was required for analysis of epitopes in low immunogenicity domains.

Peptide Size. This defines the minimal size of the epitope in your output. By default, you select "and above", which means that any domain with immunogenicity being above the threshold and of size 7 and above will be presented in the results output. If you are looking for just a regular epitope to develop antibody against, keep "and above" on.

Sort Report by. You may choose the sequences in Report to be sorted according to:

1) Order in the sequence
2) By cumulative immuogencity (AGI - Antigenicity Index) or
3) APR (antigenicity per residue), which is AGI/Length.

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Results Output: Graphical

Start

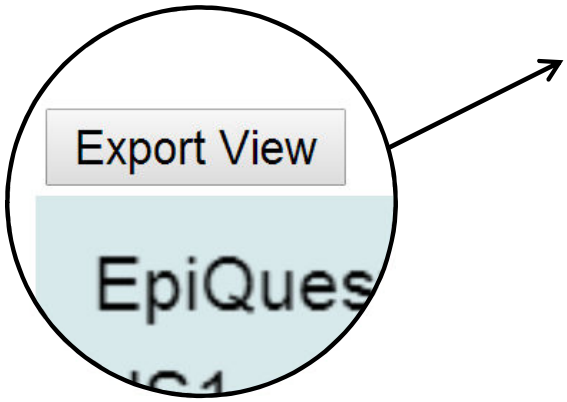
To run the analysis, press the “start” button

The report will appear on the same page, updated and re-loaded automatically.

The program will generate the overview profile for the protein, individual graph for each parameter (*on the left*).

Each graph is fully documented, contains the analysis type, matrix used, and codes for analysis parameters used.

By clicking the “export view” button near the top of each image you may export it and save. The default format of the images is PNG



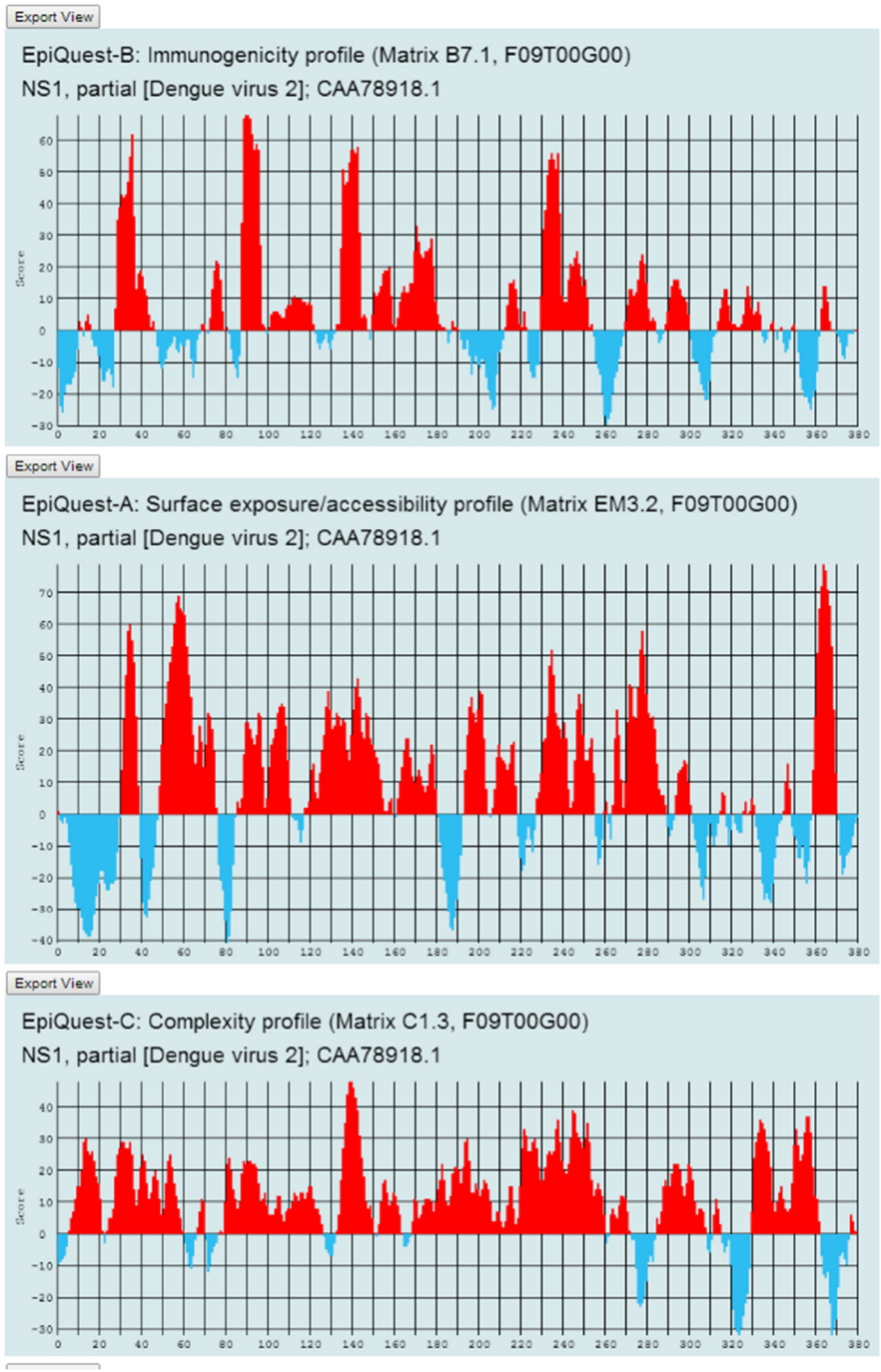
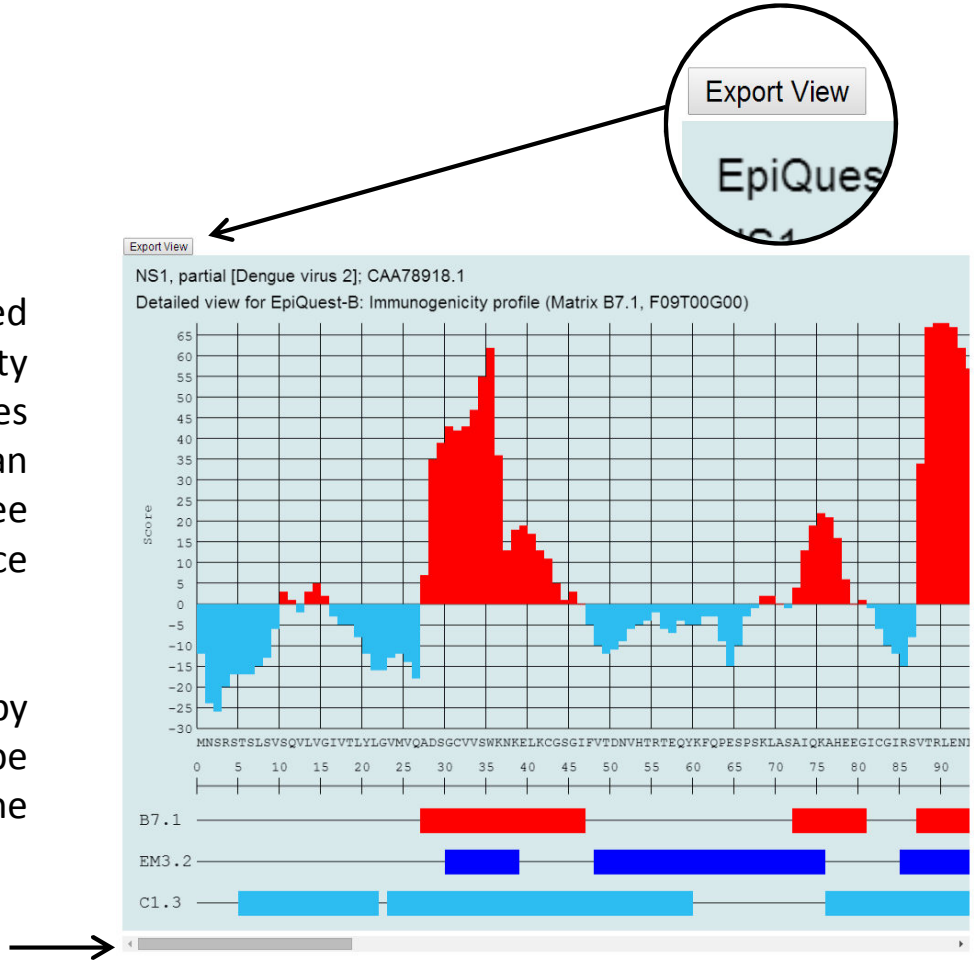


Table of content...

Output: Graphical, Detail

Further on, the results are presented in detailed graph. This image contains the immunogenicity profile of the protein, as well as the sequences which are positive and are not shorter than requested (in “Peptide Size”) length. All three graphs are presented along with the sequence and position scale.

The detailed graphical output may be viewed by moving the slider; the entire graph can be exported by clicking “save view”, and saving the graph in high-resolution PNG (below).



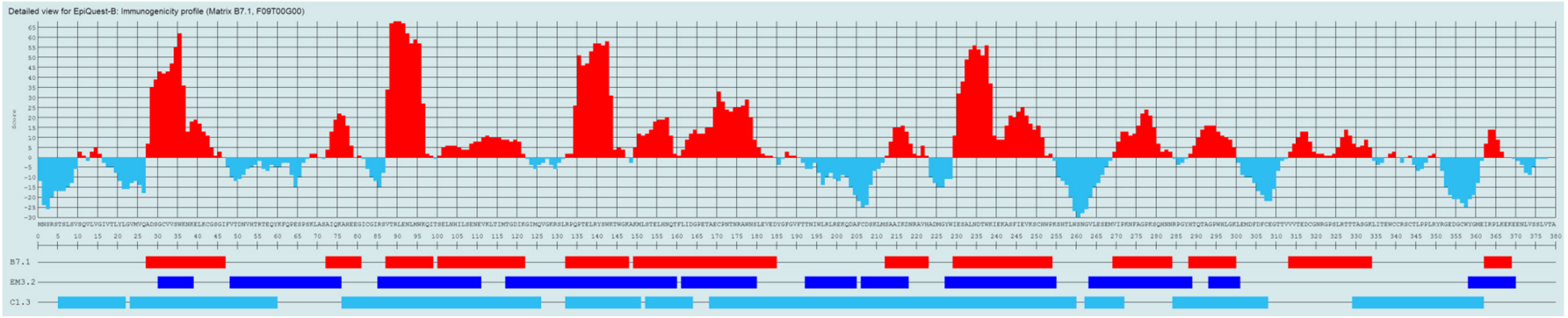


Table of content...

Output: Table format

View Save **Report for "B-epitopes":**

Start	End	Length	Sequence	AGI	AGR
28	47	20	ADSGCVVSWKNKELKCGSGI	509	25
73	81	9	AIQKAHEEG	102	11
88	99	12	VTRLENLMWKQI	569	47
101	122	22	SELNHILSENEVKLTIMTGDIK	156	7
133	148	16	RPQPTELRYSWKTWGK	499	31
150	185	36	KMLSTELHNQTFLIDGPETAECPNTNRAWNSLEVED	513	14
213	223	11	SAAIKDNRAVH	85	7
230	254	25	IESALNDTWKIEKASFIEVKSCHWP	653	26
270	284	15	VIPKNFAGPKSQHNN	175	11
289	300	12	HTQTAGPWHLGK	128	10
314	334	21	VVTEDCGNRGPSLRTTTASGK	137	6
363	369	7	IRPLKEK	47	6

View Save **Report for "B-epitopes":**

Start	End	Length	Sequence	AGI	AGR
88	99	12	VTRLENLMWKQI	569	47
133	148	16	RPQPTELRYSWKTWGK	499	31
230	254	25	IESALNDTWKIEKASFIEVKSCHWP	653	26
28	47	20	ADSGCVVSWKNKELKCGSGI	509	25
150	185	36	KMLSTELHNQTFLIDGPETAECPNTNRAWNSLEVED	513	14
270	284	15	VIPKNFAGPKSQHNN	175	11
73	81	9	AIQKAHEEG	102	11
289	300	12	HTQTAGPWHLGK	128	10
213	223	11	SAAIKDNRAVH	85	7
101	122	22	SELNHILSENEVKLTIMTGDIK	156	7
363	369	7	IRPLKEK	47	6
314	334	21	VVTEDCGNRGPSLRTTTASGK	137	6

The results are also given in table format, where they are sorted according to the requested. If you requested the output by “Start”, all positive sequences will be presented in their order in the protein, from N- to C- terminus. If the sequences were requested to be sorted by AGR (Antigenicity by residue), the more immunogenic sequences will be followed by less immunogenic.

Please, always refer the tabular results to graph, as a main immunogenic peak may be, without interruption, flanked by less immunogenic, but positive regions and presented in the results as one fragment.

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Saving Tabular Results

Report: Analysis of Immunogenicity profile (EpiQuest-B)

Date & Time: 21.01.2015 21:17:53

Protein Name: NS1, partial [Dengue virus 2]

Accession Number: CAA78918.1

Sequence:
MNSRSTSLSVSQVLVGIVTLYLGVMVQADSGCVVSWKNKELKCGSGIFVTDNVHTRTEQYKFQPESPSKL
ASAIQKAHEEGICGIRSVTRLENLMWKQITSELNHILSENEVKLTIMTGDIKGIMQVGKRSLRPQPTELR
YSWKTWGKAKMLSTELHNQTFLIDGPETAECPNTNRAWNSLEVEDYGFGVFTTNIWLRLREKQDAFCDSK
LMSAAIKDNRAVHADMGYWIESALNDTWKIEKASFIEVKSCHWPKSHTLWSNGVLESEMVIPKNFAGPKS
QHNNRPGYHTQTAGPWHLGKLEMDFDFCEGTTVVVTEDCGNRGPSLRTTTASGKLITEWCCRSCTLPPLR
YRGEDGCWYGMEIRPLKEKEENLVSSLVTA

Matrix: B7.1

Frame size: 9

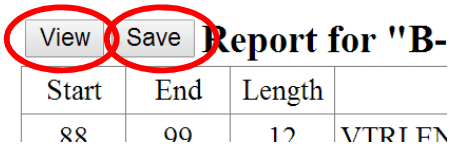
Gap: 0

Threshold: 0

Peptide size: 7 and above

Sorted by: Start

Start	End	Length	Sequence	AGI	AGR
28	47	20	ADSGCVVSWKNKELKCGSGI	509	25
73	81	9	AIQKAHEEG	102	11
88	99	12	VTRLENLMWKQI	569	47
101	122	22	SELNHILSENEVKLTIMTGDIK	156	7
133	148	16	RPQPTELRYSWKTWGK	499	31
150	185	36	KMLSTELHNQTFLIDGPETAECPNTNRAWNSLEVED	513	14
213	223	11	SAAIKDNRAVH	85	7
230	254	25	IESALNDTWKIEKASFIEVKSCHWP	653	26
270	284	15	VIPKNFAGPKSQHNN	175	11
289	300	12	HTQTAGPWHLGK	128	10
314	334	21	VVTEDCGNRGPSLRTTTASGK	137	6
363	369	7	IRPLKEK	47	6



By clicking “view” button at the top of the table, you will obtain the full report containing the date and time of analysis, Protein name, accession number, matrix that was used, as well as the conditions of the analysis. You may save the result (in Google Chrome you may right click on the page and ask Print, followed by “Save as PDF”).

You may also click the button “Save” at the top of the table, and the same view will be exported and downloaded as one page HTML. This file may be opened using MS Word or other editor, as well as copied and pasted into Excel.

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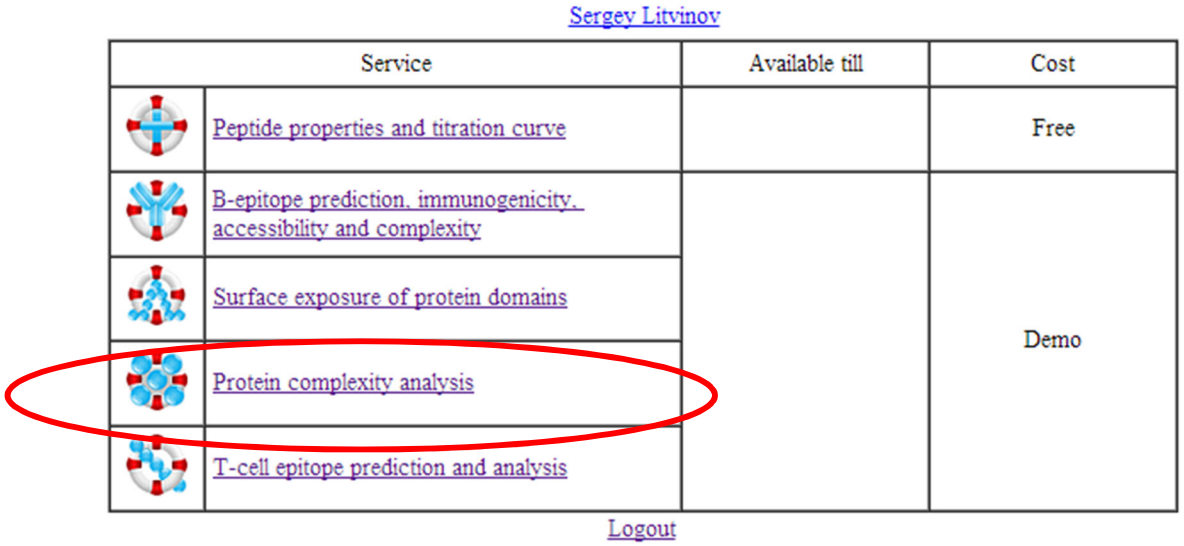
Analysing Protein Complexity

Proteins are not evenly organized on their full length. For different areas the function may be more general, and more specific: i.e. transmembrane domain is less organized and unique in its structure than ligand-binding centre of the receptor. The program EpiQuest-C allows you to analyse the relative local complexity of various regions and thus access their uniqueness for this particular protein.

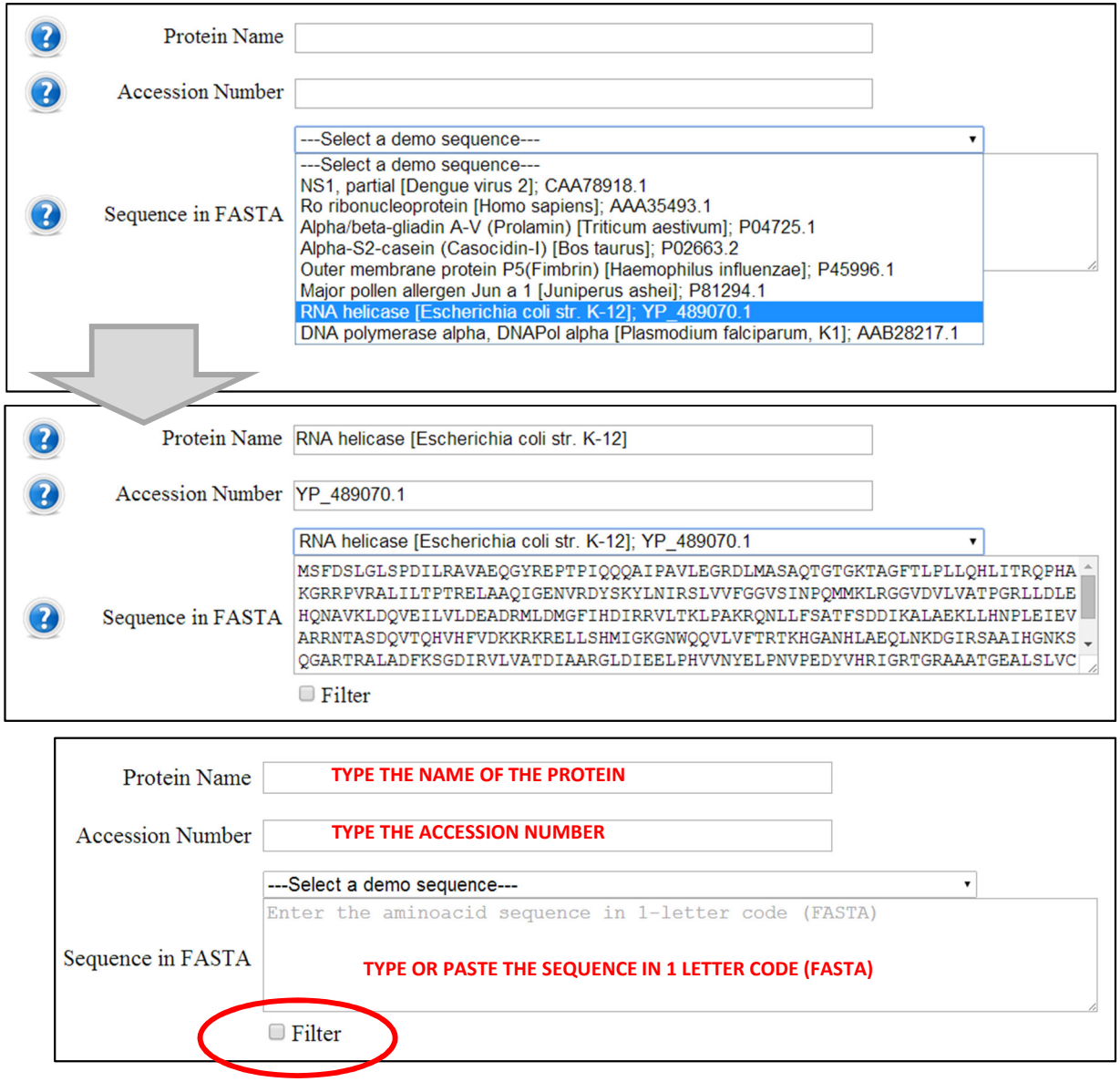


On average, the higher is the complexity – the more unique for this molecule is the domain. Accessing the complexity of the areas is highly important when selecting epitopes for raising specific antibodies, selecting determinants that are to be recognized by specific immune assay, selecting the regions to be included into recombinant protein, either to be used for immunization, vaccination or as an antigen in the assay.

To access EpiQuest-C, please click the link “Protein complexity analysis” at the entry page.

Table of content...

Choosing or Entering the Protein Sequence



In *Demo mode*, simply select one of the demo sequences by clicking on it in drop-down list.
The *sequence name, Accession number* and the *Sequence* will be automatically placed into respective windows.
In *Full Mode*, when enter your own sequence in a 1 letter code (Capitals or lower case) into the window.
We also strongly recommend entering the Acc. Number for the sequence, as it will appear in all reports and will later help you to keep the accurate records of your analysis.

The sequence you are entering will be validated for the presence of other symbols than 20 aa code letters; if such will be found, they will be removed if you will check the *Filter* box. Otherwise you will be warned about the presence of illegal symbols.

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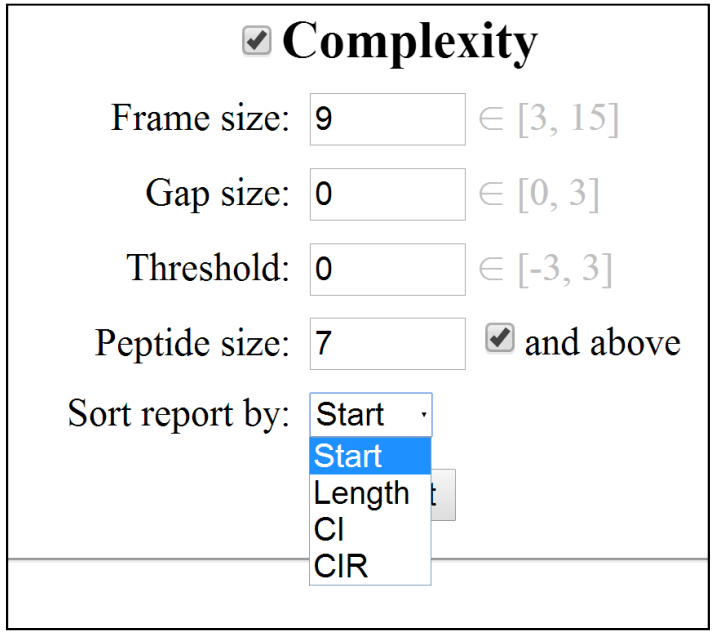
Settings for the Complexity Analysis



Before the analysis you have to define the parameters. You can (and we recommend it) keep the Default parameters. You can always reset the parameters to the default ones by clicking the *Default* button below.

You can also define how you want your tabular results to be sorted: by first aminoacid, by size, or by Complexity Index (CI) or by complexity per residue, which gives you the most organized and unique sequences first.

Overall, the ***Frame size*** 9 is optimal, but you may want to reduce it (to 5-6) if you are looking for shorter, highly organized fragments or increase, if you are aiming to get overview of the larger unique and less unique domains.

Again, if you are looking for larger continuous complex domains, you may allow ***gap size*** up to 3 negative amino acids within the otherwise positive fragment, Then such fragment will be presented in Results without interruption.

For convenience, you may increase or decrease the ***threshold*** which determines what is to be considered as negative areas. By increasing the Th you raise the cut-off level; decreasing the threshold allows you to analyze details of less complex sequences.

Peptide size determines the minimal size of positive fragment that still will be included into results output. If you will click box “and above”, the program will produce all unique positive fragments in their length. If the box “and above” will be unclicked, the program will report all peptides, overlapping, of the defined size that are positive.

To run the analysis, press the “start” button

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Results & Report

The program produces results in graphical and tubular way. The results can be exported by clicking the “save button”. The image will be available for saving in PNG from (will apprear in new window), and the tabular report will be suggested to download as HTML file, which can be printed as PDF, or saved and further manipulated using MS Word or Excel.

In the presented example, the report was requested to be produced in the order of sequences presence in the molecule, from N to C ternimus.

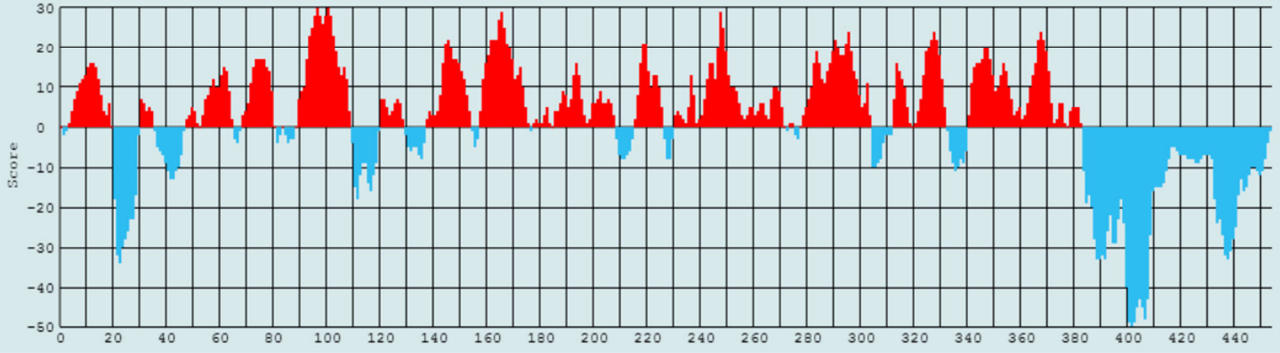
As can be seen in the report, the most complext regions are with CIR index of 18 and 16, and the fragments of CI 432 and 390 have longest sequences of highest complexity.

Whole Molecule Profile

Export View

EpiQuest-C: Complexity profile (Matrix C1.3, F09T00G00)

RNA helicase [Escherichia coli str. K-12]; YP_489070.1



View Save **Report for "Complexity":**

Start	End	Length	Sequence	CI	CIR
4	20	17	DSLGLSPDILRAVAEQG	152	8
48	65	18	GTGKTAGFTLPLLQHLIT	128	7
69	81	13	HAKGRRPVRALIL	145	11
89	109	21	AQIGENVRDYSKYLNIRSLVV	390	18
121	129	9	KLRGGVDVL	47	5
138	154	17	DLEHQNAVKLDQVEILV	191	11
158	176	19	ADRMLDMGFIHDIRRVLTK	311	16
178	208	31	PAKRQNLLFSATFSDDIKALAEKLLHNPLEI	168	5
216	226	11	ASDQVTQHVHF	133	12
231	272	42	RKRELLSHMIGKGNWQQVLVFTRTKHGANHLAEQLNKDGIRS	332	7
278	304	27	NKSQGARTRALADFKSGDIRVLVATDI	349	12
313	332	20	ELPHVVNYELPNVPEDYVHR	232	11
341	383	43	ATGEALSLVCVDEHKLLRDIEKLLKKEIPRIAIPGYEPDPSIK	432	10

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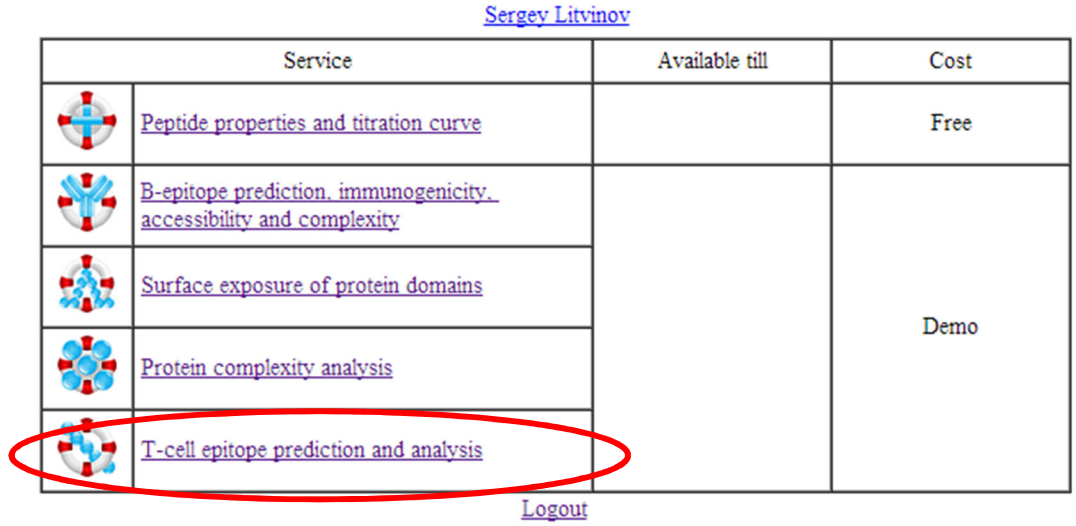
Predicting the Immunodominant T-epitopes

A good T-cell epitiope is defined by at least three factors: 1) whether this peptide can bind strongly the respective MHC molecule, 2) whether the recipient can develop a TCR that will bind to this complex strongly, and 3)whether the epitope will be utilized – depending on the presence of appropriate enzymes in MHC epitope processing compartment.
Most of epitopes predicted on the basis of their potential binding to MHC do not have strong functionality; testing them in functional assays results in either no or weak response.

In contrast to other programs, EpiQuest-T predicts fragments that contain sequences that may perform as immunodominant T-epitope. Usually there is a limited number of such sequences in a tested antigen, in contrast to the ones potentially capable of MHC-binding.



Please click the respective link at the entry screen to stat the program.

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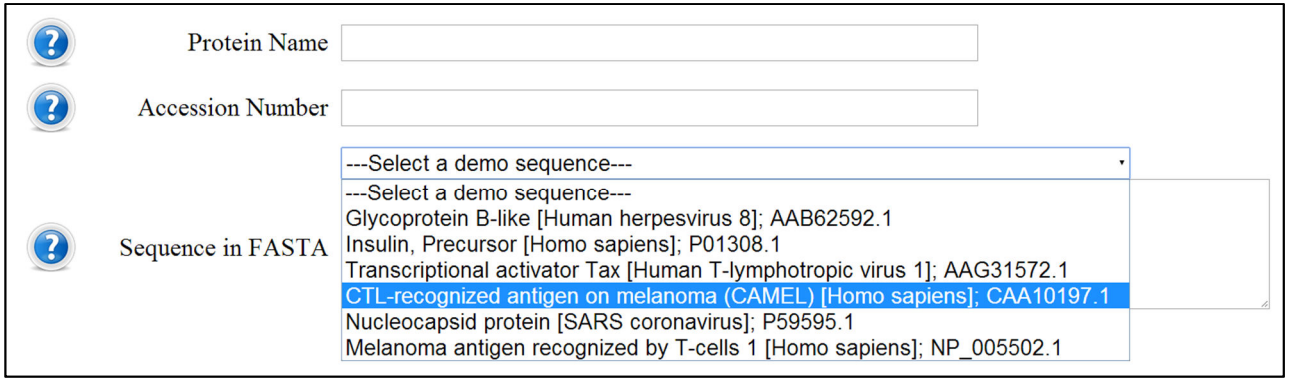
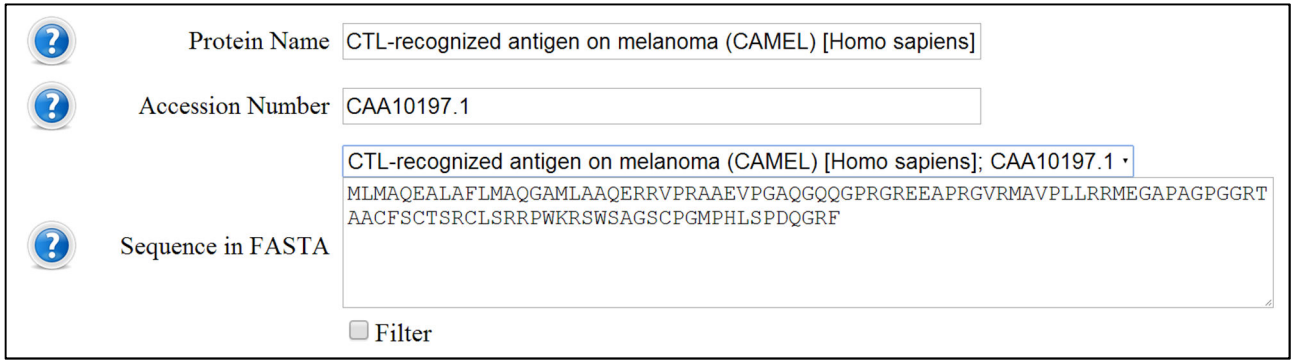
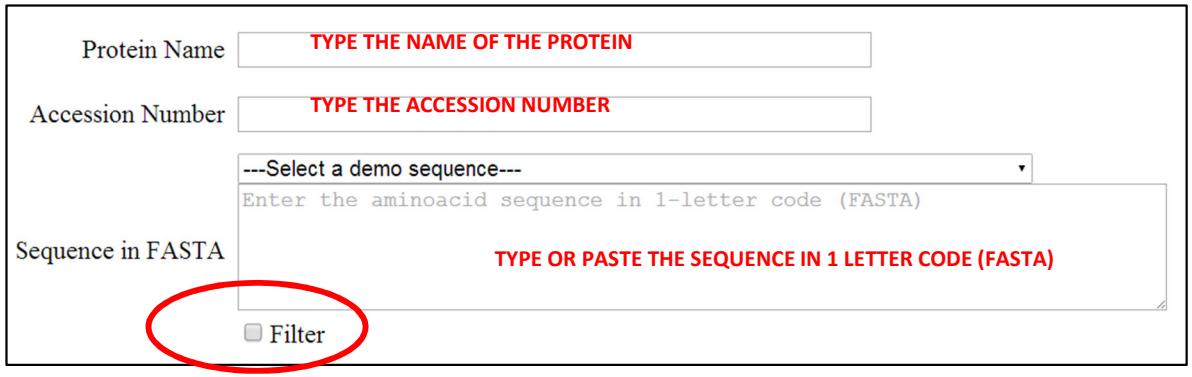
Choosing or Entering the Protein Sequence







In *Demo mode*, simply select one of the demo sequences by clicking on it in drop-down list.
The *sequence name, Accession number* and the *Sequence* will be automatically placed into respective windows.
In *Full Mode*, when enter your own sequence in a 1 letter code (Capitals or lower case) into the window.
We also strongly recommend entering the Acc. Number for the sequence, as it will appear in all reports and will later help you to keep the accurate records of your analysis.

The sequence you are entering will be validated for the presence of other than 20 aa code letters; if such will be found, they will be removed if you will check the *Filter* box. Otherwise, you will be warned about the presence of

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Settings for the analysis

EpiQuest-T uses individual matrixes for various classes and haplotypes of MHC. At the moment, only the matrix for human HLA A2 is available, but the matrixes for mouse MHC class one are coming soon.

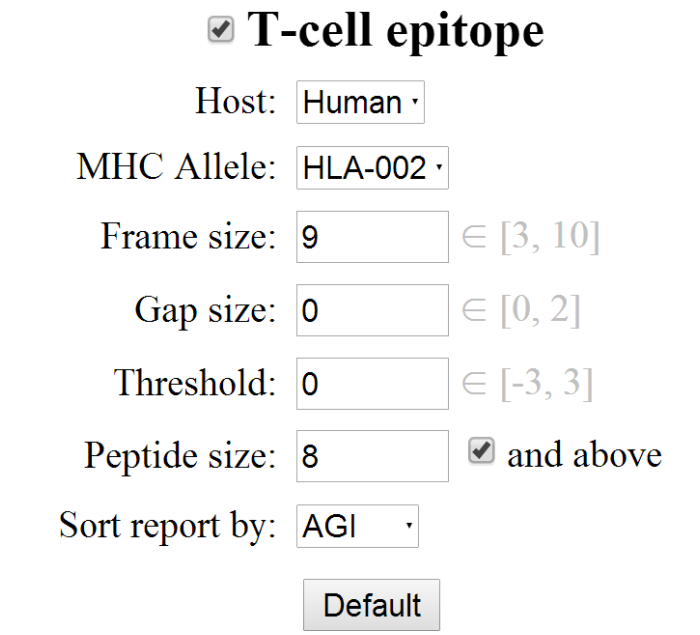
The default ***Frame size*** is 9 amino acids, but you may also lower it till 8.

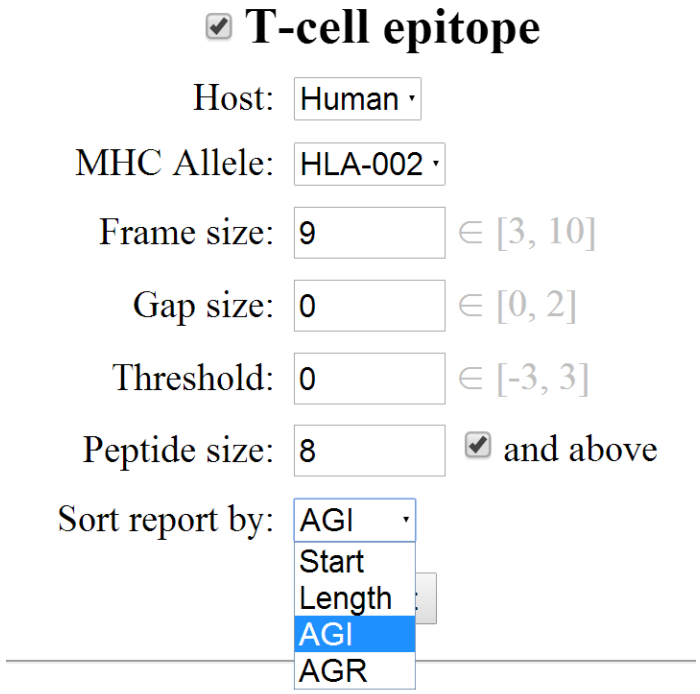
We do not recommend introducing any Gap, but there is such possibility.

The ***threshold*** (by default = zero) is calibrated in such a way that it allows discriminating all sequences that may contain a strong T-epitope from all others. You may lower the Threshold to -1 if you think that the sequence contains low immunpogenic sequences, but then also lowers the accuracy of the prediction, as the program is optimised for highly immunogenic/functionally active T-epitopes

Peptide size defines the minimal size of the positive peptides that will be included in the report.

We suggest sorting the output by AGI (relative immunogenicity index), which can be selected from the drop-down list.



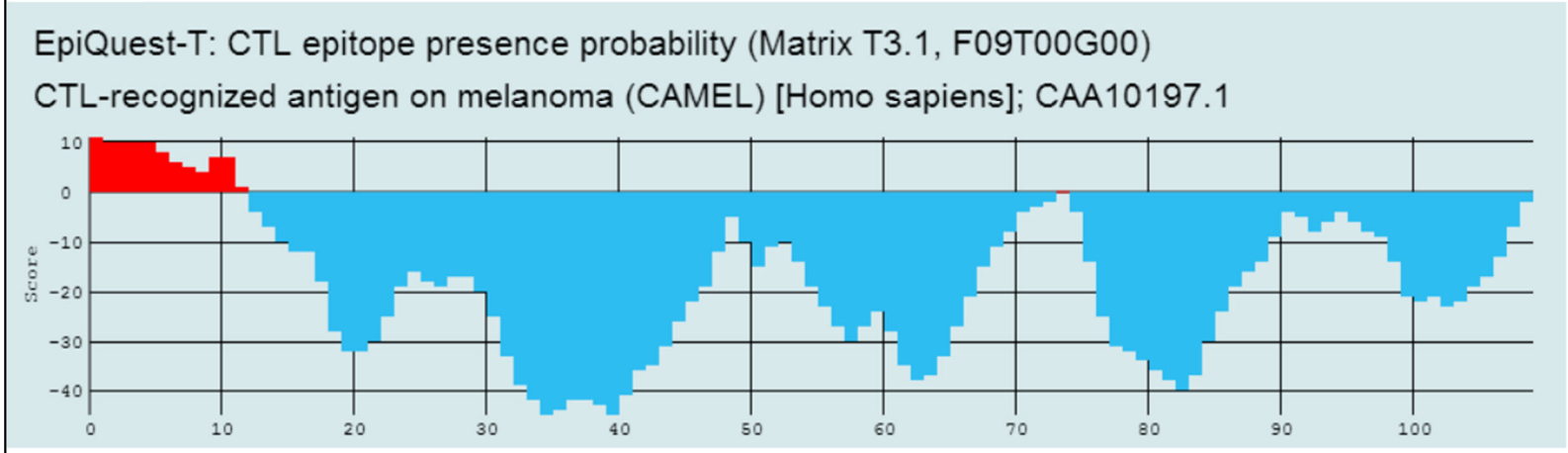


Results & Report

The program produces results in a graphical and tubular way. The results can be exported by clicking the “save button”. The image will be available for saving in PNG from (will appear in new window), and the tabular report will be suggested to download as an HTML file, which can be printed as a PDF, or saved and further manipulated using MS Word or Excel.

Whole Molecule Profile

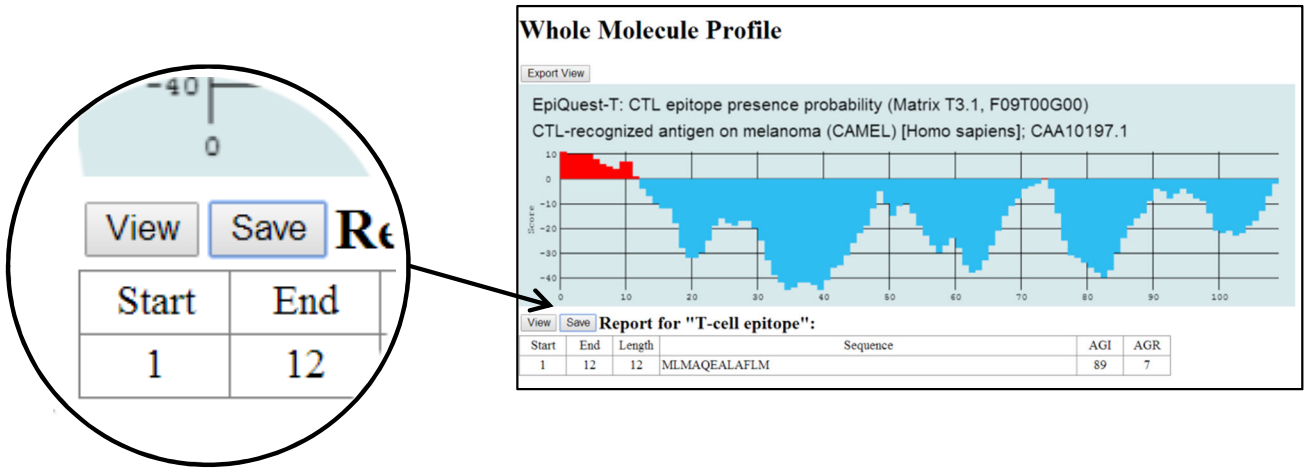
Export View



View Save **Report for "T-cell epitope":**

Start	End	Length	Sequence	AGI	AGR
1	12	12	MLMAQEALAFLM	89	7

Table of content...



Report: Analysis of CTL epitope presence probability (EpiQuest-T)

Date & Time: 12.01.2015 20:48:06

Protein Name: CTL-recognized antigen on melanoma (CAMEL) [Homo sapiens]

Accession Number: CAA10197.1

Sequence: MLMAQEALAFLMAQGAMLAAQERRVPRAAEVPGAQGQQGPRGREEAPRGVRMAVPLLRRMEGAPAGPGGR TAACFSCTSRCLSRRPWKRSWSAGSCPGMPHLSPDQGRF

Matrix: T3.1

Frame size: 9

Gap: 0

Threshold: 0

Peptide size: 8 and above

Sorted by: AGI

Start	End	Length	Sequence	AGI	AGR
1	12	12	MLMAQEALAFLM	89	7

Saving the Report

By clicking “view” button at the top of the table, you will obtain the full report containing the date and time of analysis, Protein name, accession number, matrix that was used, as well as the conditions of the analysis. You may save the result (in Google Chrome you may right click on the page and ask Print, followed by “Save as PDF”)

You may also click the button “Save” at the top of the table, and the same view will be exported and downloaded as one page HTML. This file may be opened using MS Word or other editor, as well as copied and pasted into Excel.

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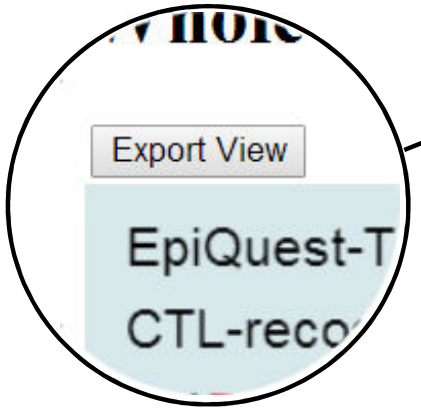
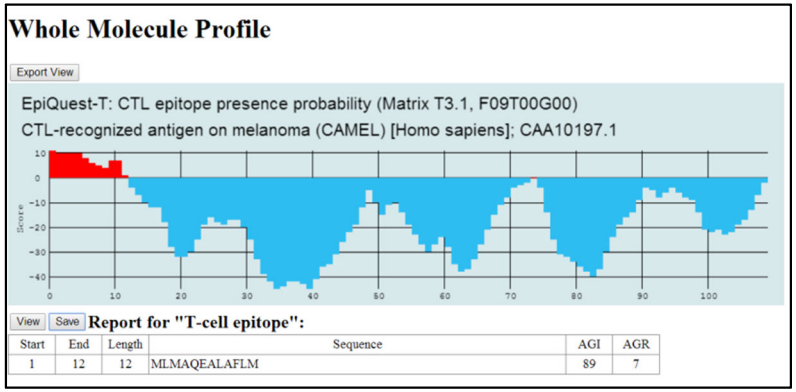
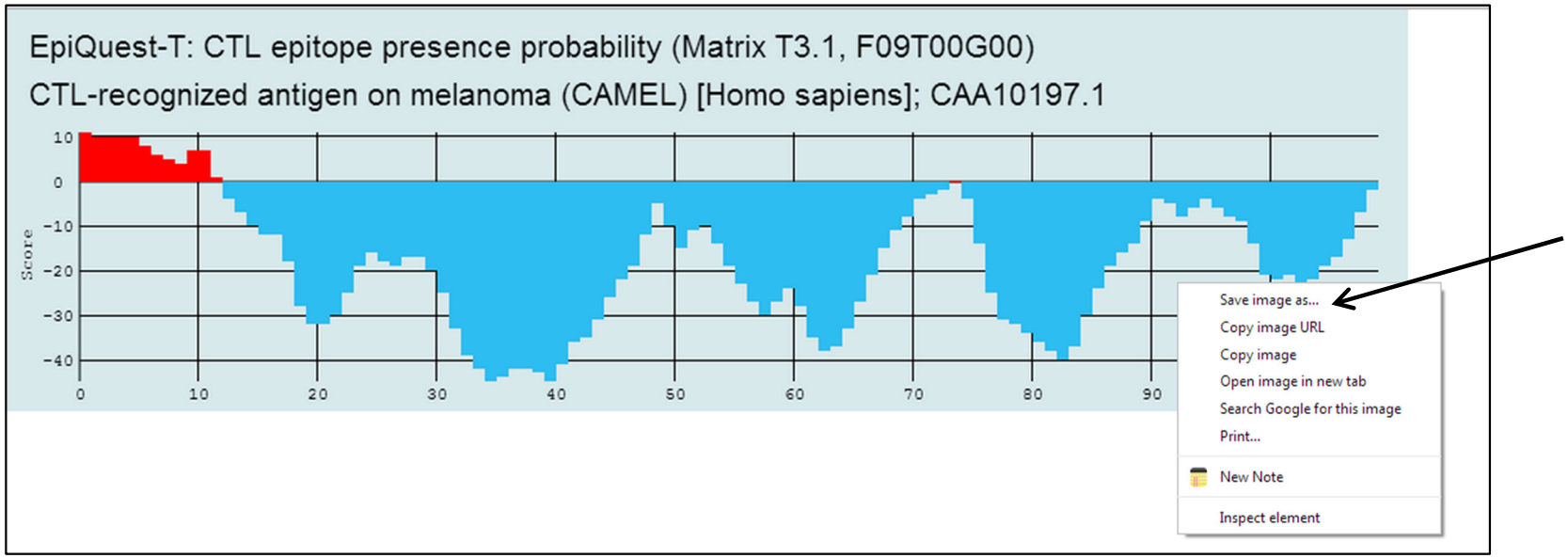
Saving the Report's Images





All images from the report may be exported by clicking "*Export view*" button on the top of the image. The image will be opened as PNG in a new window, from where it can be saved by right-clicking and using "save image as"



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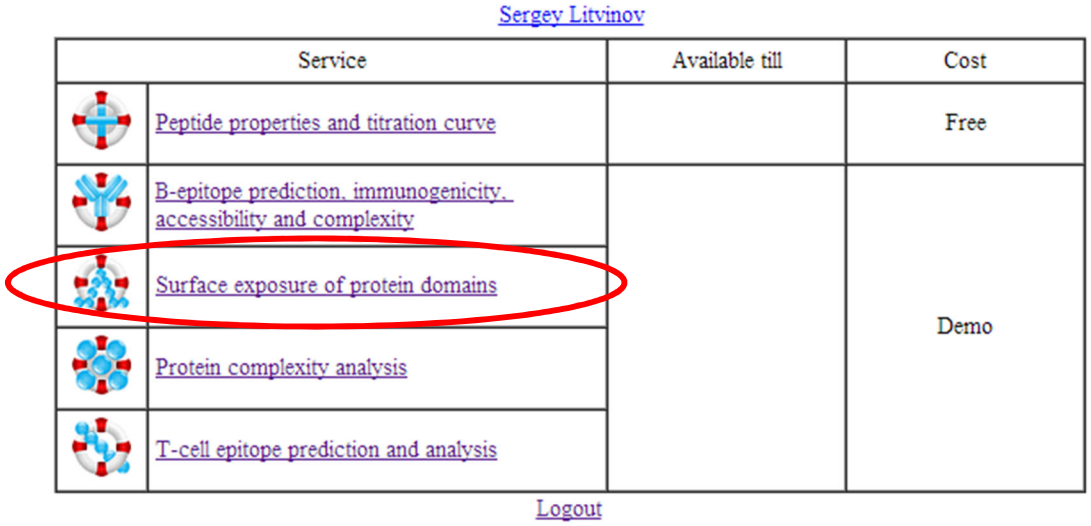
Domain Accessibility Analysis

Whenever you analyze the sequence for immunogenicity, B- epitopes, functional domains etc., you need to establish the accessibility of the protein domain/epitope at the surface of the molecule. The program EpiQuest-A, giving the ***probability*** of the domain exposure at the surface, in our benchmark tests performed better that other analogues, however, you must take into account that the program operates in terms of probability, and the threshold level is chosen as the optimal for the most of the sequences analyzed retrospectively or prospectively. For more on the subject, please refer to our web site blog and library.



To start the program on the server, please click the link.

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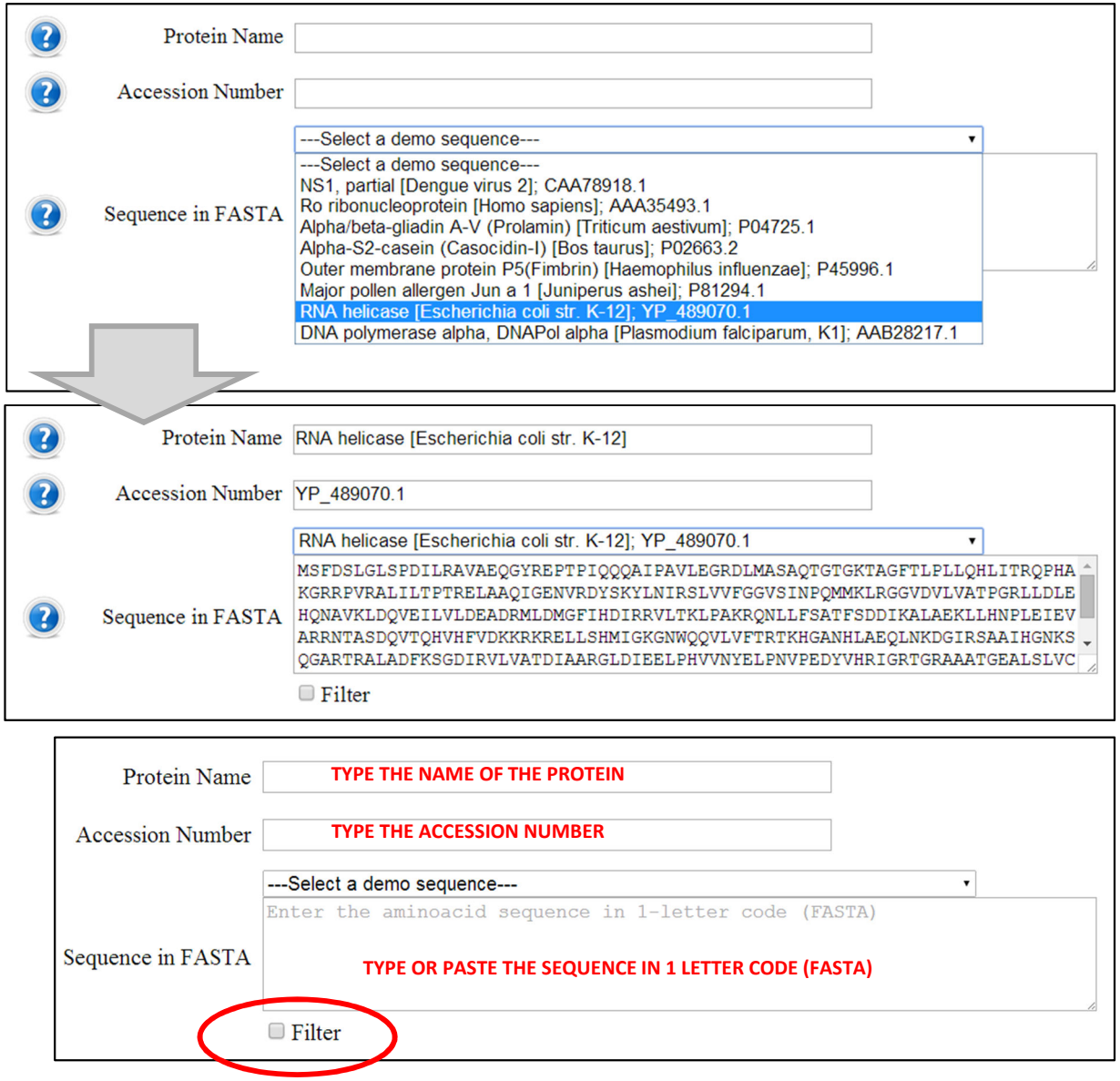
Choosing or Entering the Protein Sequence



In *Demo mode*, simply select one of the demo sequences by clicking on it in drop-down list.
The *sequence name, Accession number* and the *Sequence* will be automatically placed into respective windows.
In *Full Mode*, when enter your own sequence in a 1 letter code (Capitals or lower case) into the window.
We also strongly recommend entering the Acc. Number for the sequence, as it will appear in all reports and will later help you to keep the accurate records of your analysis.

The sequence you are entering will be validated for the presence of other symbols than 20 aa code letters; if such will be found, they will be removed if you will check the *Filter* box. Otherwise you will be warned about the presence of illegal symbols.

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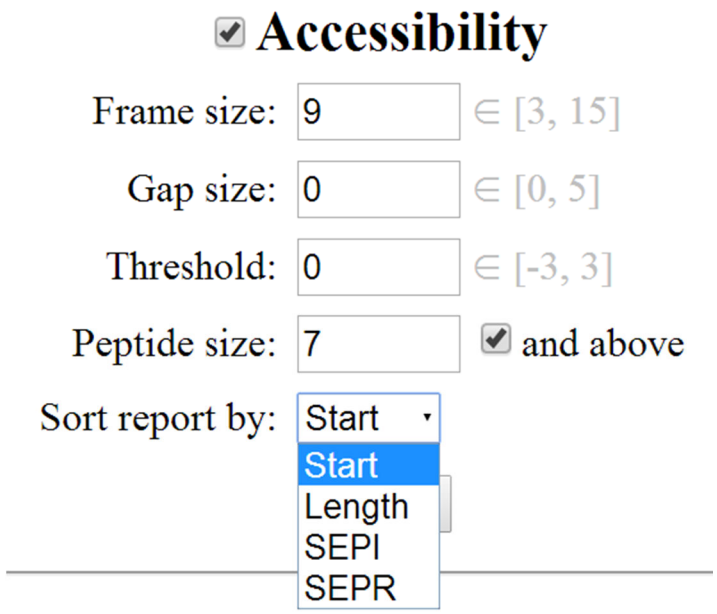
Settings for Accessibility Analysis



For a regular analysis, we advise to keep the DEFAULT settings (you can reset them by pushing the "default" button. When analysing the sequence for epitope prediction, you may want to change both the FRAME and the peptide size to 12. This may be more optimal setting for epitopes. However, please first see these explanations for how it would influence your analysis.

You can sort the text output (sequences) in the report according to different parameters. You may ask for list of positive fragments according to their sequence in the protein (START), sort them by size (Length), sort them be an absolute value (SEPI), which will sort the sequences according the their probability or exposure and long – the first will come the longest and most positive fragment, and according to relative positivity (probability per length), which will sort the fragments according to their absolute probability of surface exposure.

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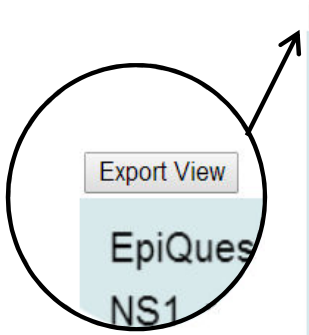
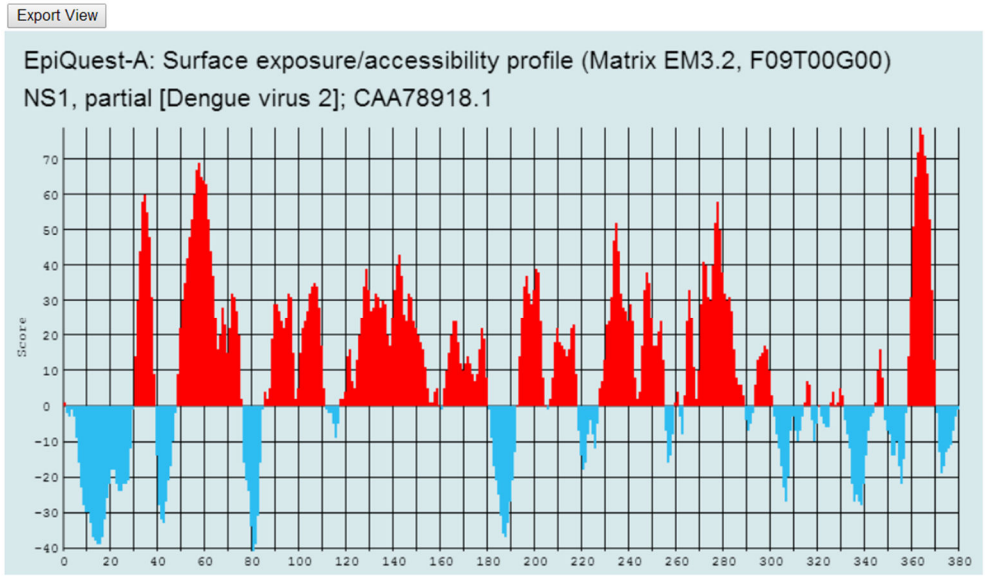
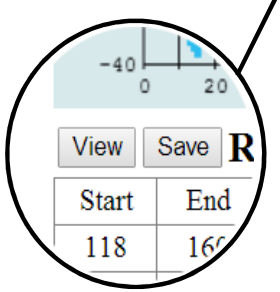
Report for Accessibility Analysis

After you push the START button, the program generates a report. In includes the graphical overview of the molecule profile and can be exported by clicking the “export view”, and saved as a PNG file (Save as). It is followed by a table report with sequences, sorted according to request criteria (see here), which can be viewed and saved. The resulting text report will contain all the key information of your analysis parameters (see further)







View Save **Report for "Accessibility":**

Start	End	Length	Sequence	SEPI	SEPR
118	160	43	TGDIKGIMQVGKRSLRPQPTELRYSWKTWGKAKMLSTELHNQT	874	20
228	255	28	YWIESALNDTWKIEKASFIEVKSCHWPK	664	23
49	76	28	VTDNVHTRTEQYKFQPESPSKLASAIQK	1022	36
264	289	26	VLESEMVIPKNFAGPKSQHNNRPGYH	676	26
86	111	26	RSVTRLENLMWKQITSELNHILSENE	544	20
162	180	19	LIDGPETAECPNTNRAWNS	266	14
193	205	13	TNIWLRLREKQDA	313	24
359	370	12	YGMEIRPLKEKE	625	52
207	218	12	CDSKLMSAAIKD	185	15
31	39	9	GCVVSWKNK	349	38
294	301	8	GPWHLGKL	94	11

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Tabular Report for probability of surface exposure

The tabular report produces a list of sequences that have probability of surface exposure >0 at given threshold, which at Th=0 (default settings) means that the identified sequences are likely exposed at the surface of the folded mature protein, and may be accessed by antibodies, ligands etc.

Depending on the settings of the parameters, the identified sequences may be listed according to their linear position in the primary sequence of the protein (from N to C terminus), or according to probability of exposure (SEPR, sequence exposure per residue). The higher is SEPR, the higher is the probability that the sequence in exposed.

Please be advices to treat with caution the very terminal sequences of the protein, the N- and C- terminal 20-30 amino acids: as they are located not inside the protein, and usually less organized than the internal domains, there is a high probability that they are exposed irrespective of their actual exposure index.

View Save **Report for "Accessibility":**

Start	End	Length	Sequence	SEPI	SEPR
31	39	9	GCVVSWKNK	349	38
49	76	28	VTDNVHTRTEQYKFQPESPSKLASAIQK	1022	36
86	111	26	RSVTRLENLMWKQITSELNHILSENE	544	20
118	160	43	TGDIKGIMQVGKRSLRPQPTELRYSWKTWGKAKMLSTELHNQT	874	20
162	180	19	LIDGPETAECPNTNRAWNS	266	14
193	205	13	TNIWLRLREKQDA	313	24
207	218	12	CDSKLMSAAIKD	185	15
228	255	28	YWIESALNDTWKIEKASFIEVKSCHWPK	664	23
264	289	26	VLESEMVIPKNFAGPKSQHNNRPGYH	676	26
294	301	8	GPWHLGKL	94	11
359	370	12	YGMEIRPLKEKE	625	52

View Save **Report for "Accessibility":**

Start	End	Length	Sequence	SEPI	SEPR
359	370	12	YGMEIRPLKEKE	625	52
31	39	9	GCVVSWKNK	349	38
49	76	28	VTDNVHTRTEQYKFQPESPSKLASAIQK	1022	36
264	289	26	VLESEMVIPKNFAGPKSQHNNRPGYH	676	26
193	205	13	TNIWLRLREKQDA	313	24
228	255	28	YWIESALNDTWKIEKASFIEVKSCHWPK	664	23
118	160	43	TGDIKGIMQVGKRSLRPQPTELRYSWKTWGKAKMLSTELHNQT	874	20
86	111	26	RSVTRLENLMWKQITSELNHILSENE	544	20
207	218	12	CDSKLMSAAIKD	185	15
162	180	19	LIDGPETAECPNTNRAWNS	266	14
294	301	8	GPWHLGKL	94	11

Table of content...

Saving Tabular Report

By clicking “save” at the top left corner of tabular results you generate a full report (below, left) in a new window. This report may be saved as HTML file (to be later opened in any browser on any OS device, or exported to one of MS office formats.

Report: Analysis of Surface exposure/accessibility profile (EpiQuest-A)

Date & Time: 04.02.2015 14:34:17

Protein Name: Ro ribonucleoprotein [Homo sapiens]

Accession Number: AAA35493.1

Sequence:
MEESVNQMQPLNEKQIANSQDGYVWQVTDMNRLHRFLCFGSEGGTYYIKEQKLGLENAEALIRLIEDGRG
CEVIQEIKSFSQEGRTTKQEPMLFALAICSQCSDISTKQAAFKAVSEVCRIPTHLFTFIQFKKDLKESMK
CGMWGRALRKAIADWYNEKGGMALALAVTKYKQRNGWSHKDLLRLSHLKPSSEGLAIVTKYITKGWKEVH
ELYKEKALSVETEKLLKYLEAVEKVKRTKDELEVIHLIEEHRLVREHLLTNHLKSKEVWKALLQEMPLTA
LLRNLGKMTANSVLEPGNSEVSLVCEKLCNEKLLKKARIHPFHILIALETYKTGHGLRGKLKWRPDEEIL
KALDAAFYKTFKTVEPTGKRFLLAVDVSASMNQRVLGSILNASTVAAAMCMVVTRTEKDSYVVAFSDEMV
PCPVTTDMTLQQVLMAMSQIPAGGTDCSLPMIWAQKTNTPADVFIVFTDNETFAGGVHPAIALREYRKKM
DIPAKLIVCGMTSNGFTIADPDDRGMLDMCGFDTGALDVIRNFTLDMI

Matrix: EM3.2

Frame size: 9

Gap: 0

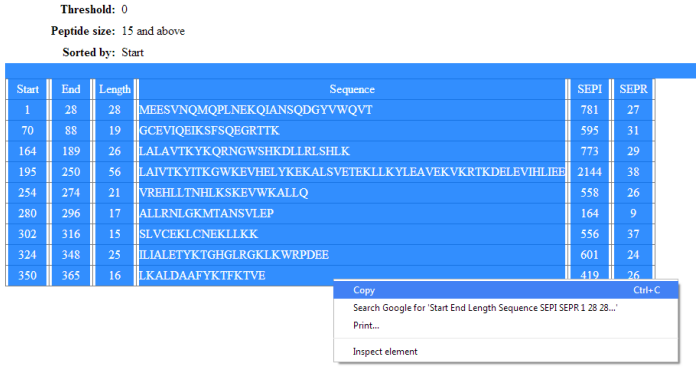
Threshold: 0

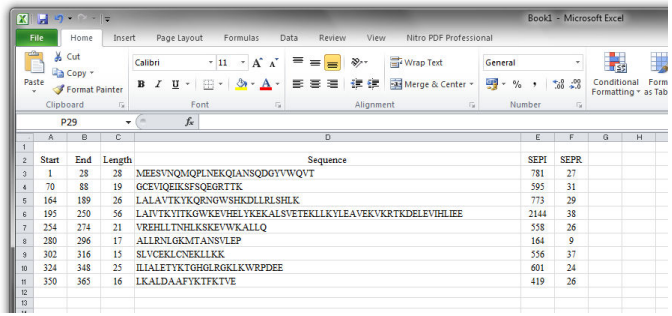
Peptide size: 15 and above

Sorted by: Start

Start	End	Length	Sequence	SEPI	SEPR
1	28	28	MEESVNQMQPLNEKQIANSQDGYVWQVT	781	27
70	88	19	GCEVIQEIKSFSQEGRTTK	595	31
164	189	26	LALAVTKYKQRNGWSHKDLLRLSHLK	773	29
195	250	56	LAIVTKYITKGWKEVHELYKEKALSVETEKLLKYLEAVEKVKRTKDELEVIHLIEE	2144	38
254	274	21	VREHLLTNHLKSKEVWKALLQ	558	26
280	296	17	ALLRNLGKMTANSVLEP	164	9
302	316	15	SLVCEKLCNEKLLKK	556	37
324	348	25	ILIALETYKTGHGLRGKLKWRPDEE	601	24
350	365	16	LKALDAAFYKTFKTVE	419	26

You may also copy (as shown below) the results’ table and save it in Excel sheet by selecting the table and simply pasting it to a chosen Worksheet.





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In Charge

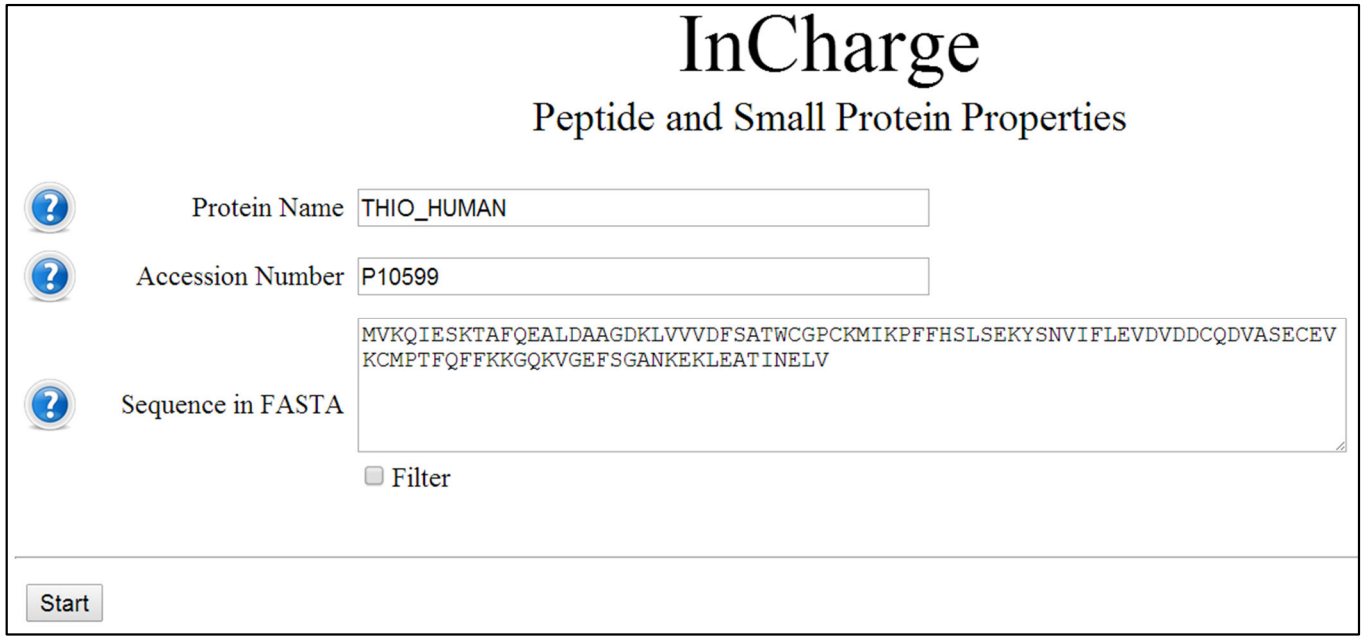
Program In Charge is at the moment the most accurately predicting the peptide charge software (by the results of benchmarking). A few additional features for characterization of peptides and short proteins will be added soon

Sergey Litvinov

Service	Available till	Cost
Peptide properties and titration curve		Free
B-epitope prediction, immunogenicity, accessibility and complexity		Demo
Surface exposure of protein domains		
Protein complexity analysis		
T-cell epitope prediction and analysis		

Logout

To use the program, click the respective link at the login screen, and fill the sequence, name of the protein/ peptide and accession or code number. Click the “Start Button”.



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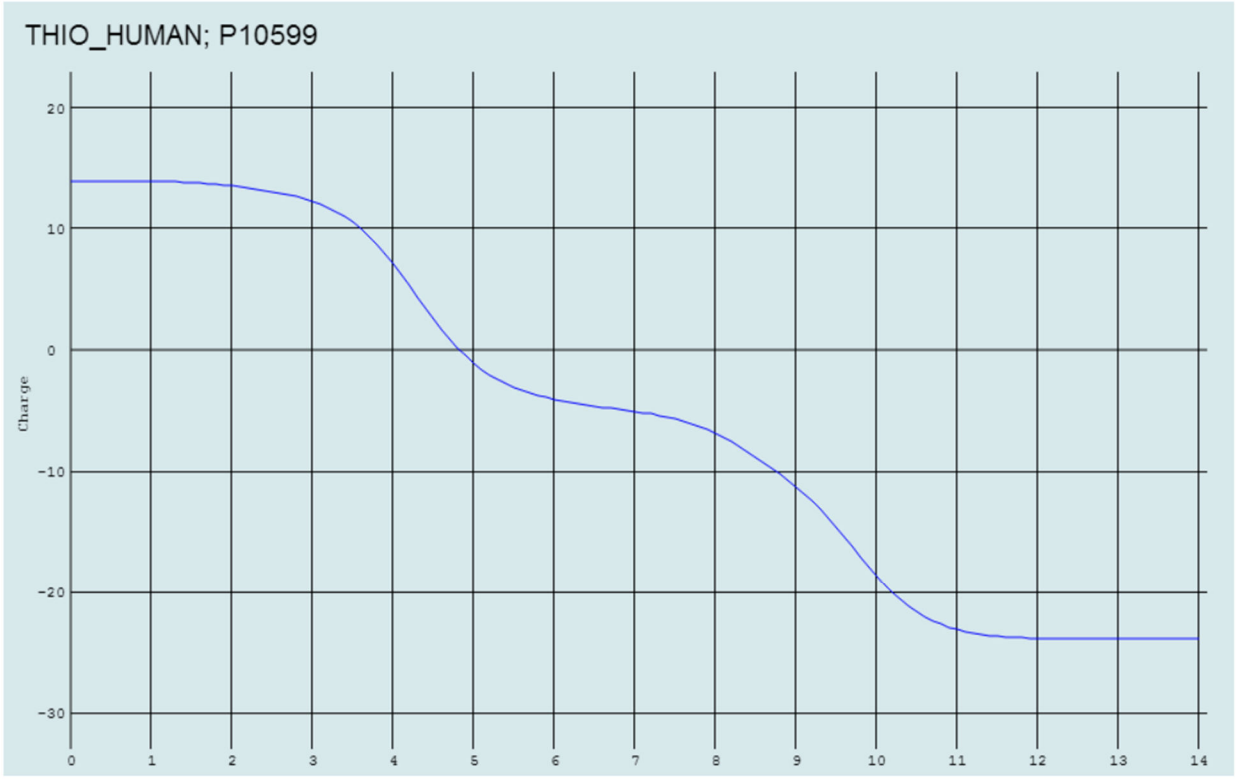
In Charge: Results

The program will built a calibration profile for the protein as a graph and as the numerical column with the charge of the molecule provided for every pH meaning with a step of 0.1. The pH at which the charge of the molecule is close to 0 is the isoelectric point. The tabular data allows you to predict the charge of the molecule at various pH (which is important when you prepare i.e. solutions, mixes of the peptide).

Export View **Calibration curve and isoelectic point (pI)**



pH = 3,9; C = 8,00
pH = 4,0; C = 7,17
pH = 4,1; C = 6,30
pH = 4,2; C = 5,38
pH = 4,3; C = 4,44
pH = 4,4; C = 3,51
pH = 4,5; C = 2,59
pH = 4,6; C = 1,71
pH = 4,7; C = 0,89
pH = 4,8; C = 0,14
pH = 4,9; C = -0,54
pH = 5,0; C = -1,14
pH = 5,1; C = -1,67
pH = 5,2; C = -2,13
pH = 5,3; C = -2,52
pH = 5,4; C = -2,86
pH = 5,5; C = -3,15
pH = 5,6; C = -3,40
pH = 5,7; C = -3,62
pH = 5,8; C = -3,81
pH = 5,9; C = -3,98
pH = 6,0; C = -4,13
pH = 6,1; C = -4,26
pH = 6,2; C = -4,39
pH = 6,3; C = -4,50
pH = 6,4; C = -4,60
pH = 6,5; C = -4,70
pH = 6,6; C = -4,79
pH = 6,7; C = -4,88
pH = 6,8; C = -4,96
pH = 6,9; C = -5,04

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