APEX

Quantitative Proteomics Tool

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Welcome to APEX

The APEX Quantitative Proteomics Tool is designed to generate APEX, Absolute Protein Expression Estimates, according to the technique described in:

Peng Lu, Christine Vogel, Rong Wang, Xin Yao, Edward M. Marcotte. Absolute Protein Expression Profiling Estimates the Relative Contributions of Transcriptional and Translational Regulation. Nature Biotech. 25(1):117-124, 2007.

John C Braisted, Srilatha Kuntumalla, Christine Vogel, Edward M Marcotte, Alan R Rodrigues, Rong Wang, Shih-Ting Huang, Erik S Ferlanti, Alexander I Saeed, Robert D Fleischmann, Scott N Peterson, Rembert Pieper. **The APEX Quantitative Proteomics Tool: Generating protein quantitation estimates from LC-MS/MS proteomics results.** ***BMC Bioinformatics 2008.***

The APEX technique uses a modified spectral counting technique that utilizes machine learning techniques to arrive at protein abundance values with improved accuracy over traditional spectral counting techniques. The APEX Tool provides computational support for this technique through a set of interfaces designed to guide the user through the process of generating APEX protein abundance estimates.

A Note on this Manual and the Companion Tutorial Document

This manual describes the use of the APEX Tool in detail. This manual includes a description of resource requirements including file format descriptions, details on the major processing tasks and related parameter selections. The APEX technique paper, cited above, is also a very good source for details on the theory behind the APEX quantitation method.

It is highly recommended to read the overview section of this manual and then run through the brief tutorial that is described in a companion document in the documentation folder. The tutorial will lead you through the three major functions of the tool using a provided data set. The tutorial will provide hands-on experience and provide a first opportunity to become familiar with the APEX user interface. The tutorial will also serve to make the processing tasks more concrete and will enhance understanding of how the tasks fit together to arrive at APEX protein abundance values.

License and Copyright

License

The APEX Quantitative Proteomics Tool is a free open source software application that is intended to support the needs of the general proteomics research community. The APEX Tool is licensed under the GNU General Public License version 3 (GPLv3). A text file copy of the GPLv3 license is included in the main APEX directory and is labeled *license_gpl-3.0.txt*. The terms of this license are also found on-line at: http://www.gnu.org/copyleft/gpl.html.

Copyright

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Computer Requirements

Java

The APEX Tool was written in the Java programming language which means that the program will run on computers running Microsoft™ Windows® (2000, XP or Vista), Linux®, and Mac® OSX (1.4 or 1.5) operating systems. APEX requires a Java Runtime Environment (JRE) installation of version 1.5 or greater. Most computers will have an installation of Java but if Java has not been installed it can be downloaded and installed from http://java.sun.com/downloads. You can determine your JRE version using the command 'java –version' on the command prompt in a dos window or in a terminal window (Mac and Linux).

Hardware

Being developed in Java, the APEX Tool is compatible with Windows, Linux, and Mac operating systems. APEX has been tested on machines with as little as 256MB of RAM however, 512MB to 1GB of memory is recommended. The processor speed will impact the time required to complete tasks. APEX has been tested on machines with processor speeds below 1 GHz but faster processors that run above 2 GHz will help speed processing.

Launching APEX for the First Time

The APEX tool will run on major operating systems however the process for launching APEX differs based on the operating system being used. The following table describes the procedure for launching APEX on Windows, Linux, and Mac OSX operating systems.

Operating System	APEX Launching Instructions
Windows XP, Windows Vista	Double click on the apex.bat file. (If file extensions are hidden, the icon looks like a window with a gear in it.)
Linux (various builds)	Use a terminal window to navigate to the directory containing apex.sh and enter 'apex.sh' or 'sh apex.sh' on the command line prompt.
Max OSX, 1.4 or 1.5	Double Click on the 'MacAPEX' application bundle OR use a terminal window, navigate to the APEX Tool folder containing apex.sh and enter 'apex.sh' or 'sh apex.sh'.

Tips on Common Problems and Getting Help

APEX Won't Launch

If APEX fails to launch, the DOS prompt window (for Windows OS), command line (for Linux), or the Console (for Mac) will log an error. Often the error will be something like *java command not found* or *version error*. This can be usually fixed by installing the latest Java Runtime Environment (JRE) by using the link above.

APEX Presented an Error Message During Processing

If an error message arises during APEX computational processes, a small window with a text error message will appear. This window will list the process and approximate processing step that generated the error as well as a more detailed error message generated from the Java code. You can copy and paste (select text, ctrl-c, then ctrl-v) this text or save the error messages to an error log file. If you save the error, a standard file browser will be presented for output file specification. Initially the file will be labeled as "error_log_" plus a randomly generated error log id number and be ready to place into the main APEX folder. You can change the destination file and location if you desire.

Getting Help

The performance of the APEX tool and your satisfaction with the tool is very important to us. We would like to help resolve any problem or question you might have regarding the APEX tool and its use. We are happy to field questions regarding software problems, questions about the underlying computations, or features that you think would enhance the tool's abilities in ways that would help the research community.

The APEX menu contains a *Report a Problem* menu option that will open an HTML page that includes a hyperlink to start a mail message to APEX Tool support. You can click on the email link on that page or simply use your email system to send us an email. Here are the key points:

1.) Our address: apex@jcvi.org
2.) Please include **[APEX]** on the subject line.
3.) In the body of the email, please describe the problem or question in as much detail as you can. The following list describes some of the information that could be useful to us when addressing your question. These are just some ideas, not all will apply in all cases.

* Operating System (Windows XP, Vista, Linux, Mac OSX)
* Operating System version (Vista, XP, Linux type, OSX version, etc.)
* Computer hardware information, amount of memory can be an important consideration
* The process being run when an error was raised and the state of the progress log window
* Please save the error log to file if present and attach it to the email to us.
* If there are separate errors reported in the DOS window (or Terminal window or Console for Linux or Mac) please copy them and send them to us.

Data Requirements – Input File Types

Several files types are required as input to the APEX tool. Appendix A will describe the various file formats in some detail. This section is designed to clearly list resources that are required prior to analyzing your data with the APEX tool. Note that an overview of the basic functions of the APEX Tool will be covered in the next section and will describe how each of these files is used in the APEX tool. Note each process within the tool has different requirements that are described in more details in later sections.

Protein Accession List

This file contains a list of protein accessions that correspond to a known set of high abundance proteins within the proteome under study. The identifiers are typically NCBI gi numbers or SwissProt accessions. The main concern is that the identifiers match the identifiers in the FASTA Sequence file. These proteins, often between 40 and 100 in number, represent a set of proteins are very likely to be identified by your MS technique within the samples under study.

FASTA Format Protein Sequence File

This file should contain all protein sequences known to possibly be observed within the samples under study. Normally this sequence file covers the sequences for an entire species or strain under study.

ProteinProphet XML Protein File

The Transproteomic Pipeline (TPP) is a collection of analysis tools that sequentially processes mass spectrometric (MS) data to produce estimates of protein identification probabilities and other values. MASCOT search result dat files or SEQUEST summary HTML files are fed into the pipeline where PeptideProphet followed by ProteinProphet are applied to generate an XML format file (protXML) that contains the list of identified proteins, a probability of accurate identification, and information relating to the number and sequence of peptides observed that relate to each identified protein. **An Appendix section contains additional information on the TPP.** The main point at this stage is that PeptideProphet and ProteinProphet should be used to generate this input protein XML file.

APEX Process Overview – The Three Major Processing Tasks

The APEX tool performs three primary processing tasks toward the generation of APEX protein abundance results. The first two of these tasks are concerned with building a resource file (O_i file) that is used in the third processing task of computing apex abundance values. Note that the first two tasks which build the resource file (O_i file) only need to be run one time for a particular study. Once the resource O_i file has been built, future analyses can be run without running tasks 1 and 2 described below.

Before describing the three major processing tasks, it is important to understand the primary computational process. The equation below gives the formula for computing an APEX quantitation value for protein *i* from a collection of *N* proteins being assessed. p_i refers to the protein identification probability for protein *i* while n_i refers to the *total* number of peptides (not necessarily unique) observed by MS that are attributed to protein *i*. p_i and n_i are both input from a ProteinProphet XML file which is generated from processing MASCOT or SEQUEST result files (See Appendix B for information on ProteinProphet and the Trans-Proteomic Pipeline.). C is input by the user and is typically an estimate of the number of proteins per cell and thus puts the relative abundance value in absolute terms.

$$\mathrm{APEX}(i) = \frac{\dfrac{p_i\, n_i}{O_i}}{\displaystyle\sum_{k=1}^{N} \frac{p_k\, n_k}{O_k}} \times C$$

Figure 1. APEX Computation Formula

The first two APEX processing steps described below are concerned with computing accurate estimates of O_i values to be used in this equation during the third processing step, actual abundance computation. O_i refers to the number of peptides that are *expected* or predicted to be observed from a single molecule of protein *i*. The ratio of n_i/O_i is at the heart of the APEX computation since it relates an *observed* number of peptides to a computationally derived *expected* number of peptides. A number of peptides are derived from protein *i* during digestion, however only a subset of these peptides is detected by MS. A complex set of factors relating to the peptide’s physicochemical properties determine which peptides are likely to be detected by MS techniques. The Mallick et al. paper (see reference section for citation) describes how different physicochemical properties impact peptide detection by various MS techniques.

1.) Training Data Generation – Building the ARFF Training File

APEX uses machine learning techniques to improve quantitation accuracy. These machine learning techniques require a data set that contains peptide physicochemical property values and an indication of which peptides have been previously detected by MS. This data matrix provides information that characterizes a set of peptides and an indication of whether each peptide was observed or not by MS. This data correlates peptide detection by MS to a set of peptide physicochemical properties. In the next task, this data, training data, will be used to build or *train* a classifier to predict the probability of a peptide being detected by MS based on its physicochemical properties. Note that the output file from this process is in a standardized format called Attribute Relation File Format and is described in further detail in the Appendix on file formats.

2.) Generating O_i Estimates – Building an O_i data file

In preparation for MS, the proteins in the sample undergo a digestion. Only a subset of these resulting peptides is detected by MS because of particular physicochemical properties that favor or limit detection. O_i values refer to the *predicted* number of peptides derived from protein *i* that will be detected by the MS technique being utilized. The training data generated in the previous step is used to build a classifier to predict which peptides will be detected based on their physicochemical properties.

The process of computing the O_i values starts by reading a FASTA protein sequence file that covers the proteins under study. The protein sequences then undergo an *in silico* digestion to produce a set of peptides derived from each protein. Physicochemical properties are computed for each of these peptides and are used by the classifier to derive the peptide's probability of being detected by MS. The O_i values are the summation of these peptide MS detection probabilities for protein *i*. O_i values are stored in a file that contains protein accessions and corresponding O_i values that will be used in computing the APEX scores in the next step.

3.) Computing APEX Scores

The primary processing task in APEX is actually computing APEX scores according to the equation in figure 1. The O_i data file produced in steps 1 and 2 above covers a particular set of proteins supplied in the FASTA file in step 2. This O_i file is suitable for all studies that have proteins that are a subset of those in the file. This means that tasks 1 and two are run very infrequently.

The APEX computation panel is the first panel presented in the interface. The process requires a ProteinProphet result XML file, an O_i file from step 2, a specified output file name, and the C factor. During computation a table will be presented that lists all proteins in the input XML file. Either the p_i or the false positive error rate can be used as a criterion to select a subset of this protein list to enter APEX abundance computation. Clicking on a row in the table will select that protein an all proteins with higher p_i values. After APEX computation, a table view will be presented to display the results as well as the specified output file.

Visual Representation of the Processing Tasks

The following figure summarizes the file inputs and outputs for each of the three primary APEX processing tasks. The main point to take from this figure is that the first task builds the training data (ARFF file), the second task takes this training data and creates the Oi file. The Oi file is then fed into subsequent APEX score computations.

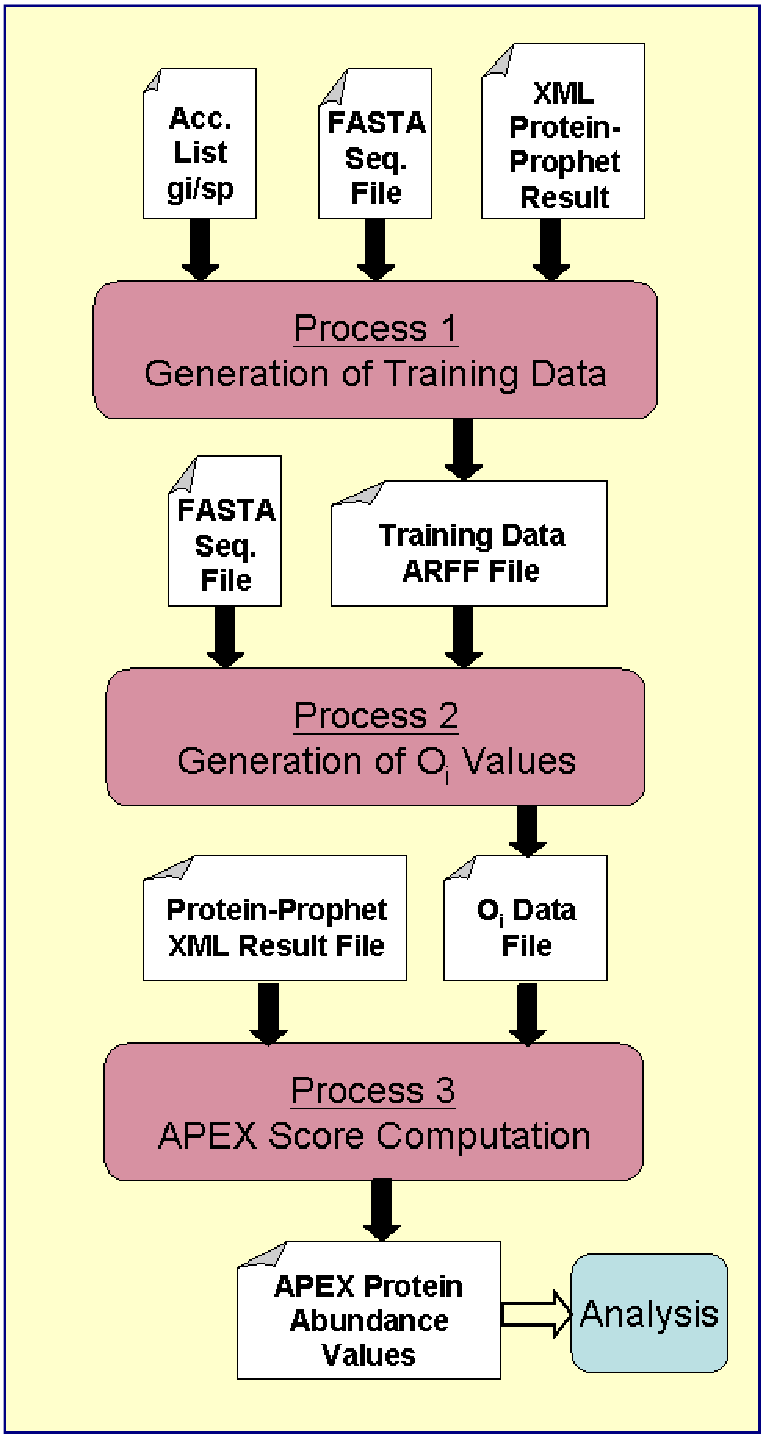
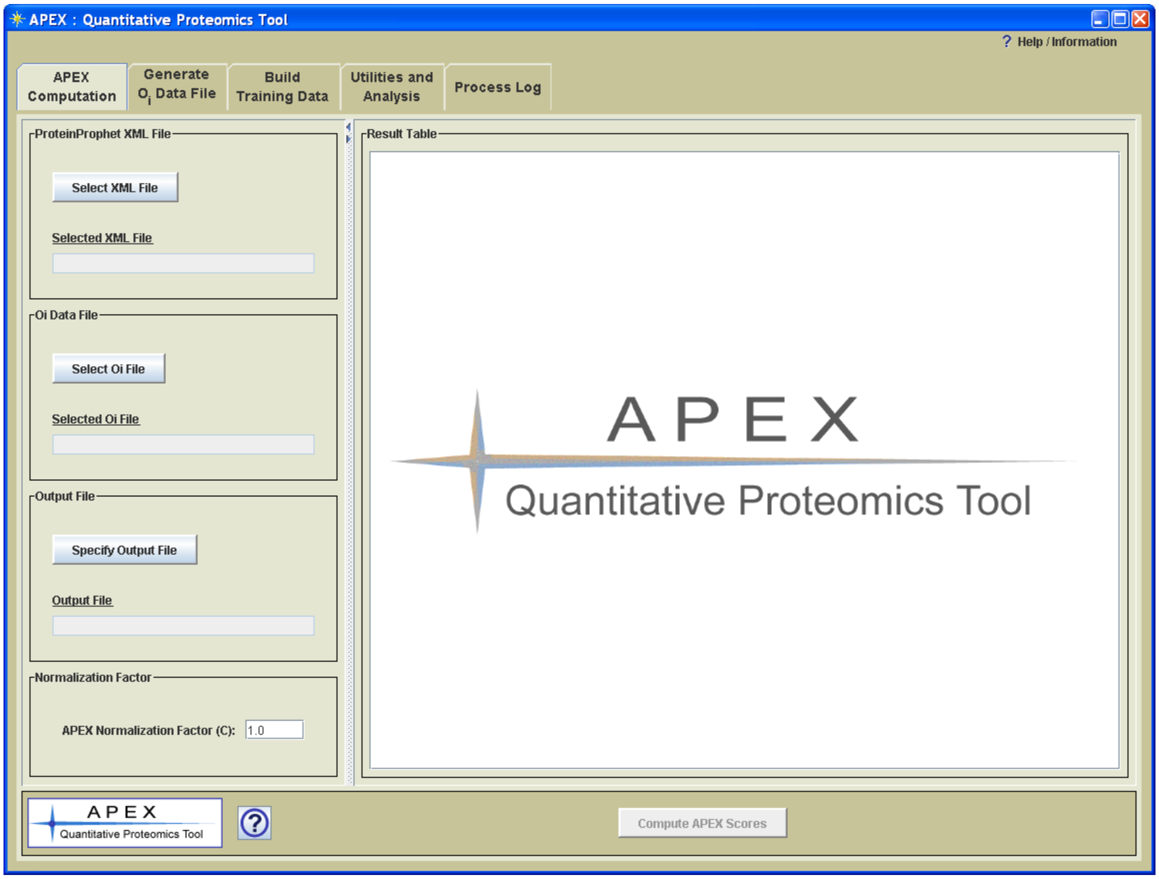


Figure 2. Graphical Representation of APEX Processing Tasks

APEX Interface Overview



APEX Normalization Factor (C): 1.0

Figure 3. APEX Interface

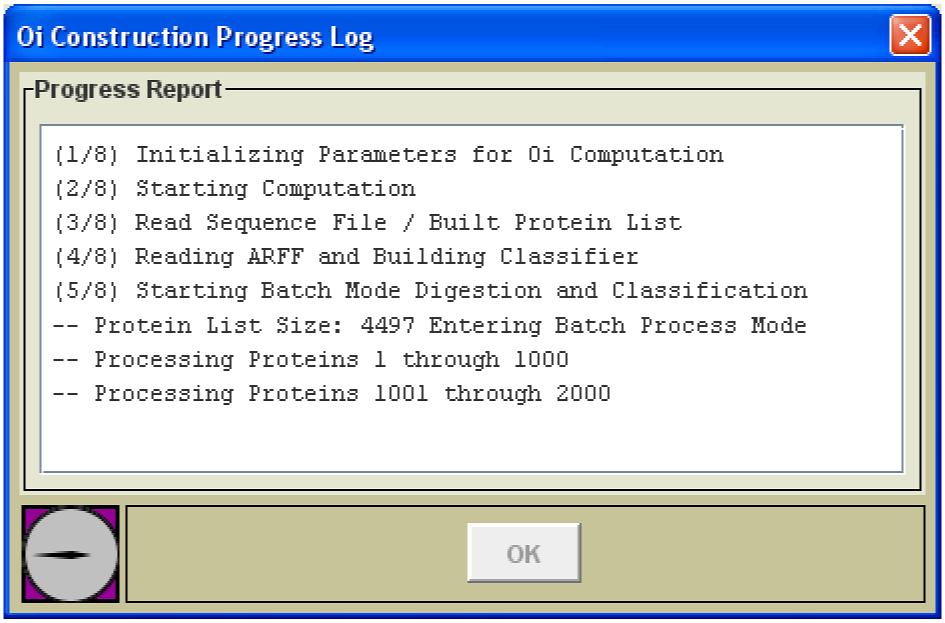
Process Panels

The APEX interface is organized into a window where processing tasks are separated and handled on separate tabbed panes (Figure 3.). Each tabbed pane contains a *process panel* which is designed to encapsulate parameter selections and controls for a particular APEX processing task. The processing panels are ordered such that the processes that are most frequently used are toward the front. Table 1 provides a brief overview of each processing panel.

The execution button on each process page is disabled until all required selections have been made. Once the file selections and parameters have been specified, the excecution button at the bottom of the panel will be enabled. During processing a progress log window (Figure 4.) will be opened and will log key progress events. On completion of the task, the progress dialog will display a message indicating completion and will enable the ‘OK’ button to allow dismissal of the progress dialog.

Table 1. Description of APEX Process Panels

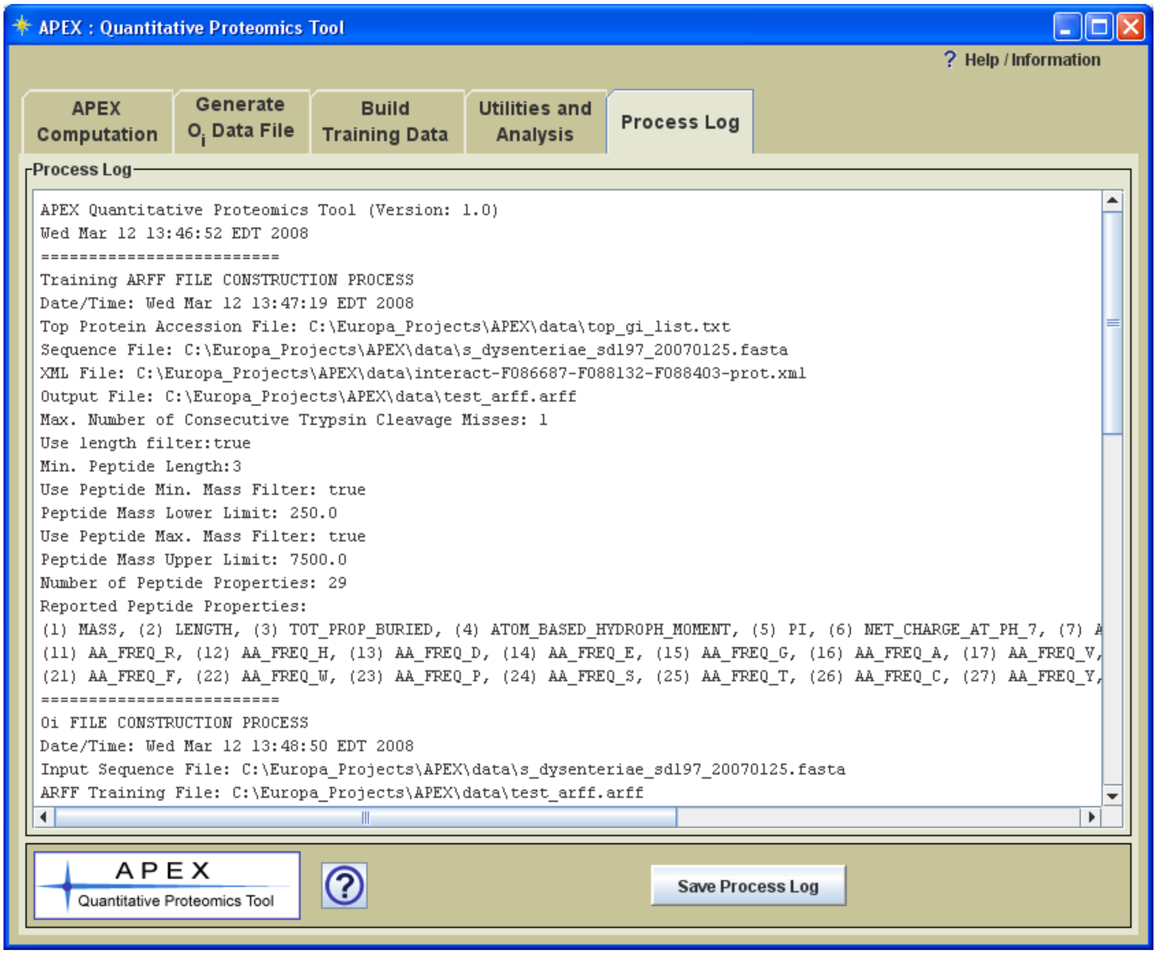
Process Panel Title	Description
APEX Computation	This panel is used to process ProteinProphet XML data files and an O_i file to produce APEX protein abundance values.
Generate Oi Data File	This panel supports the generation of an O_i file that covers the set of proteins under study.
Build Training Data	This panel constructs a training data set, ARFF file, to be used in O_i generation. Since the training data is constructed based on prior results, the training set reflects the nature of the results derived from the researcher's specific MS technique and instrumentation.
Utilities and Analysis	This panel handles utilities such as merging APEX results into a single output file for comparison, the two sample z-test for differential expression, and classifier cross validation testing. The capabilities of this area will expand in future versions.
Processing Log	This panel captures the details of each processing action. The panel captures input and output file names as well as all parameter selections.



```
(1/8) Initializing Parameters for Oi Computation
(2/8) Starting Computation
(3/8) Read Sequence File / Built Protein List
(4/8) Reading ARFF and Building Classifier
(5/8) Starting Batch Mode Digestion and Classification
-- Protein List Size: 4497 Entering Batch Process Mode
-- Processing Proteins 1 through 1000
-- Processing Proteins 1001 through 2000
```

Figure 4. Progress Log

Note that a complete description of the processes that have been run, including file and parameter selections, is written to a text log panel labeled 'Process Log' (Figure 5). This log can be saved as a text file for future reference.



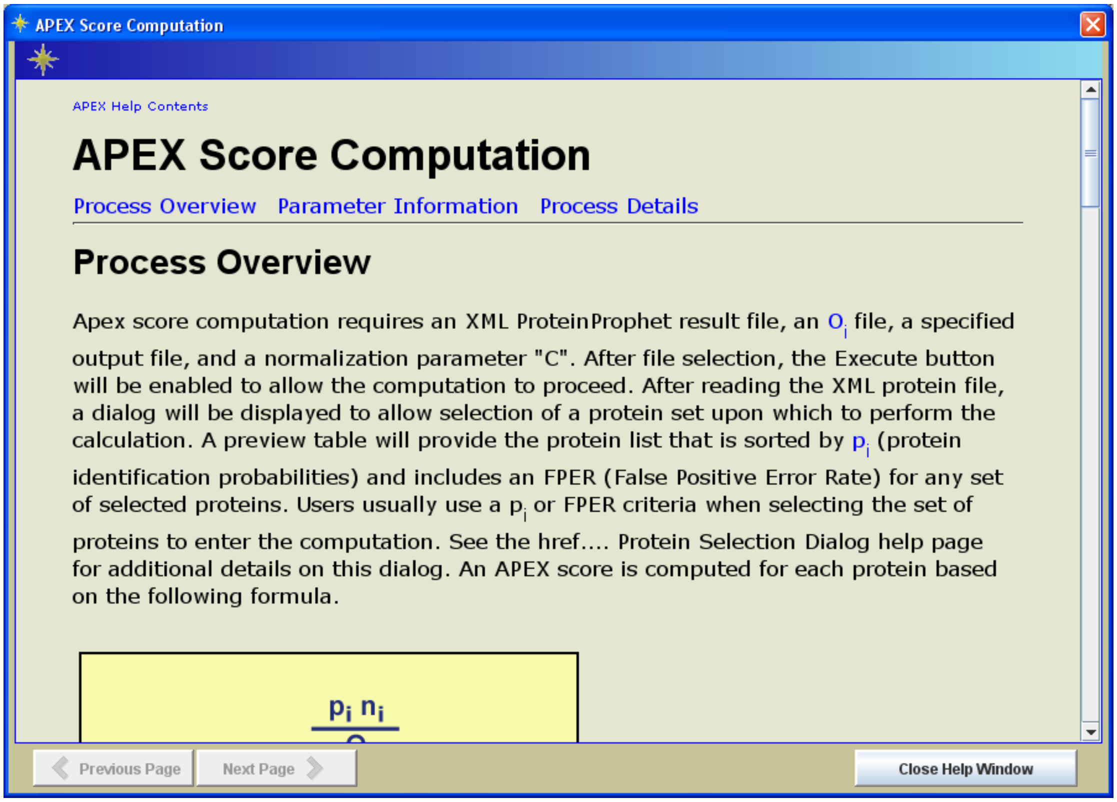
```
APEX Quantitative Proteomics Tool (Version: 1.0)
Wed Mar 12 13:46:52 EDT 2008
=========================
Training ARFF FILE CONSTRUCTION PROCESS
Date/Time: Wed Mar 12 13:47:19 EDT 2008
Top Protein Accession File: C:\Europa_Projects\APEX\data\top_gi_list.txt
Sequence File: C:\Europa_Projects\APEX\data\s_dysenteriae_sd197_20070125.fasta
XML File: C:\Europa_Projects\APEX\data\interact-F086687-F088132-F088403-prot.xml
Output File: C:\Europa_Projects\APEX\data\test_arff.arff
Max. Number of Consecutive Trypsin Cleavage Misses: 1
Use length filter:true
Min. Peptide Length:3
Use Peptide Min. Mass Filter: true
Peptide Mass Lower Limit: 250.0
Use Peptide Max. Mass Filter: true
Peptide Mass Upper Limit: 7500.0
Number of Peptide Properties: 29
Reported Peptide Properties:
(1) MASS, (2) LENGTH, (3) TOT_PROP_BURIED, (4) ATOM_BASED_HYDROPH_MOMENT, (5) PI, (6) NET_CHARGE_AT_PH_7, (7) A
(11) AA_FREQ_R, (12) AA_FREQ_H, (13) AA_FREQ_D, (14) AA_FREQ_E, (15) AA_FREQ_G, (16) AA_FREQ_A, (17) AA_FREQ_V,
(21) AA_FREQ_F, (22) AA_FREQ_W, (23) AA_FREQ_P, (24) AA_FREQ_S, (25) AA_FREQ_T, (26) AA_FREQ_C, (27) AA_FREQ_Y,
=========================
Oi FILE CONSTRUCTION PROCESS
Date/Time: Wed Mar 12 13:48:50 EDT 2008
Input Sequence File: C:\Europa_Projects\APEX\data\s_dysenteriae_sd197_20070125.fasta
ARFF Training File: C:\Europa_Projects\APEX\data\test_arff.arff
```

Figure 5. Process Log Captures Process Parameter Information

Help System

All process panels have an information button in the lower left area of the interface (see sample help page in Figure 6.). The help pages for the three primary processing panels are divided into three major areas: *Process Overview*, *Parameter Information*, and *Process Details*. The Process Overview provides a descriptive overview of the process that is handled by the currently selected process panel. The *Parameter Information* section describes the various files and parameters that can be selected to control the current process. This section contains important information regarding the options presented on the current processing panel. The last section, *Process Details*, provides information about how data is handled and processed in more detail. This last section provides a step-by-step description of what is happening to the data during each step of processing.

All help pages have a link back to the main *Help Contents* page. This page provides links to each of the main process panels' help pages as well as a link to the APEX overview page which provides a slightly condensed overview of the tool's major functions and how they work together. The content of this page is very similar to the *Process Overview* provided earlier within this manual.



APEX Help Contents

APEX Score Computation

Process Overview Parameter Information Process Details

Process Overview

Apex score computation requires an XML ProteinProphet result file, an O_i file, a specified output file, and a normalization parameter "C". After file selection, the Execute button will be enabled to allow the computation to proceed. After reading the XML protein file, a dialog will be displayed to allow selection of a protein set upon which to perform the calculation. A preview table will provide the protein list that is sorted by p_i (protein identification probabilities) and includes an FPER (False Positive Error Rate) for any set of selected proteins. Users usually use a p_i or FPER criteria when selecting the set of proteins to enter the computation. See the href.... Protein Selection Dialog help page for additional details on this dialog. An APEX score is computed for each protein based on the following formula.

$p_i n_i$

Figure 6. Sample Information Page

APEX Menu Options

The APEX menu only plays a supporting role in the tool’s interface in providing access to the help system and general information.

Open Help System and *Open APEX Overview* open help pages that display the main help contents page or the APEX overview page.

APEX Tutorial opens an APEX tutorial. This is a good way to become familiar with the APEX tool after reading the overview pages. A PDF version of the tutorial can also be found within the *documentation* folder.

The *Report a Problem* menu option will attempt to open the default web browser to display a help system information page. The help information page provides the email address for support as well as some tips on key pieces of information to report when requesting help. If APEX cannot open the browser, an abbreviated message window will be displayed with instructions.

APEX Citations is a quick link to a list of references related to the APEX tool including the original paper cited in the *Welcome* section at the start of this manual.

Process Descriptions: More Details

This section of the manual describes each of the main functions of the APEX tool. Each section describes the input files, the parameters, and the output files.

Building Training Data

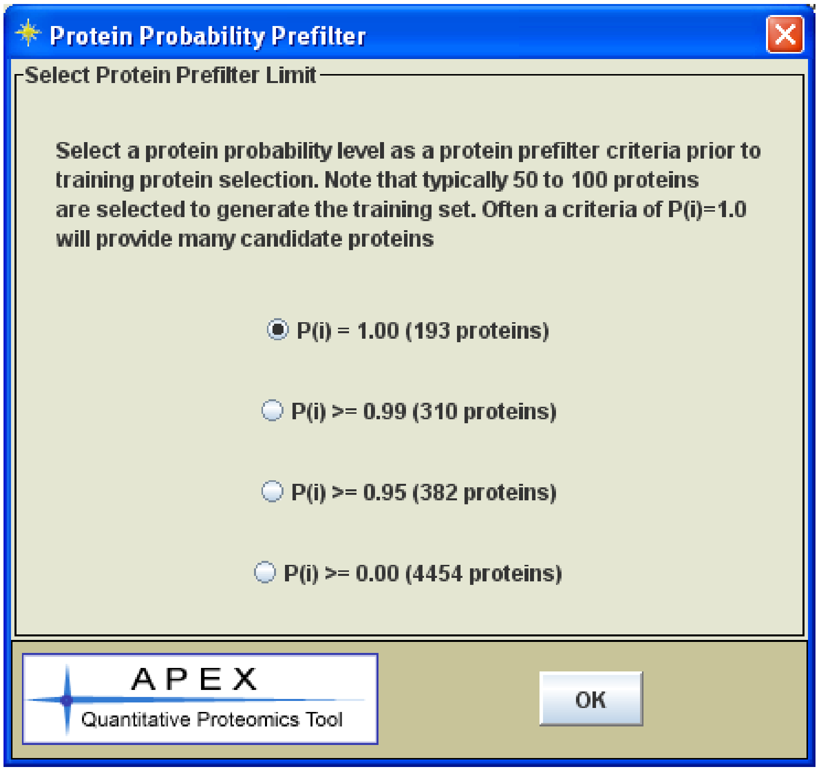
Process Panel Title: Build Training Data

Process: Constructs a training data set in ARFF format that will be used for O_i generation.

Input Files:

1.) A list of accessions of high abundance proteins likely to be in the sample (usually 40-100 protein accessions) determines which proteins will be used to generate peptides for the training data set. This protein list is required to be input either by a selection from a ProteinProphet XML file/MS Result (input by step 3 below) based on setting a prefilter criteria, or by supplying a Protein Accession List File, which is a simple text file with a column of accessions, one accession per line.

If the option to Select Proteins from prot.XML File / MS Result is desired, after the Sequence Fasta File, ProteinProphet XML File and Output File are specified, a Protein Probability Prefilter dialog (see Figure 7.) followed by an APEX Protein List Selection dialog (see Figure 8.) will be displayed during ARFF File Construction.



Protein Probability Prefilter

Select Protein Prefilter Limit

Select a protein probability level as a protein prefilter criteria prior to training protein selection. Note that typically 50 to 100 proteins are selected to generate the training set. Often a criteria of P(i)=1.0 will provide many candidate proteins

● P(i) = 1.00 (193 proteins)

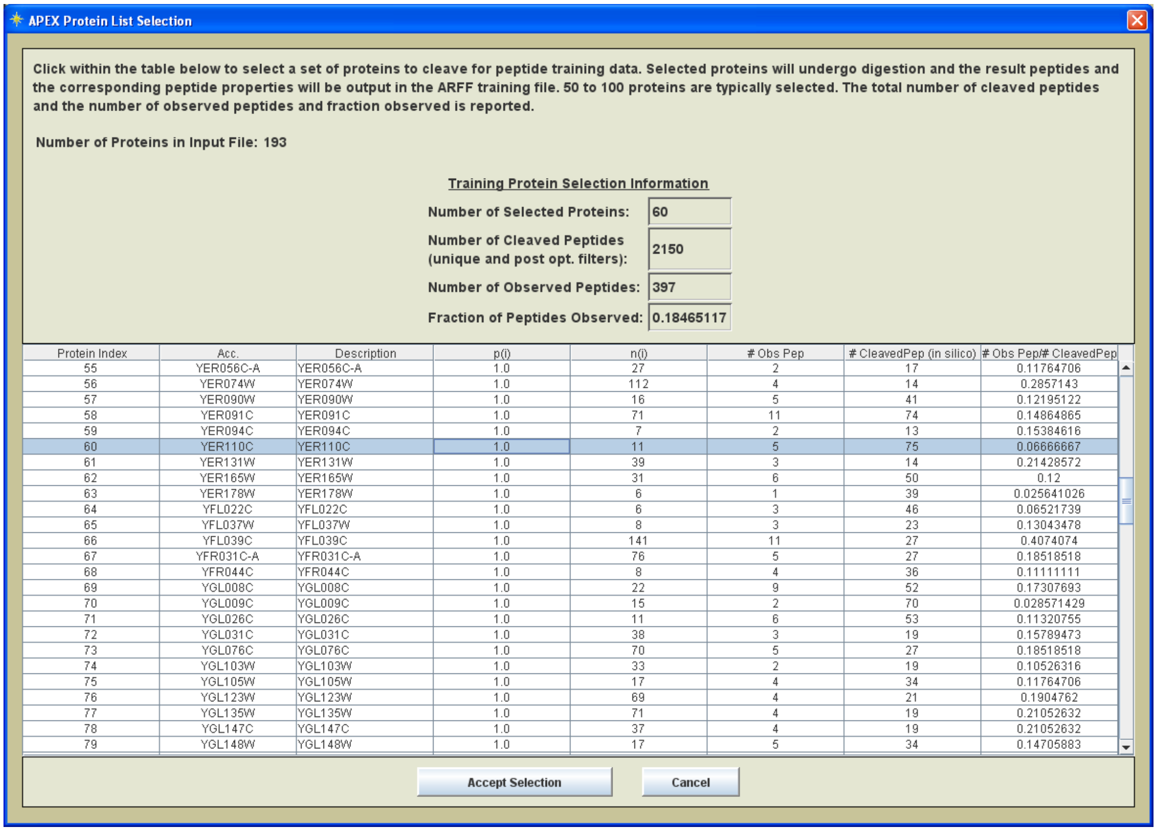
○ P(i) >= 0.99 (310 proteins)

○ P(i) >= 0.95 (382 proteins)

○ P(i) >= 0.00 (4454 proteins)

Figure 7. Protein Probability Prefilter Dialog

In the Protein Probability Prefilter dialog, a protein probability prefilter limit is required to be set by selecting from a list of criteria. The number of proteins that will pass the criteria is calculated and presented. Note that typically 50 to 100 proteins are needed to generate the training set. Often a criteria of P(i) = 1.0 will provide many candidate proteins.



Click within the table below to select a set of proteins to cleave for peptide training data. Selected proteins will undergo digestion and the result peptides and the corresponding peptide properties will be output in the ARFF training file. 50 to 100 proteins are typically selected. The total number of cleaved peptides and the number of observed peptides and fraction observed is reported.

Number of Proteins in Input File: 193

Training Protein Selection Information

Number of Selected Proteins: 60

Number of Cleaved Peptides (unique and post opt. filters): 2150

Number of Observed Peptides: 397

Fraction of Peptides Observed: 0.18465117

Protein Index	Acc.	Description	p(i)	n(i)	# Obs Pep	# CleavedPep (in silico)	# Obs Pep/# CleavedPep
55	YER056C-A	YER056C-A	1.0	27	2	17	0.11764706
56	YER074W	YER074W	1.0	112	4	14	0.2857143
57	YER090W	YER090W	1.0	16	5	41	0.12195122
58	YER091C	YER091C	1.0	71	11	74	0.14864865
59	YER094C	YER094C	1.0	7	2	13	0.15384616
60	YER110C	YER110C	1.0	11	5	75	0.06666667
61	YER131W	YER131W	1.0	39	3	14	0.21428572
62	YER165W	YER165W	1.0	31	6	50	0.12
63	YER178W	YER178W	1.0	6	1	39	0.025641026
64	YFL022C	YFL022C	1.0	6	3	46	0.06521739
65	YFL037W	YFL037W	1.0	8	3	23	0.13043478
66	YFL039C	YFL039C	1.0	141	11	27	0.4074074
67	YFR031C-A	YFR031C-A	1.0	76	5	27	0.18518518
68	YFR044C	YFR044C	1.0	8	4	36	0.11111111
69	YGL008C	YGL008C	1.0	22	9	52	0.17307693
70	YGL009C	YGL009C	1.0	15	2	70	0.028571429
71	YGL026C	YGL026C	1.0	11	6	53	0.11320755
72	YGL031C	YGL031C	1.0	38	3	19	0.15789473
73	YGL076C	YGL076C	1.0	70	5	27	0.18518518
74	YGL103W	YGL103W	1.0	33	2	19	0.10526316
75	YGL105W	YGL105W	1.0	17	4	34	0.11764706
76	YGL123W	YGL123W	1.0	69	4	21	0.1904762
77	YGL135W	YGL135W	1.0	71	4	19	0.21052632
78	YGL147C	YGL147C	1.0	37	4	19	0.21052632
79	YGL148W	YGL148W	1.0	17	5	34	0.14705883

Figure 8. Protein List Selection Dialog

In the APEX Protein List Selection dialog, those proteins that have passed the protein probability prefilter criteria are listed in a tabular form. Click within the table to select a set of proteins to cleave for peptide training data. The selected proteins will undergo digestion and the resulting peptides and the corresponding peptide properties will be output in the ARFF training file. 50 to 100 proteins are typically selected. The total number of cleaved peptides and the number of observed peptides as well as the fraction observed are reported in the top section of the dialog. This allows users to verify the number of cleaved peptides and the fraction of those peptides that are observed in the protXML file.

2.) A protein sequence FASTA file is required which includes sequences that correspond to the proteins in the accession list file. Note that this file often covers thousands of proteins. The key point is to include the proteins in the accession list file.

3.) A ProteinProphet protein protXML file is required for input. This file provides a list of peptides that were observed during one or more MS runs. The Appendix on the Trans-Proteomic Pipeline will provide information on the construction of this file.

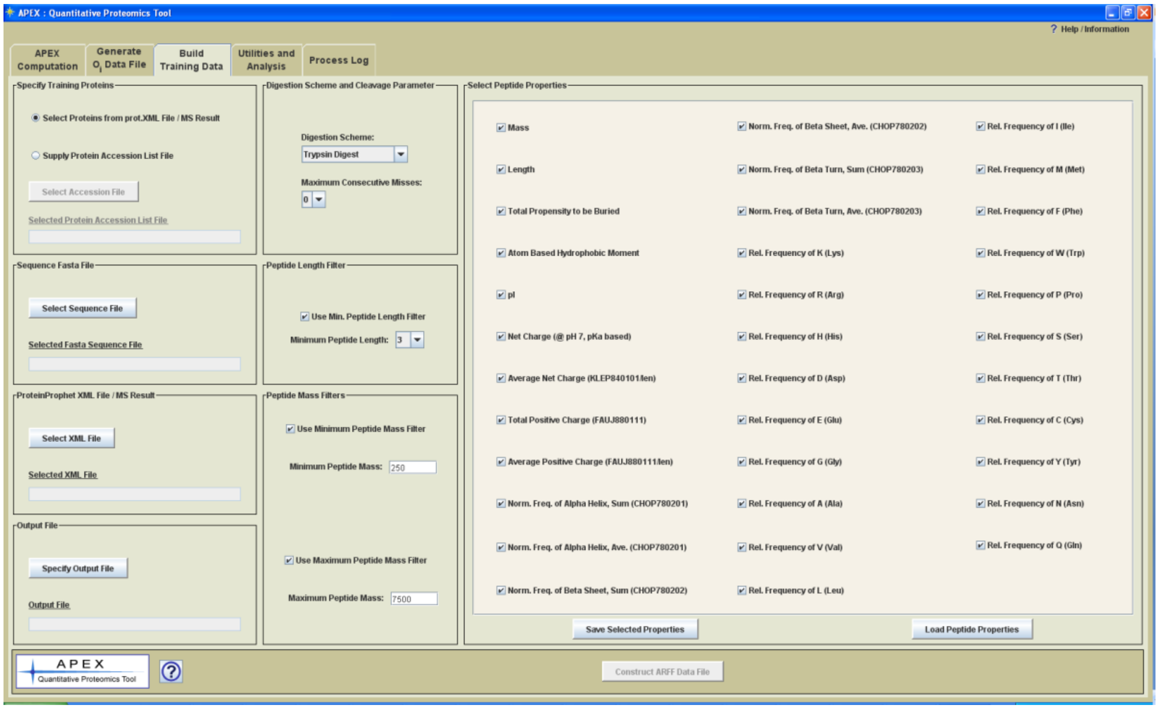
Parameters:

Maximum Consecutive Cleavage Misses: This parameter indicates the number of cleavage misses permitted during *Insilico* trypsin digestion of the input proteins. At this stage it makes sense to focus on peptides that are generated based on zero misses since these are most likely to be observed, or not observed, based mostly upon physicochemical properties. If the miss rate is higher then peptides will be evaluated so that the result will include possible missed cleavages. These peptides may not be observed because the cleavage miss was rare. At this point we want to focus on peptides that were not detected purely based on physicochemical properties and so a miss rate of 0 or 1 seems appropriate.

Minimum Length Filter: This optional filter eliminates or disregards peptides below a certain sequence length. Peptides smaller than a certain sequence length are likely not to be detected by MS. Since we want to focus on physicochemical properties as a determinant of detection, it makes sense to exclude very short peptides. The original Lu paper on APEX allowed a minimum peptide length of 3 amino acid residues.

Peptide Mass Filters: These optional filters eliminate peptides that are smaller than a lower cutoff and greater than an upper mass cutoff. Usually this is set to mimic the range of settings applied during the MS analysis and searches.

Peptide Properties: A number of check boxes allow one to select a set of peptide properties to report in the training data set. *At this stage it is acceptable to select to report on **all** possible peptide properties available within the APEX tool. A subset of these peptide properties will work as predictors of whether a peptide will be detected or not in the O_i generation process. These properties characterize the input peptides that are derived from the input proteins and that pass the peptide filters.



Specify Training Proteins

● Select Proteins from prot.XML File / MS Result

○ Supply Protein Accession List File

Digestion Scheme and Cleavage Parameter

Digestion Scheme: Trypsin Digest

Maximum Consecutive Misses: 0

Peptide Length Filter

☑ Use Min. Peptide Length Filter

Minimum Peptide Length: 3

Peptide Mass Filters

☑ Use Minimum Peptide Mass Filter

Minimum Peptide Mass: 250

☑ Use Maximum Peptide Mass Filter

Maximum Peptide Mass: 7500

Select Peptide Properties

☑ Mass
☑ Length
☑ Total Propensity to be Buried
☑ Atom Based Hydrophobic Moment
☑ pI
☑ Net Charge (@ pH 7, pKa based)
☑ Average Net Charge (KLEP840101/len)
☑ Total Positive Charge (FAUJ880111)
☑ Average Positive Charge (FAUJ880111/len)
☑ Norm. Freq. of Alpha Helix, Sum (CHOP780201)
☑ Norm. Freq. of Alpha Helix, Ave. (CHOP780201)
☑ Norm. Freq. of Beta Sheet, Sum (CHOP780202)
☑ Norm. Freq. of Beta Sheet, Ave. (CHOP780202)
☑ Norm. Freq. of Beta Turn, Sum (CHOP780203)
☑ Norm. Freq. of Beta Turn, Ave. (CHOP780203)
☑ Rel. Frequency of K (Lys)
☑ Rel. Frequency of R (Arg)
☑ Rel. Frequency of H (His)
☑ Rel. Frequency of D (Asp)
☑ Rel. Frequency of E (Glu)
☑ Rel. Frequency of G (Gly)
☑ Rel. Frequency of A (Ala)
☑ Rel. Frequency of V (Val)
☑ Rel. Frequency of L (Leu)
☑ Rel. Frequency of I (Ile)
☑ Rel. Frequency of M (Met)
☑ Rel. Frequency of F (Phe)
☑ Rel. Frequency of W (Trp)
☑ Rel. Frequency of P (Pro)
☑ Rel. Frequency of S (Ser)
☑ Rel. Frequency of T (Thr)
☑ Rel. Frequency of C (Cys)
☑ Rel. Frequency of Y (Tyr)
☑ Rel. Frequency of N (Asn)
☑ Rel. Frequency of Q (Gln)

Figure 9. *Build Training Data* Process Panel

Process Details:

1.) The accession list of high abundance proteins is loaded from an accession file or selected from a protein list of proteins from the prot.XML file. Next the FASTA sequence file is read. The accession list (supplied by the input file or selected from the prot.XML file) is used to dictate which protein sequences are retained for further processing.

2.) The retained protein sequences then undergo an *in silico* trypsin digestion to produce peptides. The maximum miss rate is taken into account during this stage. Increased miss rate will increase the number of peptides. For instance, if a protein naturally cleaves into three peptides, A, B, and C with zero misses, one miss would produce two additional peptides, AB and BC. Note that at this stage we want to focus on reporting on peptides that were very likely to be present during the MS run but were detected or not detected based on physicochemical properties of the peptides.

3.) The optional length and mass filters are applied to remove peptides that fall outside these constraints.

4.) The PeptideProphet XML file is read and all peptide sequences are loaded based on the *peptide_sequence* tag in the XML file. This peptide list includes all peptides that were observed by MS in previous runs. This observed peptide list is checked for the existence of each of the peptides in the peptide list derived from *in silico* digestion.

5.) The rows in the output file consist of peptide properties for each peptide from *in silico* digestion and an indication of whether the peptide was observed or not in the XML file of MS results. The file is produced one row at a time by taking a peptide, computing its peptide property values, then checking the XML file supplied peptide list for whether the peptide was observed by MS. Each line then consists of a comma delimited set of peptide property values followed with the text tags **Obs** or **Not** to indicate whether the peptide was detected by MS or not.

See the Appendix on file formats and the sample .arff file to better understand the output format of this step.

Generating O_i Values

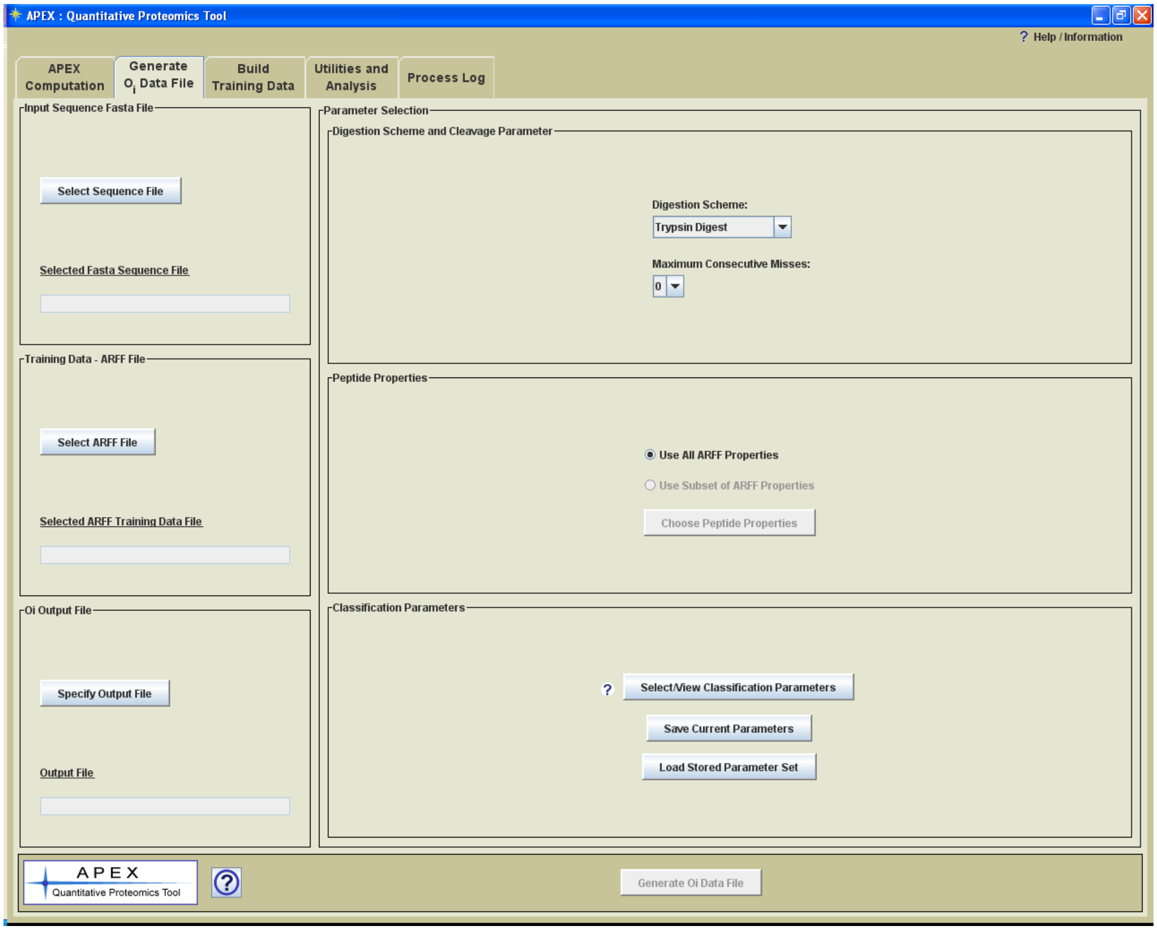
Process Panel Title: Generate Oi Data File

Process: This process produces an O_i data file for the proteins within an input FASTA sequence file.

Input Files:

1.) A protein sequence FASTA file is required to supply protein sequences. An O_i value will be generated for each protein sequence in the file.

2.) The ARFF Training data file generated in the previous step will be used to train the classifier that generates O_i values in this step.


APEX : Quantitative Proteomics Tool
? Help / Information
APEX Computation
Generate O_i Data File
Build Training Data
Utilities and Analysis
Process Log
Input Sequence Fasta File
Select Sequence File
Selected Fasta Sequence File
Training Data - ARFF File
Select ARFF File
Selected ARFF Training Data File
Oi Output File
Specify Output File
Output File
Parameter Selection
Digestion Scheme and Cleavage Parameter
Peptide Properties
Choose Peptide Properties
Classification Parameters
?
Select/View Classification Parameters
Save Current Parameters
Load Stored Parameter Set
APEX
Quantitative Proteomics Tool
?
Generate Oi Data File

Digestion Scheme: Trypsin Digest

Maximum Consecutive Misses: 0

● Use All ARFF Properties

○ Use Subset of ARFF Properties

Figure 10. O_i Generation Process Panel

Parameters:

Digestion Scheme: This parameter indicates the *in silico* digestion scheme that each protein undergoes to form peptide sequences, which are fed into a classifier trained on previously constructed training data. Note that the selected digestion scheme should mimic that used in the lab to process samples for MS. Five default digestion options are presented for selection.

- *Trypsin Digest*: Proteins are only cleaved by trypsin.

- *Chymotrypsin Digest*: Proteins are only cleaved by chymotrypsin.

- *Trypsin>Chymotrypsin*: Sequential or serial digestion, proteins are first cleaved by trypsin and then cleaved by chymotrypsin.

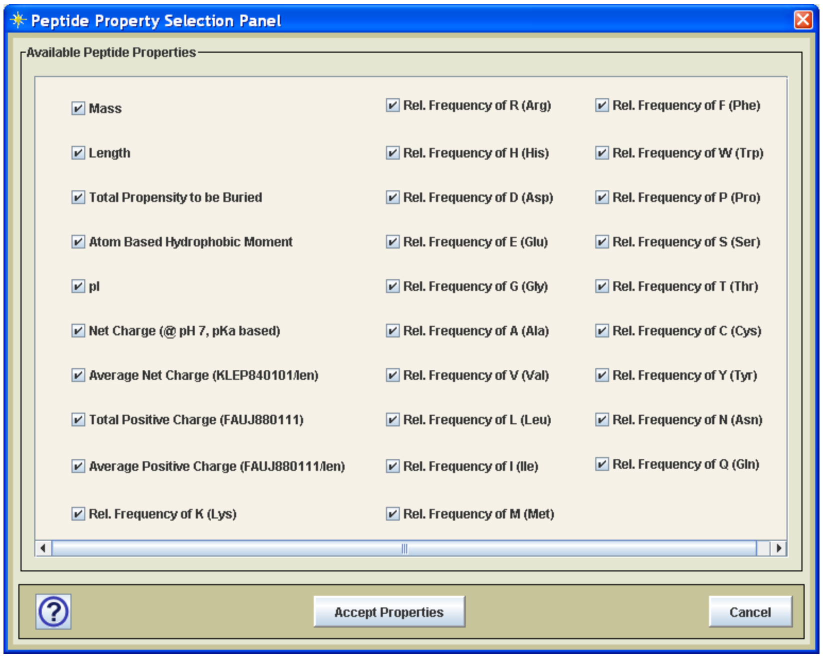
- *Trypsin+Chymotrypsin*: Parallell digestion, combination of the cleavage results of Typsin Digestion and Chymotrypsin digestion as if the proteins are digested by the two enzymes in two separate aliquots then later combined.

- *TrypsinChymotrypsin*: Combination of the cleavage results of trypsin and chymotrypsin. This is a combination of digestion rules for trypsin and chymotrypsin and matches an option that is found in some popular peptide search software packages.

The digestion schemes presented in APEX are determined based on a configuration file found in the apex_config folder labeled, *enzyme_rules.config*. Users can modify this file to define digestion schemes that are needed to model those used in the lab. An appendix section, *APEX Digestion Schemes – Defining Custom Schemes*, contains information on how to add new digestion schemes or modify existing rules. Note that trypsin is the most common enzyme used and is presented as the default option.

Maximum Consecutive Cleavage Misses: This parameter indicates the number of cleavage misses permitted during *in silico* digestion of the input proteins. This setting should match the setting used during previous MS searches. If the MS technique is set up to identify peptides that result from missed cleavages, this parameter should be set to match.

Peptide Properties: A set of peptide properties will be computed for each peptide cleaved from the input proteins. These peptide properties will be used in conjunction with the classifier to determine a probability of MS detection for each peptide. Many classifiers select random sets of properties (attributes) to find a set that can be used as a predictor of peptide MS detection. The various peptide properties are described in a section of the appendix.


Peptide Property Selection Panel

Available Peptide Properties

☑ Mass
☑ Length
☑ Total Propensity to be Buried
☑ Atom Based Hydrophobic Moment
☑ pI
☑ Net Charge (@ pH 7, pKa based)
☑ Average Net Charge (KLEP840101/len)
☑ Total Positive Charge (FAUJ880111)
☑ Average Positive Charge (FAUJ880111/len)
☑ Rel. Frequency of K (Lys)
☑ Rel. Frequency of R (Arg)
☑ Rel. Frequency of H (His)
☑ Rel. Frequency of D (Asp)
☑ Rel. Frequency of E (Glu)
☑ Rel. Frequency of G (Gly)
☑ Rel. Frequency of A (Ala)
☑ Rel. Frequency of V (Val)
☑ Rel. Frequency of L (Leu)
☑ Rel. Frequency of I (Ile)
☑ Rel. Frequency of M (Met)
☑ Rel. Frequency of F (Phe)
☑ Rel. Frequency of W (Trp)
☑ Rel. Frequency of P (Pro)
☑ Rel. Frequency of S (Ser)
☑ Rel. Frequency of T (Thr)
☑ Rel. Frequency of C (Cys)
☑ Rel. Frequency of Y (Tyr)
☑ Rel. Frequency of N (Asn)
☑ Rel. Frequency of Q (Gln)

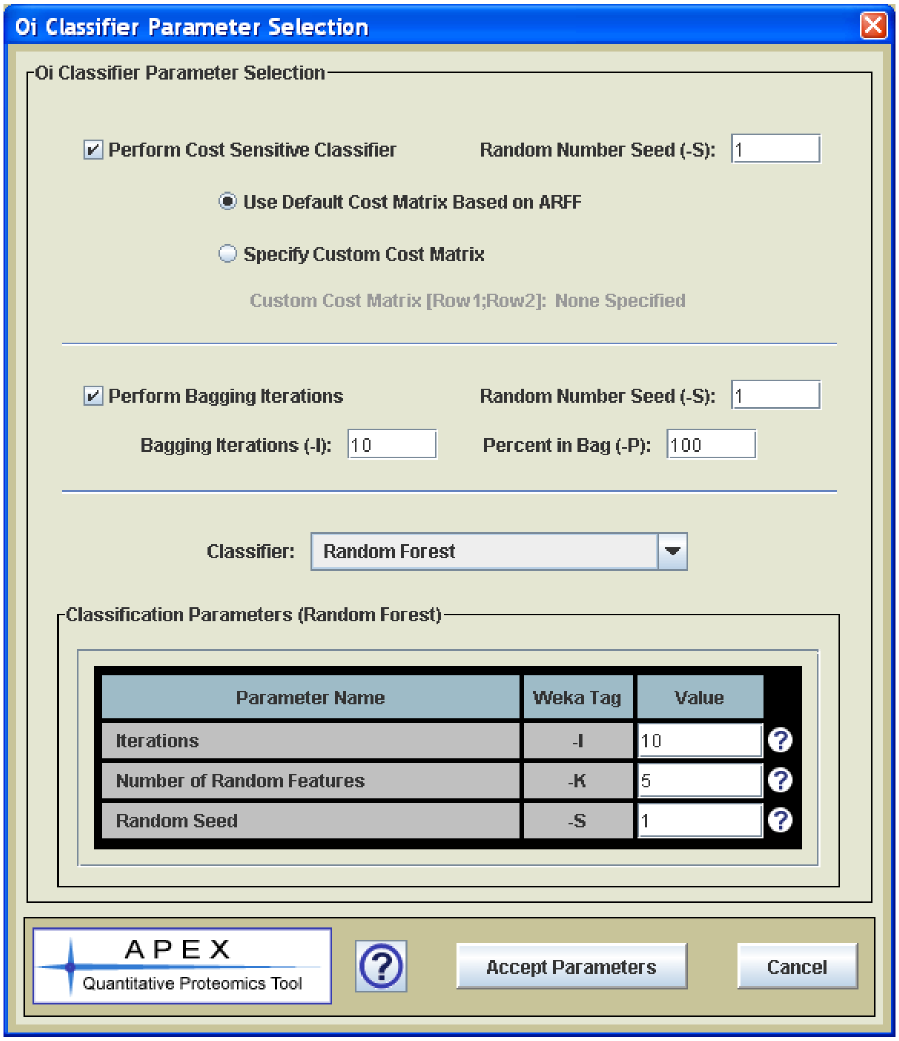
Figure 11. Protein Property Selection Panel

The user has two options with regard to these properties. One option is to use all peptide properties contained in the selected training data ARFF file while the other allows one to select a subset of the protein properties using the peptide property selection dialog as shown in Figure 11. Unless one has specific information about which properties are most important to determine which peptides will be detected by MS, it is best to just include all peptide properties and allow the classifier (e.g. Random Forest) to select attributes.

Classifier Options: A classifier is trained to determine the probability that a peptide will be detected (observed) by MS. APEX uses a Java data mining package, Weka, which contains many different classifiers. **The Lu et al. paper used a Random Forest classifier with good success and for that reason Random Forest is the default classifier used by the APEX Tool. The Random Forest parameters match those used by Lu et al. and most will find that these default parameters work fine.** Two other classifiers, RIDOR, and J48 Trees, also supported by Weka, are included in APEX. Each classifier algorithm has distinct parameter options that help to control the process of building the classifier. Additional information on Weka is found in the Appendices.

The APEX tool has a configuration file that lists the available classifiers and their parameters. This file is read when APEX starts and initializes a set of classifiers that can be used by APEX. Experienced users that are familiar with machine learning classifiers and the Weka data mining tool set, can customize the entries in the *weka_parameters.config* file in the *apex_config* folder. The comments in this configuration file include instructions on how to modify this file. Note that it is necessary to be familiar with the Weka classifier and specific parameter tags in order to add a classifier and new parameters to those already supported.

*As previously mentioned, the Lu paper found that the Random Forest classifier worked well with particular options set such as running as a cost sensitive classifier with Bagging which averages results. It is beyond the scope of this manual to describe classifier algorithms and related parameters in detail but the Lu paper and Weka documentation provides additional information on classifiers and their specific parameter selections.



Oi Classifier Parameter Selection

Oi Classifier Parameter Selection

☑ Perform Cost Sensitive Classifier   Random Number Seed (-S): 1

● Use Default Cost Matrix Based on ARFF

○ Specify Custom Cost Matrix

Custom Cost Matrix [Row1;Row2]: None Specified

☑ Perform Bagging Iterations   Random Number Seed (-S): 1

Bagging Iterations (-I): 10   Percent in Bag (-P): 100

Classifier: Random Forest

Classification Parameters (Random Forest)

Parameter Name	Weka Tag	Value
Iterations	-I	10
Number of Random Features	-K	5
Random Seed	-S	1

Figure 12. Classifier Parameter Selection Dialog

The Select/View Classifier Parameters button is used to open the classifier parameter selection dialog. This dialog, shown in Figure 12, allows one to select a classifier for APEX from a drop-down menu. When the classifier is selected, the parameter list updates to support the parameters that are specific to the selected classifier. The value types and value constraints (valid value ranges if applicable) are enforced based on Weka's requirement for these parameters.

Process Details:

1.) The first step is reading the input FASTA sequence file. This file contains all protein sequences of interest and for smaller genomes such as prokaryotes it typically covers all proteins known to exist. The file is read and the protein accession, annotation, and sequence is stored in a data structure.

2.) The loaded proteins are then subjected to the *in silico* digestion to produce a set of peptides for each protein. This process takes into account the consecutive cleavage miss rate parameter.

3.) Next the classifier is constructed. The ARFF training file is read, the selected peptide parameters (often all parameters in the ARFF) are used to build a classifier. At this stage the selected classifier parameters come into play to control the construction of the classifier.

4.) The peptides generated during digestion are then assigned probabilities (probability of being observed, 0-1) by the trained classifier. Each peptide is first characterized by a set of computed peptide properties. The assigned probability for each peptide is based on these computed physicochemical properties.

5.) The O_i values are computed by summing all peptide probabilities associated with the peptides derived from protein *i*. The O_i values as well as all of the file and parameter selections are output to a file. These O_i values will later be used during APEX abundance computation.

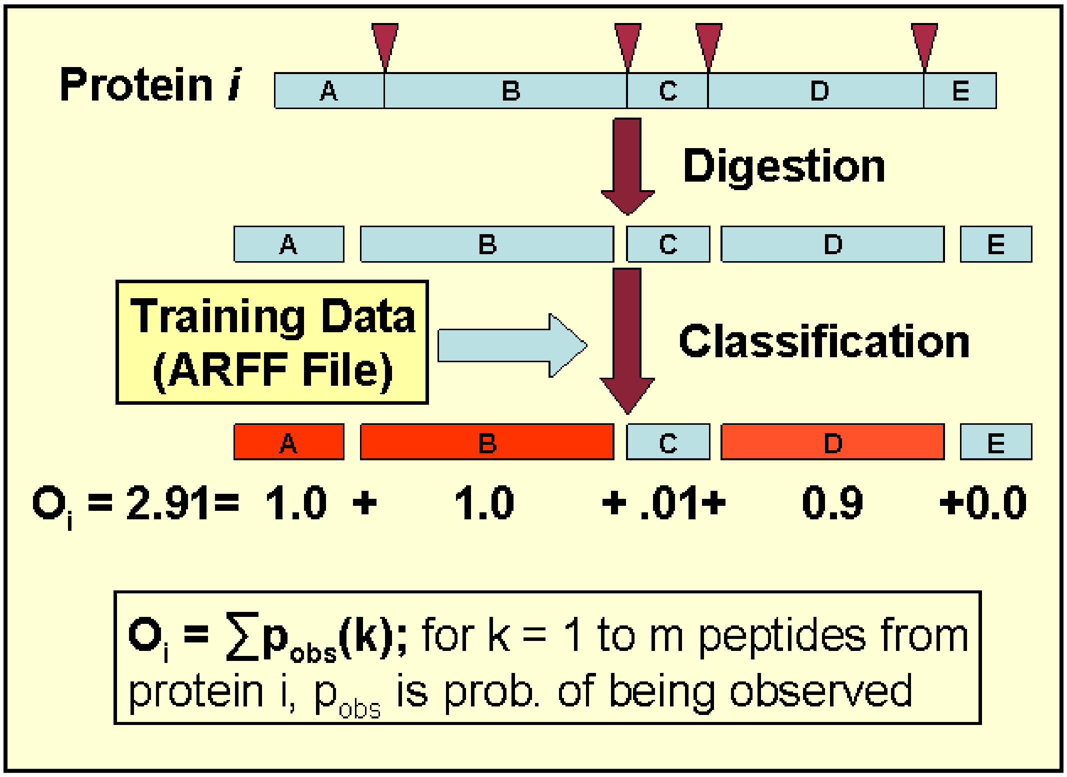

Protein *i*
A
B
C
D
E
Digestion
A
B
C
D
E
Training Data (ARFF File)
Classification
A
B
C
D
E
O_i = 2.91= 1.0 + 1.0 + .01+ 0.9 +0.0
$O_i = \sum p_{obs}(k)$; for k = 1 to m peptides from protein i, p_{obs} is prob. of being observed

Figure 13. O_i Generation Figure.

Computing APEX Abundance Values

Process Panel Title: APEX Computation

Process: This process computes APEX protein abundance values using an XML protein file and the generated O_i values.

Input Files:

1.) A ProteinProphet protein XML result file is required to supply the protein list, p_i, and n_i values for the computation shown in figure 1.

2.) An O_i file is required that has O_i values that cover the proteins within the sample.

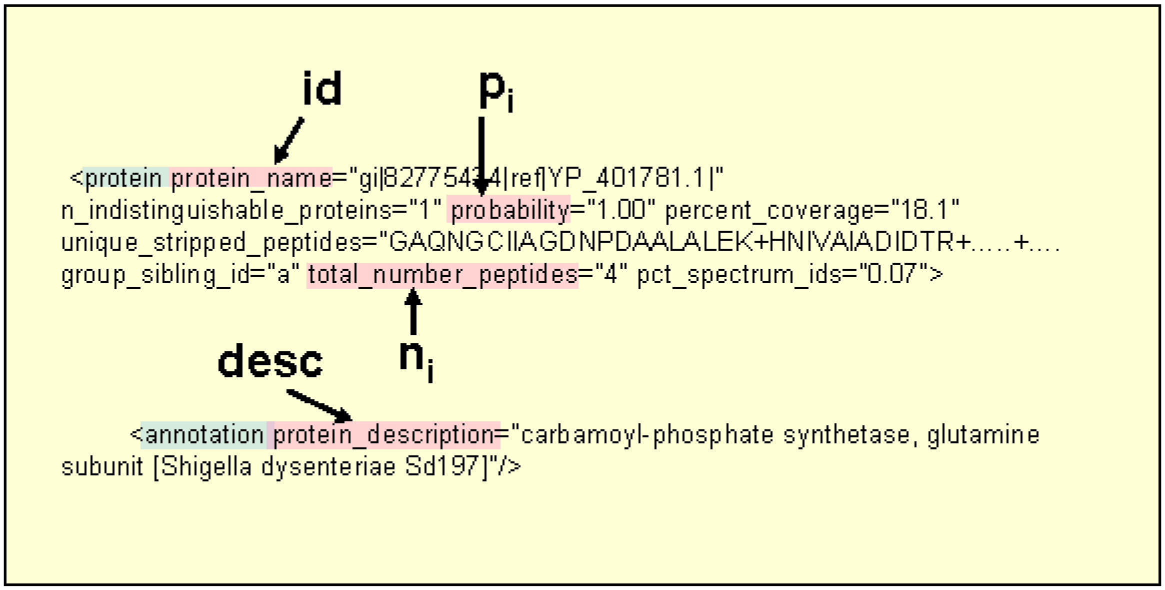
Parameters:

C value: A numerical value, denoted as *C*, is used in the equation in figure 1. This value converts the relative APEX score to absolute terms. Up to this point this value has usually been an estimate of # proteins/cell. Lu et al. presented reported estimates of 5 x 10^7 proteins/cell for yeast and 2-3 x 10^7 proteins/cell for E. coli based on Futcher et al. 1999 and Neidhardt et al. 1996 respectively.

Process Details:

Once the O_i file has been generated, the process of computing is quite simple, following the equation given in figure 1.

1.) The input ProteinProphet XML file is input and protein id, p_i, n_i, and description are pulled from the file according to the tag labels shown in figure 14 which shows a section of a sample XML file.



```
 <protein protein_name="gi|82775434|ref|YP_401781.1|"
n_indistinguishable_proteins="1" probability="1.00" percent_coverage="18.1"
unique_stripped_peptides="GAQNGCIIAGDNPDAALALEK+HNIVAIADIDTR+.....+....
group_sibling_id="a" total_number_peptides="4" pct_spectrum_ids="0.07">

        <annotation protein_description="carbamoyl-phosphate synthetase, glutamine
subunit [Shigella dysenteriae Sd197]"/>
```

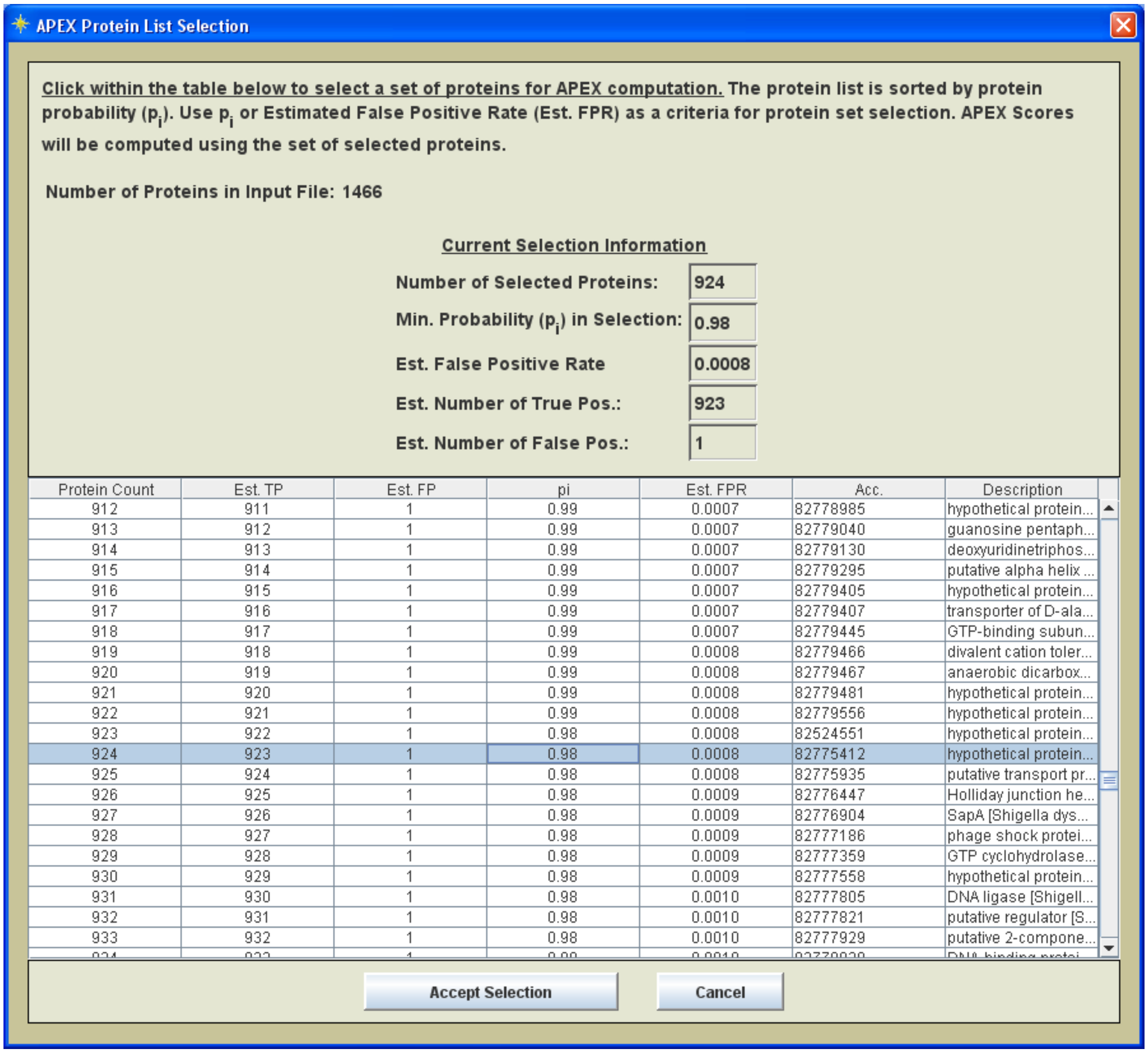
Figure 14. Sample section of ProteinProphet XML file showing the fields that are parsed to supply information for computation.

2.) The protein list from the XML file is ordered by p_i (protein identification probability) and the estimated false positive rate is computed for the N ordered subsets of proteins. The estimated false positive error rate for a set of *n* proteins that is ordered by p_i is given by:

$$Est\_FPR = \frac{1}{n}\sum_{i=1}^{n}(1 - p_i);\ \ n = \#\ proteins\, in\, the\ list$$

The FPR can be used to estimate the number of proteins in a list that are false positives.

3.) The ordered set of proteins are displayed in a table as shown in figure 15. This table is used to select a set of proteins to enter APEX computation. FPR or p_i can be used as a criteria. The information at the top of the table viewer is updated when you click on a row in the table. Once you have selected the proteins to enter APEX the *Accept Selection* button is hit and computation begins.



APEX Protein List Selection

Click within the table below to select a set of proteins for APEX computation. The protein list is sorted by protein probability (p_i). Use p_i or Estimated False Positive Rate (Est. FPR) as a criteria for protein set selection. APEX Scores will be computed using the set of selected proteins.

Number of Proteins in Input File: 1466

Current Selection Information

Number of Selected Proteins: 924

Min. Probability (p_i) in Selection: 0.98

Est. False Positive Rate 0.0008

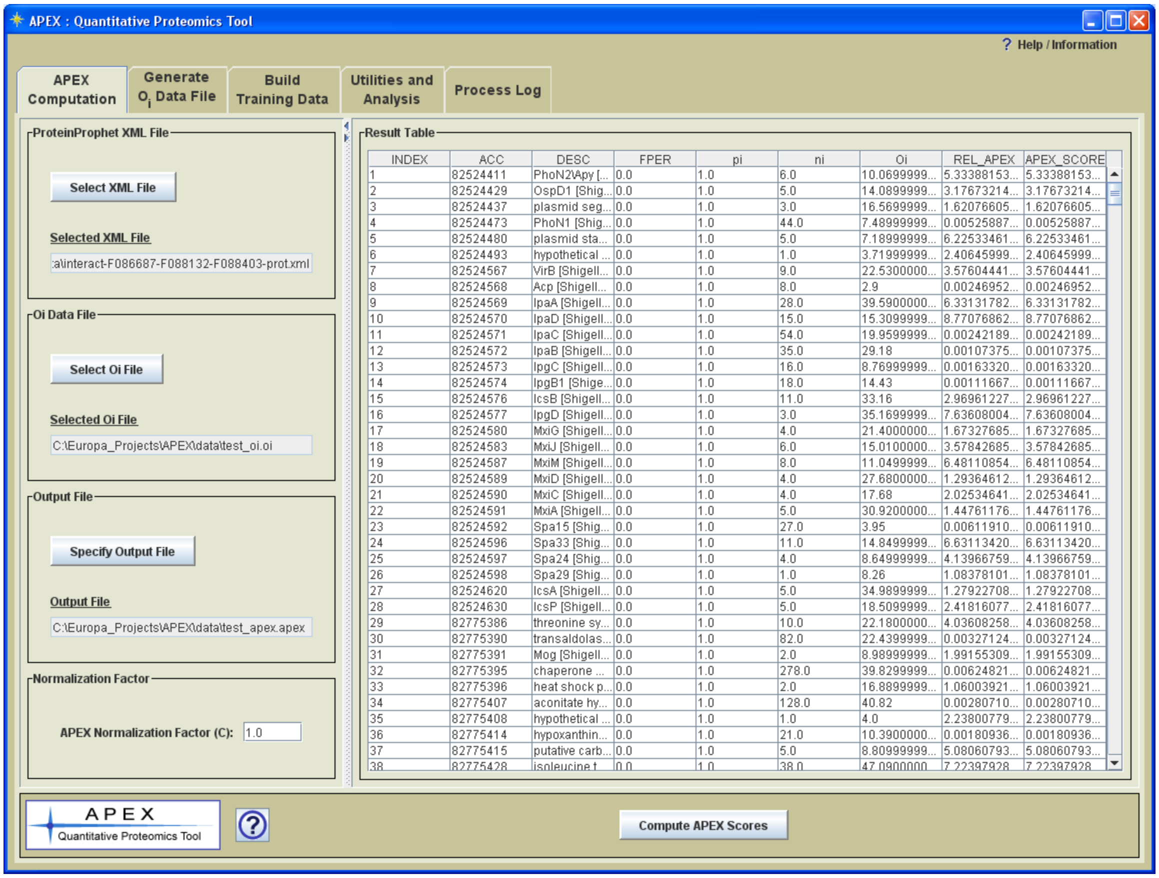
Est. Number of True Pos.: 923

Est. Number of False Pos.: 1

Protein Count	Est. TP	Est. FP	pi	Est. FPR	Acc.	Description
912	911	1	0.99	0.0007	82778985	hypothetical protein...
913	912	1	0.99	0.0007	82779040	guanosine pentaph...
914	913	1	0.99	0.0007	82779130	deoxyuridinetriphos...
915	914	1	0.99	0.0007	82779295	putative alpha helix ...
916	915	1	0.99	0.0007	82779405	hypothetical protein...
917	916	1	0.99	0.0007	82779407	transporter of D-ala...
918	917	1	0.99	0.0007	82779445	GTP-binding subun...
919	918	1	0.99	0.0008	82779466	divalent cation toler...
920	919	1	0.99	0.0008	82779467	anaerobic dicarbox...
921	920	1	0.99	0.0008	82779481	hypothetical protein...
922	921	1	0.99	0.0008	82779556	hypothetical protein...
923	922	1	0.98	0.0008	82524551	hypothetical protein...
924	923	1	0.98	0.0008	82775412	hypothetical protein...
925	924	1	0.98	0.0008	82775935	putative transport pr...
926	925	1	0.98	0.0009	82776447	Holliday junction he...
927	926	1	0.98	0.0009	82776904	SapA [Shigella dys...
928	927	1	0.98	0.0009	82777186	phage shock protei...
929	928	1	0.98	0.0009	82777359	GTP cyclohydrolase...
930	929	1	0.98	0.0009	82777558	hypothetical protein...
931	930	1	0.98	0.0010	82777805	DNA ligase [Shigell...
932	931	1	0.98	0.0010	82777821	putative regulator [S...
933	932	1	0.98	0.0010	82777929	putative 2-compone...

Figure 15. Protein selection list ordered by p_i

4.) APEX values are computed as described by the equation in figure 1. The results are output to file and are also displayed in the interface as shown in Figure 16.



Selected XML File: a\interact-F086687-F088132-F088403-prot.xml

Selected Oi File: C:\Europa_Projects\APEX\data\test_oi.oi

Output File: C:\Europa_Projects\APEX\data\test_apex.apex

APEX Normalization Factor (C): 1.0

INDEX	ACC	DESC	FPER	pi	ni	Oi	REL_APEX	APEX_SCORE
1	82524411	PhoN2\Apy [...	0.0	1.0	6.0	10.0699999...	5.33388153...	5.33388153...
2	82524429	OspD1 [Shig...	0.0	1.0	5.0	14.0899999...	3.17673214...	3.17673214...
3	82524437	plasmid seg...	0.0	1.0	3.0	16.5699999...	1.62076605...	1.62076605...
4	82524473	PhoN1 [Shig...	0.0	1.0	44.0	7.48999999...	0.00525887...	0.00525887...
5	82524480	plasmid sta...	0.0	1.0	5.0	7.18999999...	6.22533461...	6.22533461...
6	82524493	hypothetical ...	0.0	1.0	1.0	3.71999999...	2.40645999...	2.40645999...
7	82524567	VirB [Shigell...	0.0	1.0	9.0	22.5300000...	3.57604441...	3.57604441...
8	82524568	Acp [Shigell...	0.0	1.0	8.0	2.9	0.00246952...	0.00246952...
9	82524569	IpaA [Shigell...	0.0	1.0	28.0	39.5900000...	6.33131782...	6.33131782...
10	82524570	IpaD [Shigell...	0.0	1.0	15.0	15.3099999...	8.77076862...	8.77076862...
11	82524571	IpaC [Shigell...	0.0	1.0	54.0	19.9599999...	0.00242189...	0.00242189...
12	82524572	IpaB [Shigell...	0.0	1.0	35.0	29.18	0.00107375...	0.00107375...
13	82524573	IpgC [Shigell...	0.0	1.0	16.0	8.76999999...	0.00163320...	0.00163320...
14	82524574	IpgB1 [Shige...	0.0	1.0	18.0	14.43	0.00111667...	0.00111667...
15	82524576	IcsB [Shigell...	0.0	1.0	11.0	33.16	2.96961227...	2.96961227...
16	82524577	IpgD [Shigell...	0.0	1.0	3.0	35.1699999...	7.63608004...	7.63608004...
17	82524580	MxiG [Shigell...	0.0	1.0	4.0	21.4000000...	1.67327685...	1.67327685...
18	82524583	MxiJ [Shigell...	0.0	1.0	6.0	15.0100000...	3.57842685...	3.57842685...
19	82524587	MxiM [Shigell...	0.0	1.0	8.0	11.0499999...	6.48110854...	6.48110854...
20	82524589	MxiD [Shigell...	0.0	1.0	4.0	27.6800000...	1.29364612...	1.29364612...
21	82524590	MxiC [Shigell...	0.0	1.0	4.0	17.68	2.02534641...	2.02534641...
22	82524591	MxiA [Shigell...	0.0	1.0	5.0	30.9200000...	1.44761176...	1.44761176...
23	82524592	Spa15 [Shig...	0.0	1.0	27.0	3.95	0.00611910...	0.00611910...
24	82524596	Spa33 [Shig...	0.0	1.0	11.0	14.8499999...	6.63113420...	6.63113420...
25	82524597	Spa24 [Shig...	0.0	1.0	4.0	8.64999999...	4.13966759...	4.13966759...
26	82524598	Spa29 [Shig...	0.0	1.0	1.0	8.26	1.08378101...	1.08378101...
27	82524620	IcsA [Shigell...	0.0	1.0	5.0	34.9899999...	1.27922708...	1.27922708...
28	82524630	IcsP [Shigell...	0.0	1.0	5.0	18.5099999...	2.41816077...	2.41816077...
29	82775386	threonine sy...	0.0	1.0	10.0	22.1800000...	4.03608258...	4.03608258...
30	82775390	transaldolas...	0.0	1.0	82.0	22.4399999...	0.00327124...	0.00327124...
31	82775391	Mog [Shigell...	0.0	1.0	2.0	8.98999999...	1.99155309...	1.99155309...
32	82775395	chaperone ...	0.0	1.0	278.0	39.8299999...	0.00624821...	0.00624821...
33	82775396	heat shock p...	0.0	1.0	2.0	16.8899999...	1.06003921...	1.06003921...
34	82775407	aconitate hy...	0.0	1.0	128.0	40.82	0.00280710...	0.00280710...
35	82775408	hypothetical ...	0.0	1.0	1.0	4.0	2.23800779...	2.23800779...
36	82775414	hypoxanthin...	0.0	1.0	21.0	10.3900000...	0.00180936...	0.00180936...
37	82775415	putative carb...	0.0	1.0	5.0	8.80999999...	5.08060793...	5.08060793...
38	82775428	isoleucine t	0.0	1.0	38.0	47.0900000	7.22397928	7.22397928

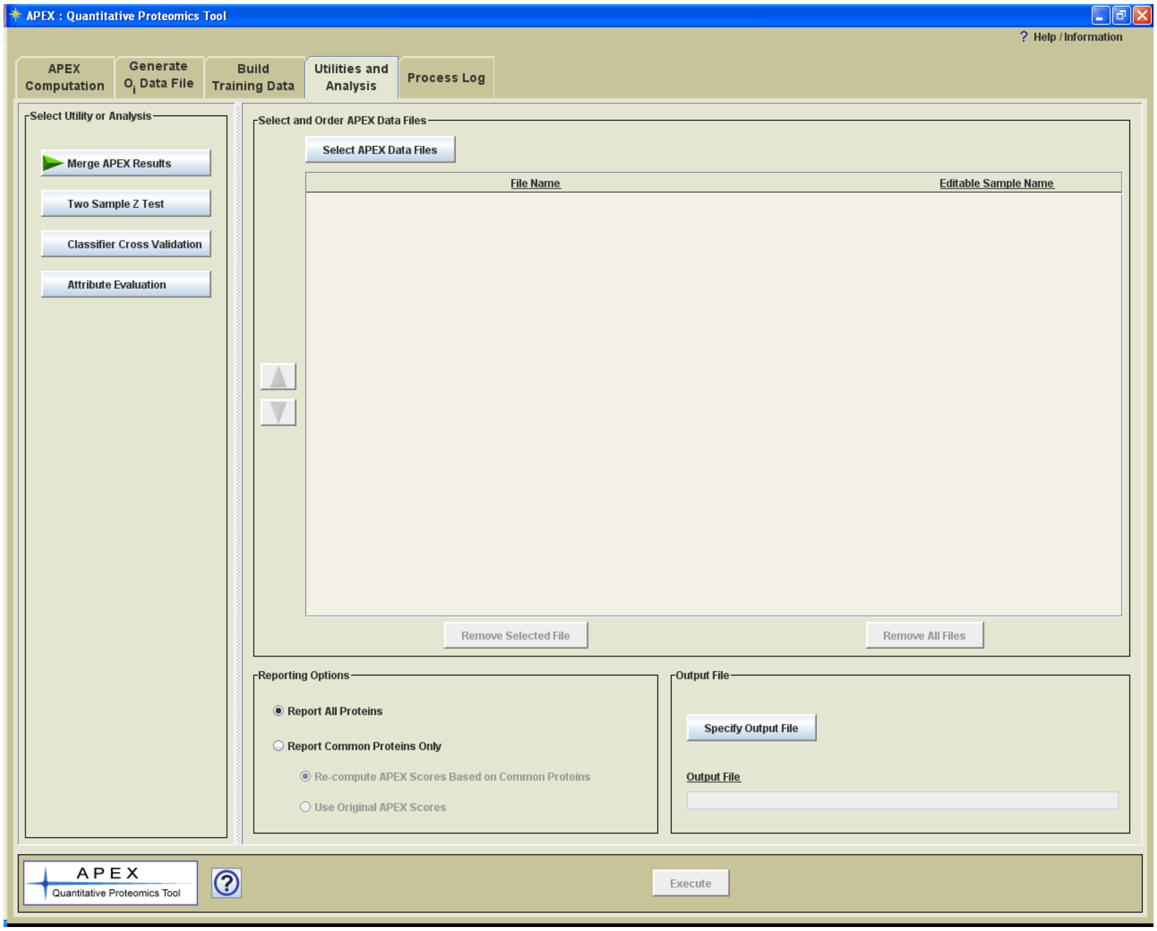
Figure 16. APEX Computation Panel with APEX Quantitation Results

The output file has a standardized output format where each data row includes and index, protein accession, description, false positive rate (relates to the set of proteins with index <= to the observed row), p_i, n_i, O_i, relative APEX, and absolute APEX score. The comments section in the file lists the analysis date, the input files, and the C value.

Utilities and Analysis

Merging APEX Results

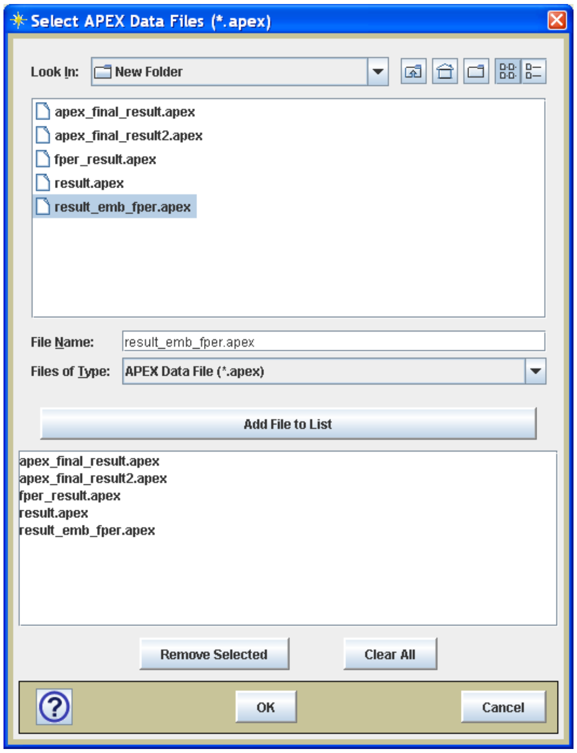
The Utilities and Analysis panel includes four options for APEX version 1.1.0. The first option is a utility that merges two or more APEX data files into a tab delimited text file that summarizes multiple experiments. The output file format is a tabular format where data columns represent different apex results, perhaps different experimental conditions, and the rows contain protein data including annotation and apex scores. The file format conforms to the TDMS (Tab Delimited Multiple Sample) format specification of the MultiExperiment viewer (of the TM4 suite of tools, www.tm4.org/mev.html) also known as MeV. MeV is a clustering and statistical analysis application that was originally produced for microarray data but has more recently been used with quantitative proteomics data for finding proteins that share trends in protein expression over the conditions under study.



● Report All Proteins
○ Report Common Proteins Only
● Re-compute APEX Scores Based on Common Proteins
○ Use Original APEX Scores

Figure 17. Utilities and Analysis Panel

Once files have been selected using the multi-file chooser (Figure 18), the files can be ordered (Figure 19) according to the experimental design to place time points in the proper order or to group samples based on common experimental conditions. Note that one can assign a descriptive sample identifier by clicking in the text field to the right of the file name as shown in Figure 19. This descriptive sample name is useful when data mining as it provides a meaningful label for each sample.



Look In: New Folder

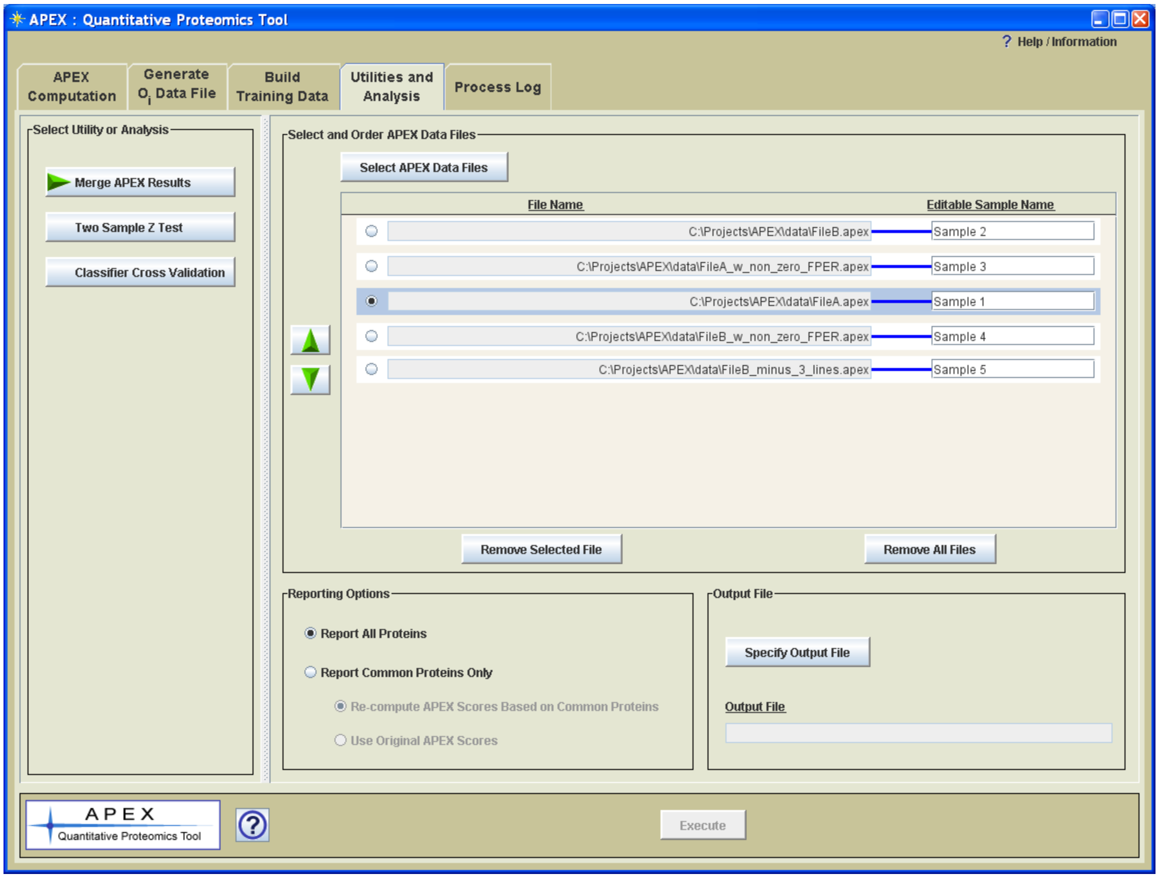
apex_final_result.apex
apex_final_result2.apex
fper_result.apex
result.apex
result_emb_fper.apex

File Name: result_emb_fper.apex
Files of Type: APEX Data File (*.apex)

apex_final_result.apex
apex_final_result2.apex
fper_result.apex
result.apex
result_emb_fper.apex

Figure 18. Multi-file selection dialog.

It is likely that the selected files have many proteins in common but that the protein lists from the files may not overlap entirely. The default option is to report all proteins, the union set of all proteins in all lists. It may be the case that for a particular protein that only 1 sample has an APEX value. In that case a placeholder text value will be inserted for each missing value. This option allows one to see a record of which proteins were only reported under certain conditions. The alternative reporting option is to take an intersection of common proteins and only report on those found in all lists. This option will result in a smaller protein set but will not have any missing values. If only common proteins are reported, there is an option to re-compute APEX (by equation in Figure 1) using just the common proteins. The APEX equation denominator will be smaller since this common protein set is smaller and thus will achieve relatively higher APEX scores. The difference is that the APEX scores are computed for the same set of proteins across all input samples. The alternative to re-computing the APEX scores is to report the original APEX scores that were based on analysis within a different protein set. The reporting options are found on the file merge utility panel (Figure 17 or Figure 19).



	File Name	Editable Sample Name
○	C:\Projects\APEX\data\FileB.apex	Sample 2
○	C:\Projects\APEX\data\FileA_w_non_zero_FPER.apex	Sample 3
●	C:\Projects\APEX\data\FileA.apex	Sample 1
○	C:\Projects\APEX\data\FileB_w_non_zero_FPER.apex	Sample 4
○	C:\Projects\APEX\data\FileB_minus_3_lines.apex	Sample 5

● Report All Proteins

○ Report Common Proteins Only

● Re-compute APEX Scores Based on Common Proteins

○ Use Original APEX Scores

Figure 19. File Merge Utility Panel with Loaded Samples.

Two Sample z-score Test

The Lu et al. paper presented a formula for computing a z-score for investigating differential protein expression between two samples. The formula is shown here for reference. Please refer to the Lu reference (full citation is at the end of this manual) for further information on this statistic and the assumptions made regarding the normality of the distribution of *f* values.

$$z = \frac{f_{i,1} - f_{i,2}}{\sqrt{f_{i,0}(1 - f_{i,0})/N_1 + f_{i,0}(1 - f_{i,0})/N_2}}$$

$$f_{i,1} = n_{i,1}/N_1$$

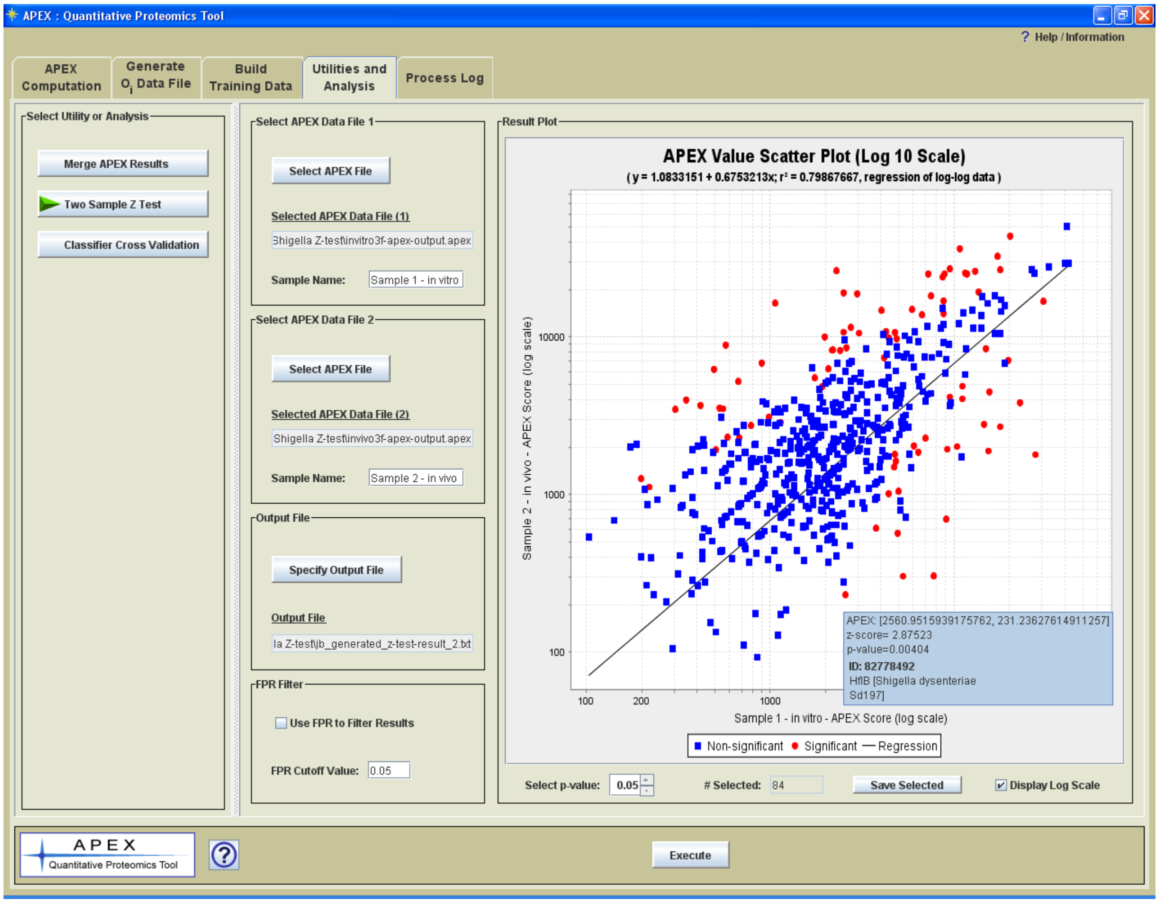
$$f_{i,2} = n_{i,2}/N_2$$

$$f_{i,0} = (n_{i,1} + n_{i,2})/(N_1 + N_2)$$

Figure 20. Z score computation.

Note that if the experimental design contains multiple biological replicate samples of one or more experimental conditions, that it is advisable to use the data merge utility and use the parametric or non-parametric statistical tests in MeV to find proteins that show significant changes in abundance between experimental groups. The z-test shown here is specifically for the case where there are exactly two samples to compare.

The z-score test processing panel (Figure 21) includes controls to select the two input files, specify the output file name, and a control to filter the result based on the estimated false positive rate (FPR) computed from p_i values. If the FPR filter is applied, all proteins are reported for which at least one of the two samples includes the protein in the subset of proteins with FPR lower than the supplied cutoff. For example if Protein A is present in both samples but is in the list of sorted proteins that has an FPR greater than the cutoff, protein A would be excluded from the report file. In this case protein A would have had a relatively low p_i in both files. If the FPR for a list of peptides in at least one file was lower than the cutoff, then the protein would be reported. In this case both samples contained the protein but one sample had a relatively high p_i and the other had a low p_i. The point here is that only proteins in common are reported and if the FPR filter is applied, only one of the two proteins needs to pass the filter criteria.


APEX : Quantitative Proteomics Tool
? Help / Information
APEX Computation
Generate O_i Data File
Build Training Data
Utilities and Analysis
Process Log
Select Utility or Analysis
Merge APEX Results
Two Sample Z Test
Classifier Cross Validation
Select APEX Data File 1
Select APEX File
Select APEX Data File 2
Select APEX File
Output File
Specify Output File
FPR Filter
Result Plot
APEX Value Scatter Plot (Log 10 Scale)
(y = 1.0833151 + 0.6753213x; r² = 0.79867667, regression of log-log data)
Sample 2 - in vivo - APEX Score (log scale)
10000
1000
100
100
200
1000
Sample 1 - in vitro - APEX Score (log scale)
■ Non-significant ● Significant — Regression
Save Selected
Execute
APEX
Quantitative Proteomics Tool

Selected APEX Data File (1): Shigella Z-test\invitro3f-apex-output.apex

Sample Name: Sample 1 - in vitro

Selected APEX Data File (2): Shigella Z-test\invivo3f-apex-output.apex

Sample Name: Sample 2 - in vivo

Output File: la Z-test\jb_generated_z-test-result_2.txt

☐ Use FPR to Filter Results

FPR Cutoff Value: 0.05

APEX: [2560.9515939175762, 231.23627614911257]
z-score= 2.87523
p-value=0.00404
ID: 82778492
HflB [Shigella dysenteriae
Sd197]

Select p-value: 0.05   # Selected: 84   ☑ Display Log Scale

Figure 21. Z-score Test Processing Panel with Result

In addition to the output file, the result is displayed as a graph that shows the correlation of APEX scores between the two samples. Initially the view is in log scale but the linear scale can be viewed as well. Moving the mouse cursor over points in the plot will open a tool tip that displays the APEX values, z-score, p-value, protein id, and protein description.

A p-value selector is found in the lower left area of the result plot. This cutoff value is applied to the data and will update the graph to show which points are significant based on z-score p-value. A text area to the right of the p-values selector will display the number of proteins that have p-values the fall at or below the entered criteria. The *Save Selected* button will save the proteins that pass the p-value cutoff (have p-values <= the cutoff) to file. The p-values here are two-tailed probabilities of the z-scores taken from a normal distribution.

The main output file contains information on the two input files, the sample names, and information about the source for the z-score equation and the derivation of p-values. For each data row relating to a particular protein the following information is output: protein accession, protein description, APEX scores (2), APEX fold change, n_i values (2), z-scores, and p-values.

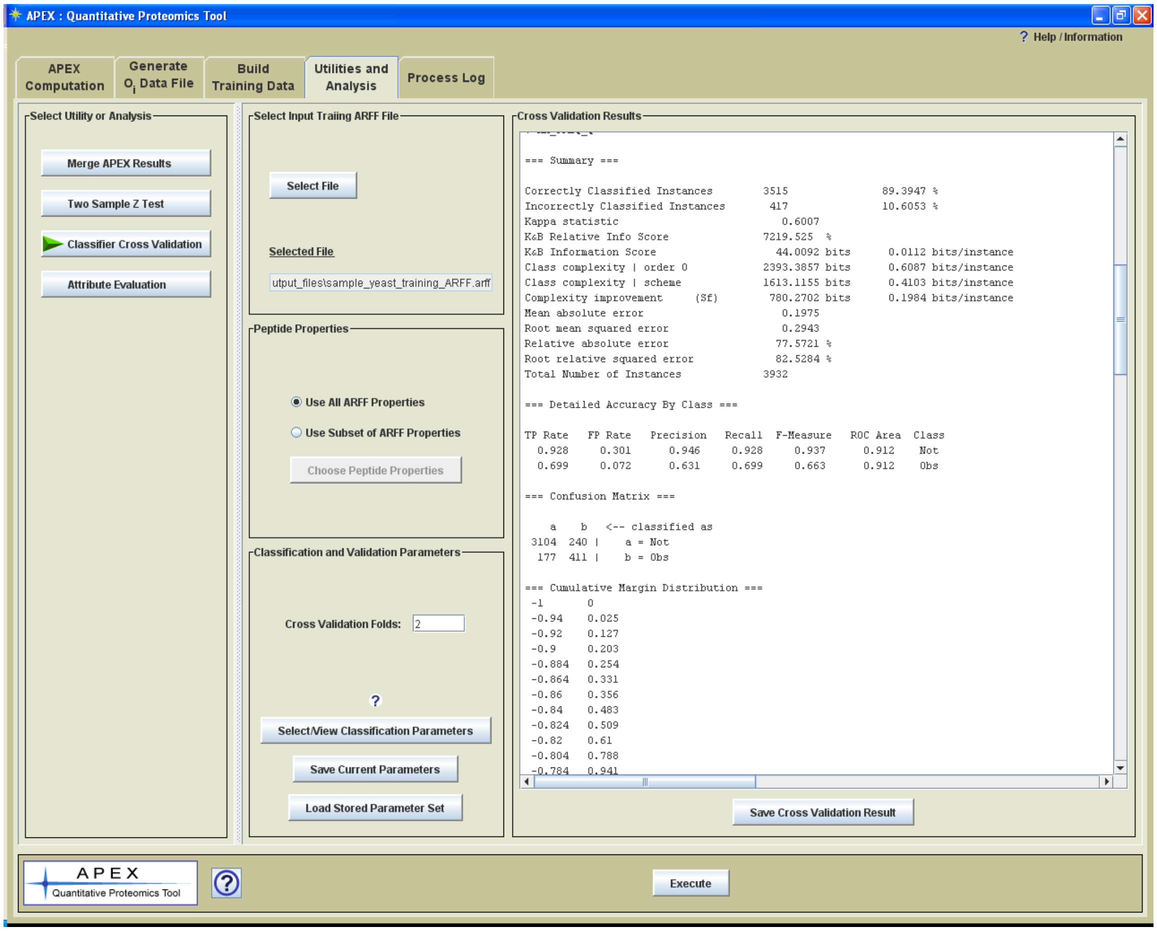
Classifier Cross Validation

The O_i generation process uses the training data (ARFF file) to build a classifier that is used to generate peptide observation probabilities which are summed to result in the O_i value. Please refer to Figure 13 and related description for more information on the role of the classifier. The improvement of the APEX technique over traditional spectral counting techniques is that a protein's observed spectral count is compared with it's expected or predicted spectral count, the O_i value. The accuracy of the O_i value is dependent on the ability of the classifier to generate accurate peptide probabilities that are based on their physicochemical properties. This cross validation utility allows one to examine classifier performance. This utility reports on how accurately the classifier partitions the observed and non-observed peptides in the training ARFF file. Improved classifier performance will presumably result in more accurate O_i values which can improve APEX quantitation results.

The cross validation process starts by selection of an APEX generated training ARFF file. This training data is used to build a classifier based on input classifier options and a selected set of peptide attributes. The number of validation iterations, Cross Validation Folds, is selected. On each iteration a number of training peptides from the ARFF file are selected to build a new classifier. The remaining data is fed into the classifier as test data. For example, suppose we select to run 10 iterations of cross validation. On each iteration, 9/10ths of the data is used to train the classifier. The remaining 1/10th of the data is then used as test data. The peptides in the test data have known classifications, each has been observed or not observed during a previously run MS experiment. The classifier tries to predict a class for each test peptide and since we know the actual classification we can assess classification accuracy. On each iteration of cross validation the subset of training data is selected such that it has nearly the same proportion of observed to not-observed peptides that is present in the data set as a whole.

Figure 22 shows the Cross Validation Parameter Panel with the text result from a cross validation run. Following the run the results are displayed in a panel on the right side of the interface. A button below the result panel allows one to save the result to a text file.

Weka code supports building the classifier and performing the cross validation steps. The output from cross validation is an extension of the typical Weka output from cross validation. The output includes the ARFF file name, classifier options, number of ARFF peptide properties, number of ARFF peptide properties selected to enter the process, the peptide property list, and a summary that includes the number and percentage of test peptides that are classified correctly and incorrectly. The output also includes details separated by class such as precision (fraction correct) and recall (sensitivity, number peptides that are captured into the proper category.) A confusion matrix is also included that lists the number of true positives, true negatives, false positives, and false negatives. The last section of the output is the cumulative margin distribution which describes the separation of probabilities between the two classes over the set of iterations.


APEX : Quantitative Proteomics Tool
? Help / Information
APEX Computation
Generate O_i Data File
Build Training Data
Utilities and Analysis
Process Log
Select Utility or Analysis
Merge APEX Results
Two Sample Z Test
Classifier Cross Validation
Attribute Evaluation
Select Input Traiing ARFF File
Select File
Peptide Properties
Choose Peptide Properties
Classification and Validation Parameters
?
Select/View Classification Parameters
Save Current Parameters
Load Stored Parameter Set
Cross Validation Results
Save Cross Validation Result
APEX
Quantitative Proteomics Tool
Execute

Selected File: utput_files\sample_yeast_training_ARFF.arff

● Use All ARFF Properties

○ Use Subset of ARFF Properties

Cross Validation Folds: 2

```
=== Summary ===

Correctly Classified Instances        3515               89.3947 %
Incorrectly Classified Instances       417               10.6053 %
Kappa statistic                          0.6007
K&B Relative Info Score               7219.525  %
K&B Information Score                   44.0092 bits      0.0112 bits/instance
Class complexity | order 0            2393.3857 bits      0.6087 bits/instance
Class complexity | scheme             1613.1155 bits      0.4103 bits/instance
Complexity improvement     (Sf)        780.2702 bits      0.1984 bits/instance
Mean absolute error                      0.1975
Root mean squared error                  0.2943
Relative absolute error                 77.5721 %
Root relative squared error             82.5284 %
Total Number of Instances             3932

=== Detailed Accuracy By Class ===

TP Rate   FP Rate   Precision   Recall  F-Measure   ROC Area  Class
  0.928     0.301      0.946     0.928     0.937      0.912    Not
  0.699     0.072      0.631     0.699     0.663      0.912    Obs

=== Confusion Matrix ===

    a    b   <-- classified as
 3104  240 |    a = Not
  177  411 |    b = Obs

=== Cumulative Margin Distribution ===
 -1       0
 -0.94    0.025
 -0.92    0.127
 -0.9     0.203
 -0.884   0.254
 -0.864   0.331
 -0.86    0.356
 -0.84    0.483
 -0.824   0.509
 -0.82    0.61
 -0.804   0.788
 -0.784   0.941
```

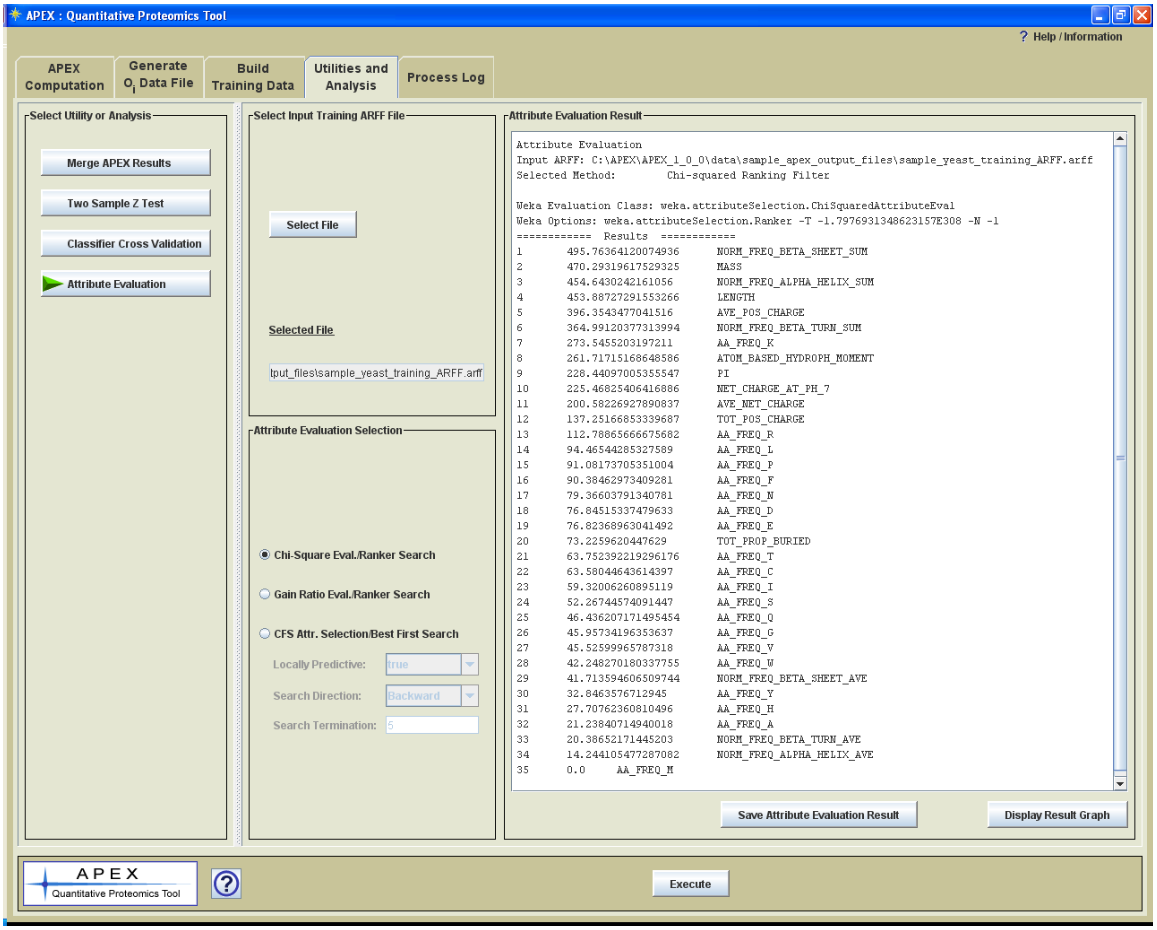
Figure 22. Cross Validation Utility Parameter Panel with a Displayed Result.

Note that APEX uses a Weka class (Evaluation.java) for cross validation and that software piece does not report on the progress of the process. This means that the APEX progress dialog will be able to indicate when the validation starts but will not be able to report on the progress of the validation iterations. As a reference, an ARFF file with 5239 peptides and 27 peptide properties, took approximately 5 minutes to complete on a relatively new PC with 1GB ram and a dual core processor. Fortunately, most users will find that Random Forest with the default parameters works well and hence extensive evaluation of classifiers will not be strictly required however, having classifier performance information is good information to convey when publishing results.

Attribute Evaluation

The APEX method uses knowledge about peptide properties and prior MS result to build a classifier to predict which peptide sequences are likely to be observed based on peptide properties.   The APEX tool has the ability to report on many different peptide properties. In data mining and classification, these properties are generically referred to as *attributes*. These attributes characterize the object, in our case peptides, that are being classified. The attributes that are computed vary in their ability to predict the class of interest, in our case the ability to detect or not to be able to detect a peptide using the procedures and MS instrumentation in use. This aspect of each attribute might be considered its predictive value. Attributes that are tightly correlated with observed and not-observed classes are generally better predictors of the class. Most classifiers use multiple attributes and it is the case that sometimes multiple attributes combine to work better at predicting the two classes (observed and not observed).

Most classifiers, like the default in APEX, Random Forest, consider subsets of attributes and evaluates for their predictive value and therefore it isn't strictly necessary for users of the tool to pre-select attributes. This utility is primarily available to provide users a set of reports that help to evaluate which attributes appear to be most critical in predicting peptide observation. Some work suggests that the critical attributes will vary based on LC or sample preparation differences and instrumentation characteristics and it is of interest to some to report this information. Figure 23 shows the Attribute Evaluation Parameter Panel with the text result from a cross validation run. Following the run the results are displayed in a panel on the right side of the interface. A button below the result panel allows one to save the result to a text file.


APEX : Quantitative Proteomics Tool
? Help / Information
APEX Computation
Generate O$_i$ Data File
Build Training Data
Utilities and Analysis
Process Log
Select Utility or Analysis
Merge APEX Results
Two Sample Z Test
Classifier Cross Validation
Attribute Evaluation
Select Input Training ARFF File
Select File
Attribute Evaluation Selection
Attribute Evaluation Result
Save Attribute Evaluation Result
Display Result Graph
APEX
Quantitative Proteomics Tool
Execute

Selected File: tput_files\sample_yeast_training_ARFF.arff

● Chi-Square Eval./Ranker Search

○ Gain Ratio Eval./Ranker Search

○ CFS Attr. Selection/Best First Search

Locally Predictive: true

Search Direction: Backward

Search Termination: 5

```
Attribute Evaluation
Input ARFF: C:\APEX\APEX_1_0_0\data\sample_apex_output_files\sample_yeast_training_ARFF.arff
Selected Method:        Chi-squared Ranking Filter

Weka Evaluation Class: weka.attributeSelection.ChiSquaredAttributeEval
Weka Options: weka.attributeSelection.Ranker -T -1.7976931348623157E308 -N -1
============  Results  ============
1       495.76364120074936      NORM_FREQ_BETA_SHEET_SUM
2       470.29319617529325      MASS
3       454.6430242161056       NORM_FREQ_ALPHA_HELIX_SUM
4       453.88727291553266      LENGTH
5       396.3543477041516       AVE_POS_CHARGE
6       364.99120377313994      NORM_FREQ_BETA_TURN_SUM
7       273.5455203197211       AA_FREQ_K
8       261.71715168648586      ATOM_BASED_HYDROPH_MOMENT
9       228.44097005355547      PI
10      225.46825406416886      NET_CHARGE_AT_PH_7
11      200.58226927890837      AVE_NET_CHARGE
12      137.25166853339687      TOT_POS_CHARGE
13      112.78865666675682      AA_FREQ_R
14      94.46544285327589       AA_FREQ_L
15      91.08173705351004       AA_FREQ_P
16      90.38462973409281       AA_FREQ_F
17      79.36603791340781       AA_FREQ_N
18      76.84515337479633       AA_FREQ_D
19      76.82368963041492       AA_FREQ_E
20      73.2259620447629        TOT_PROP_BURIED
21      63.752392219296176      AA_FREQ_T
22      63.58044643614397       AA_FREQ_C
23      59.32006260895119       AA_FREQ_I
24      52.26744574091447       AA_FREQ_S
25      46.436207171495454      AA_FREQ_Q
26      45.95734196353637       AA_FREQ_G
27      45.52599965787318       AA_FREQ_V
28      42.248270180337755      AA_FREQ_W
29      41.713594606509744      NORM_FREQ_BETA_SHEET_AVE
30      32.8463576712945        AA_FREQ_Y
31      27.70762360810496       AA_FREQ_H
32      21.23840714940018       AA_FREQ_A
33      20.38652171445203       NORM_FREQ_BETA_TURN_AVE
34      14.244105477287082      NORM_FREQ_ALPHA_HELIX_AVE
35      0.0     AA_FREQ_M
```

Figure 23. Attribute Evaluation Utility Parameter Panel with a Displayed Result.

The main input for these methods is the training ARFF file.

Chi Square Attribute Evaluation

The Chi Square Attribute Evaluation test considers each attribute independently. The attribute values are binned into two bins, high and low, according to a computed threshold, and the Chi Square score is computed. The output is sorted based on score, placing the attributes with the highest scores on top. A bar graph can be used to visualize the scores and to look for logical break points in the scores.

Gain Ratio Attribute Evaluation

Gain Ratio Attribute Evaluation reports on the worth of an attribute by measuring the information gain ratio with respect to the class where,

GainRatio(Class, Attribute) = (H(Class) - H(Class | Attribute)) / H(Attribute) where H represents the Shannon entropy, also known as information value.

CFS Attribute Selection

CFS attribute selection (Correlation-based Feature Selection) technique tends to select a subset of attributes with high correlation to the class (observed vs. not observed in our case) and low attribute-to-attribute correlation (low redundancy). For example, this means that attributes that are highly correlated with each other such as peptide mass and peptide length would tend not to be included in the selected subset of attributes. One of the two attributes might represent the pair if they are both well correlated with the predicted classes.

Locally Predictive

Identify locally predictive attributes. Iteratively adds attributes with the highest correlation with the class as long as there is not already an attribute in the subset that has a higher correlation with the attribute in question.

Search Direction

Weka's BestFirst search algorithm used in APEX can select attributes in a *forward* direction by starting with an empty set of attributes and adding attributes to the tentative subset. On each addition the subset of attributes is evaluated for it's predictive value. Another search approach direction described as *backward*, refers to starting with the full set of attributes and eliminating attributes and assessing the remaining subset of attributes. *Bidirectional* combines these approaches.

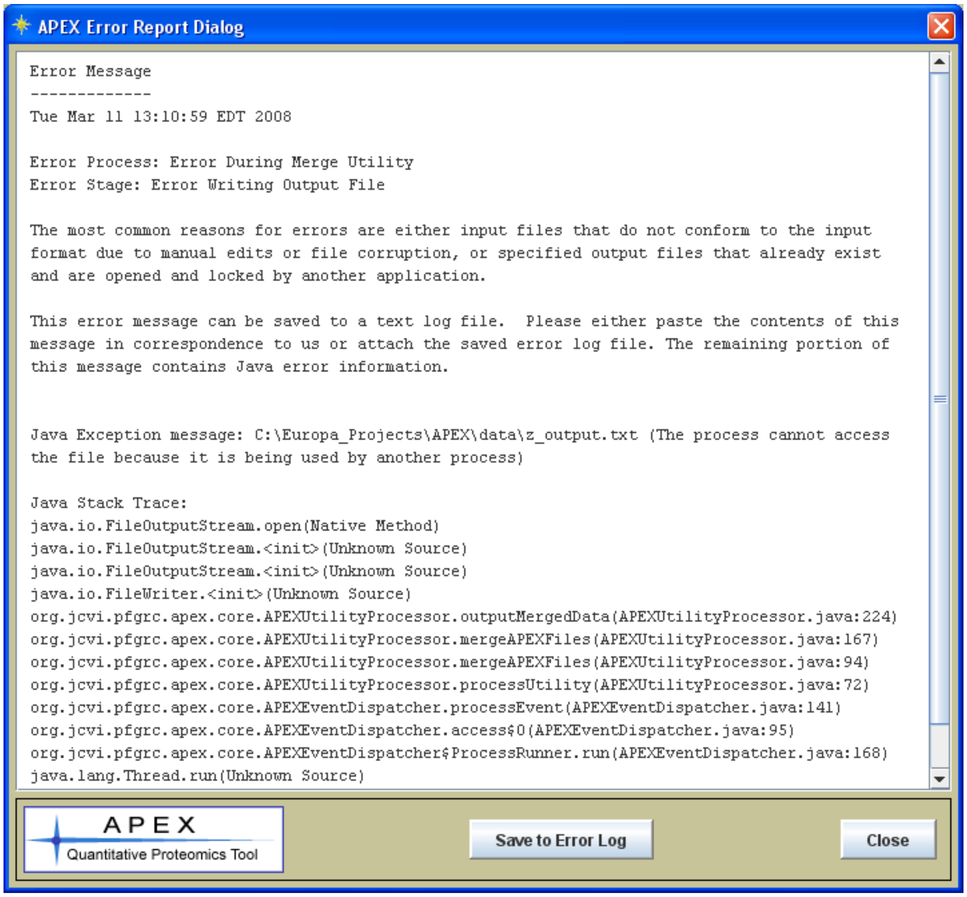
Search Termination

This option controls the amount of backtracking that can be done when evaluating attributes. Decisions are made to add (forward search) or remove (backward elimination) attributes to the subset. Each of these choices can affect future attribute additions or subtractions during the search process. This parameter controls how many steps backward the process can go (sort of an undo feature) before trying another branch in the decision tree.

Reporting Errors

The most common reasons for a process to error is if either a file is locked by another application (as reported in the sample Error Dialog shown in figure 21), or if an input file has been corrupted or manually edited such that it doesn't conform to the expected format specification. Efforts have been made to capture and report errors that occur in descriptive way in order to help the user and the APEX support team (me) to isolate and remedy the problem. Figure 24 shows an error dialog launched from APEX when a process (file merge utility) tried to write to a file that was already open and locked by another application. The main areas of the error message are the date, process, processing stage, the Java Exception Message and the Java Stack Trace. The Java Exception Message is usually descriptive and fairly explicit. The Stack Trace is primarily used as a tool for developers to find the code line associated with the error.

The error log can be saved to file and if you can't resolve the problem, the error log can be attached to an email sent to apex@jcvi.org. Please also check the DOS command prompt window (Terminal or Console for Linux or Mac) in case there is an additional error message.



```
Error Message
-------------
Tue Mar 11 13:10:59 EDT 2008

Error Process: Error During Merge Utility
Error Stage: Error Writing Output File

The most common reasons for errors are either input files that do not conform to the input
format due to manual edits or file corruption, or specified output files that already exist
and are opened and locked by another application.

This error message can be saved to a text log file.  Please either paste the contents of this
message in correspondence to us or attach the saved error log file. The remaining portion of
this message contains Java error information.


Java Exception message: C:\Europa_Projects\APEX\data\z_output.txt (The process cannot access
the file because it is being used by another process)

Java Stack Trace:
java.io.FileOutputStream.open(Native Method)
java.io.FileOutputStream.<init>(Unknown Source)
java.io.FileOutputStream.<init>(Unknown Source)
java.io.FileWriter.<init>(Unknown Source)
org.jcvi.pfgrc.apex.core.APEXUtilityProcessor.outputMergedData(APEXUtilityProcessor.java:224)
org.jcvi.pfgrc.apex.core.APEXUtilityProcessor.mergeAPEXFiles(APEXUtilityProcessor.java:167)
org.jcvi.pfgrc.apex.core.APEXUtilityProcessor.mergeAPEXFiles(APEXUtilityProcessor.java:94)
org.jcvi.pfgrc.apex.core.APEXUtilityProcessor.processUtility(APEXUtilityProcessor.java:72)
org.jcvi.pfgrc.apex.core.APEXEventDispatcher.processEvent(APEXEventDispatcher.java:141)
org.jcvi.pfgrc.apex.core.APEXEventDispatcher.access$0(APEXEventDispatcher.java:95)
org.jcvi.pfgrc.apex.core.APEXEventDispatcher$ProcessRunner.run(APEXEventDispatcher.java:168)
java.lang.Thread.run(Unknown Source)
```

Figure 24. Sample Error Dialog

The Appendices

Appendix I. File Formats

FASTA Protein Sequence File

The protein sequence files used in APEX conform to the NCBI standard format for protein sequence FASTA files. The description of the FASTA format can be found here: http://www.ncbi.nlm.nih.gov/blast/fasta.shtml

ProteinProphet Protein XML File

Appendix II describes the Trans-Proteomic Pipeline (TPP), a set of software tools which include PeptideProphet and ProteinProphet which are required to produce this protein XML file. These tools work on SEQUEST or MASCOT output files.

The document at this URL contains the schema for the protXML format: http://sashimi.sourceforge.net/schema_revision/protXML/Docs/protXML_v3.html

More important than knowing the details of this XML format is having the capability to produce files of this type as input to the APEX Tool. Please refer to Appendix II for details on generating these files.

Attribute-Relation File Format (Weka ARFF File, *.arff)

The Attribute Relation File Format is a standardized format that was developed for use with the Weka data mining tools. This file's main data area is a comma delimited matrix of values. In the case of APEX, each row contains the values (APEX peptide properties) relating to a specific peptide in the training data set. The last value in each row is the classification of the peptide indicating whether or not the peptide containing those properties (attributes) was detected by MS. Each row in the data section is an ordered list of attributes for a peptide. Just above the '@DATA' matrix in the file, there is an ordered list of attributes which identifies the columns in the data matrix. These values are preceded by the text '@ATTRIBUTE' and list the attribute name and data type.

Comments in the ARFF file are preceded with a '%' character. APEX generated ARFF files include three main comment areas:

1.) General Information - describes the basic structure of the file.
2.) Input Parameters – captures the input accession file, the FASTA sequence file, the protXML ProteinProphet input file, the number of consecutive trypsin cleavage misses allowed during in silico digestion. The length filer and mass filter limits are also captured.
3.) Output File Information – captures the number of input protein accessions in the list file, the number of computed peptide attributes, total number of tryptic peptides before and after applying length and mass filers, the number of peptides

observed and not observed by MS. A sample file that was produced by the APEX tool can be found in the *data/sample_apex_generated_files*.

A full description of the ARFF file format is found at this Weka page: http://www.cs.waikato.ac.nz/~ml/weka/arff.html

O_i File Format (*.oi)

APEX O_i files are used as input to the APEX abundance value computation. Comment lines in the file are marked by '#'. Comments include the construction date and time, input sequence file, ARFF Training File, output file, missed cleavage parameter, number and list of peptide properties, and the Weka classifier parameters. The data rows include protein accession, total cleaved peptide count (taking into account missed cleavages), and the O_i values. A sample Oi file, *sample_Oi.oi*, can be found in the *data/sample_ouput_data* folder.

Column Labels: ACC, TOT_PEP_CNT, Oi

APEX File Format (*.apex)

The APEX output file captures the construction date and time, the XML input file, the Oi input file, and the C values. The file also captures the number of proteins in the set, the minimum p_i, and the false positive rate for the set of proteins entering the computation. The data rows in the file include a sequential index, the protein accession, the protein description, the false positive error rate, pi, ni, Oi, relative APEX, APEX score.

Column Labels: INDEX, ACC, DESC, FPER, pi, ni, Oi, REL_APEX, APEX_SCORE

Appendix II. PeptideProphet and ProteinProphet: Using the Trans-Proteomic Pipeline (TPP) to Generate Protein XML (protXML) Files for use in the APEX Tool.

The Trans-Proteomic Pipeline (TPP) is a collection of software tools for the analysis of MS/MS proteomics data. The TPP tools are used to generate the input files for the APEX tool. The following steps describe the use of the TPP tools to generate the protXML files for input to APEX.

1.) The general data flow starts with a file format conversion from SEQUEST summary HTML file **or** MASCOT search result dat file to the TPP's pepXML file format. Support for this conversion is available from the TPP interface but Sequest2XML or Mascot2XML modules are actually doing the work. Note that for SEQUESST the process also requires the sequest.params file and Mascot conversion requires the search database FASTA file.

2.) The pepXML file is then processed by the TPP's PeptideProphet and ProteinProphet to produce two output XML files. One file, labeled as interact_<original_input_file_name>.xml contains PeptideProphet results while the other file, interact_<original_input_file_name>-prot.xml contains ProteinProphet results that are used as input

These pages describe the Trans-Proteomic Pipeline:
http://tools.proteomecenter.org/wiki/index.php?title=Software:TPP
http://sashimi.sourceforge.net/software_tpp.html

Refer to Keller et al., 2005 in the reference section for additional information on the TPP.

Appendix III. Peptide Physicochemical Properties

Peptide physicochemical properties are used as predictors that are used by the classifier during O_i generation to assign the probability of the peptide being detected by MS. Peptide properties such as mass, length, net charge, hydrophobicity, among others help to determine which peptides will be detected by MS. Mallick et al. 2007 characterizes several properties that are good predictors for peptide detection for four different MS technologies. The following table describes the peptide properties in available in the APEX tool. Note that some entries below represent two properties where a amino acid values are either summed or averaged.

Peptide Property	Description
Mass	Mass is a major determinant as to whether a peptide will fly during MS. Peptide mass in APEX is the sum of the *monoisotopic* amino acid masses (each minus 18.01 Da). + 18.01 for the two end residues' –OH and H. Formula: $Mass = 18.01 + \sum_{i=1}^{n} m_i$; where m_i is the mass of residue i given the particular residue at position i where m_i comes from this list: *MASS_A* = 71.04 *MASS_C* = 103.01 *MASS_D* = 115.03 *MASS_E* = 129.04 *MASS_F* = 147.07 *MASS_G* = 57.02 *MASS_H* = 137.00 *MASS_I* = 113.08 *MASS_K* = 128.09 *MASS_L* = 113.08 *MASS_M* = 131.04 *MASS_N* = 114.04 *MASS_P* = 97.05 *MASS_Q* = 128.06 *MASS_R* = 156.10 *MASS_S* = 87.03 *MASS_T* = 101.05 *MASS_V* = 99.07 *MASS_W* = 186.08 *MASS_Y* = 163.06
Sequence Length	Sequence length is also an important determinant of detection by MS as is the related (correlated) property of mass.
Relative Amino Acid Compositions	This is actually the set of 20 to amino acid frequencies where the count of each amino acid is divided by the

	sequence length. Some of these amino acid frequencies tend to be more critical. Histidine tends to be an important predictor of peptide detection by some MS techniques according to Mallick et al. 2007 (reference at end of manual).
Net Charge @ pH7 (pKa Based Charge)	This computation takes into account pKa values of charged residues. $Z = \sum_i N_i \frac{10^{pKa_i}}{10^{pH}+10^{pKa_i}} - \sum_j N_j \frac{10^{pH}}{10^{pH}+10^{pKa_j}}$ Where residues with for the first term with subscript *i* are are residues of K (pKa = 10.5), R (pKa =12.4), H (pKa=6.00), or the NH2- terminus (pKa=9.69). The second term residues (*j* index) include D (pka=3.86), E (pka = 4.25), C (pKa=8.33), Y (pKa=10.0) or the –COOH terminus (pKa=2.34).
pI (Isoelectric Point)	pH at which the net charge (pKa method above) is zero. APEX performs a bisection method to find the pH where charge is zero (within machine measurable tolerance).
Average Net Charge KLEP840101	KLEP840101 is the amino acid index (AAindex1) in the GenomeNet database for this property. This url links to a page for amino acid values for this property: http://www.genome.ad.jp/dbget-bin/www_bget?aax1:KLEP840101 This reference is provided as a reference to these values: Klein, P., Kanehisa, M. and DeLisi, C. Prediction of protein function from sequence properties: Discriminant analysis of a data base. Biochim. Biophys. Acta 787, 221-226 (1984) For this value we increment by +1 for positively charged residues (R,K) and decrement by -1 for all negatively charged residues (E,D). *Note that according to this source database, Histidine (H) is not contributing to the value. Once this summation has been completed, the value is divided by the length of the sequence. Note that this method does not take into account the pH nor pKa value of the amino acids as does our 'Net charge @ pH 7' property above. $AveNetCharge = \frac{\sum_{i=1}^{n}[+1 \mid r_i \in \{R,K\}][-1 \mid r_i \in \{E,D\}]}{n}$ To paraphrase, we add 1 for R or K, and subtract 1 for each E or D in the sequence. After account for all residues, this Net Charge is divided by the length of the sequence (n).

Total Positive Charge FAUJ880111	FAUJ880111 is the reference number (AAIndex1) for this property at GenomeNet. This url links to the amino acid values for this property: http://www.genome.ad.jp/dbget-bin/www_bget?aax1:FAUJ880111 Reference to the values: Fauchere, J.L., Charton, M., Kier, L.B., Verloop, A. and Pliska, V. Amino acid side chain parameters for correlation studies in biology and pharmacology Int. J. Peptide Protein Res. 32, 269-278 (1988) For this property we go down the sequence and increment a counter for each positively charged residue (R,K, and H) $TotPosCharge = \sum_{i=1}^{n}[+1 \mid r_i \in \{R, K, H\}]$
Average Positive Charge FAUJ880111 divided by sequence length	This is the Total Positive Charge divided by the sequence length.
Atom Based Hydrophobic Moment, EISD860102	EISD860102 is the reference number of this property at GenomeNet. URL for this property: http://www.genome.ad.jp/dbget-bin/www_bget?aaindex+EISD860102 The following reference is cited in reference to this property: Eisenberg, D. and McLachlan, A.D. Solvation energy in protein folding and binding. Nature 319, 199-203 (1986) In APEX we simply take the summation of the amino acid values for this property and divide by the sequence length. We use the amino acid level values found at the URL which match the Eisenberg values.
Total Propensity to be Buried, WERD780101	WERD780101 is the reference for this property at GenomeNet. URL: http://www.genome.ad.jp/dbget-bin/www_bget?aaindex+WERD780101 Reference: Wertz, D.H. and Scheraga, H.A. Influence of water on protein structure. An analysis of the preferences of amino acid residues for the inside or outside and for specific conformations in a protein molecule. Macromolecules 11, 9-15 (1978)

	In APEX we take the summation of the amino acid values for this property and divide by the length of the sequence.
Normalized frequency of alpha-helix (CHOP780201) (report SUM or AVE)	CHOP780201 is the reference for this property at GenomeNet. URL: http://www.genome.ad.jp/dbget-bin/www_bget?aaindex+CHOP780201 Reference: Chou, P.Y. and Fasman, G.D. Prediction of the secondary structure of proteins from their amino acid sequence Adv. Enzymol. 47, 45-148 (1978) In APEX we take the summation of the amino acid values for this property for the SUM property and divide by the length of the sequence for the corresponding AVE property.
Normalized frequency of beta-sheet (CHOP780202) (report SUM or AVE)	CHOP780202 is the reference for this property at GenomeNet. URL: http://www.genome.ad.jp/dbget-bin/www_bget?aaindex+CHOP780202 Reference: Chou, P.Y. and Fasman, G.D. Prediction of the secondary structure of proteins from their amino acid sequence Adv. Enzymol. 47, 45-148 (1978) In APEX we take the summation of the amino acid values for this property for the SUM property and divide by the length of the sequence for the corresponding AVE property.
Normalized frequency of beta-turn (CHOP780203) (report SUM or AVE)	CHOP780203 is the reference for this property at GenomeNet. URL: http://www.genome.ad.jp/dbget-bin/www_bget?aaindex+CHOP780203 Reference: Chou, P.Y. and Fasman, G.D. Prediction of the secondary structure of proteins from their amino acid sequence Adv. Enzymol. 47, 45-148 (1978) In APEX we take the summation of the amino acid values for this property for the SUM property and divide by the length of the sequence for the corresponding AVE property.

The peptide classification step within the O_i generation process is handled by Java code from Weka, a free open source data mining software project. Weka has a vast number of machine learning algorithms and is well established as a leader in the field of software for data mining. Weka is issued under the GNU General Public License (GPL). The authors of APEX are grateful for the rich capabilities supported by Weka and the fact that these powerful tools are offered to the public both within the Weka interface and behind many other tools, such as APEX, that utilize Weka for these capabilities. Please visit Weka at http://www.cs.waikato.ac.nz/ml/weka/ for additional information on Weka tools.

The following reference book (referenced below) is very useful in that it provides basic theory behind data mining with machine learning techniques and presents the information with practical examples that can be run using Weka. This book provides much more information than is necessary for most APEX users but it is a good reference to the general ideas behind machine learning.

Ian H. Witten and Eibe Frank (2005) "Data Mining: Practical machine learning tools and techniques", 2nd Edition, Morgan Kaufmann, San Francisco, 2005.

APEX presents three optional classifiers, Random Forest, RIDOR, and J48 Trees. The next appendix section will describe how users familiar with Weka and its classifiers can easily configure APEX to utilize different classifier implementations that are found in Weka. Each of these classifiers is an implementation of the Weka software. The Lu paper that introduces the APEX technique, describes the Random Forest as having superior performance during cross validation tests of peptide property data sets. During development of this tool, the Random Forest technique has also shown to give very good results in our testing. The Random Forest algorithm, coupled with an option that makes the classifier cost sensitive (weighting training instances based on the frequency of observed and not observed peptides in the training set) and Bagging (iterations of classifier training on random subsets of the training data) work fine in our hands and was found to work quite well following extensive classifier testing described in the Lu APEX paper’s supplemental information document.

The following classifier options are the default options in APEX. Note that the letter tags are option tags used within Weka to specify parameters. Within the APEX tool we present the official Weka tags as well as a descriptive parameter name.

Cost Sensitive Classification (weka.classifiers.meta.CostSensitiveClassifier)

This option weights the training data instances based on the frequency of observed and not observed peptides. This option helps the handle the situation that non-observed peptides are naturally much more prevalent than observed peptides within the training data set.

-cost-matrix arff-cost

This Weka option is used to specify the cost matrix for the cost sensitive classifier. Note that the default value, **arff-cost**, is **not** a standard Weka value but is rather a value that tells APEX to generate an appropriate cost matrix based on the frequency of observed and not observed peptides in the training set. Note that one can modify this option and supply a specific cost matrix however the matrix generated by the arff class frequencies is appropriate for our purposes.

Bagging (weka.classifiers.meta.Bagging)

The bagging option causes the classifier to be built several times on random subsets of the data. This can help to eliminate possible biases that may exist in a subset of the data. Another benefit is that bagging will average results from randomized algorithms

-I 10

This option indicates that Bagging should iterate 10 times (builds the classifier 10 times).

-P 100

This option instructs Bagging to use 100% of the data on each iteration.

-S 1

This is a random number seed. Any number will do for this parameter.

Random Forest (weka.classifiers.trees.RandomForest)

This option indicates the use of the Random Forest classifier as the primary classifier.

-I 10

This option tells random forest to construct 10 classifiers (iterations), each using a set of randomly selected features (peptide properties or attributes).

-K 5

This option instructs random forest to use 5 random features on each of the iterations.

-S 1

This is a random number seed value. Any number is fine for this parameter.

A note for Weka savy users: when using these options with Weka and in APEX, each classifier is preceded by the –W tag which marks the beginning of a new classifier. If you move the mouse over the question mark next to the Select/View Classifier Parameters button on the *O_i Processing Panel*, you will see a listing of the currently selected classification parameters.

Appendix V. Classifier Customization

The default Random Forest classifier implemented in Weka has performed well according the work reported in the Lu paper describing the APEX technique. In work at our center, this classifier also seems to perform fine for our data. If one is interested in using classifiers other than the three currently available in APEX, one can configure APEX to utilize other classifiers within the Weka collection. Classifier options are loaded when the APEX tool is launched according to the contents of the we*ka_parameters.config* file found in the *apex_config* folder. The following text has been copied from the configuration file (slightly reformatted to fix line breaks). It describes the configuration fields that are used in the file and shows the specification for Random Forest within the configuration file as an example. Each classifier has default values that can be altered. The first classifier in the configuration file is the default classifier inside the APEX tool. Note that appending additional Weka classifiers to this configuration file demands that one be familiar with the Weka option tags that are available for the classifier and have an understanding about option value types, possible value constraints, and option dependencies (one option depending on the value of another). A backup of the original file is included in the *apex_config* folder.

```
# APEX Weka Classifier Definition File
#
# This file is used to configure Weka classifier options available in
# APEX. The first classifier in this file is always taken as the
# default classifier and parameter values.  In order to add a
# classifier it is necessary to be  # familiar with Weka's command line
# tag options for the classifier of interest.  This # includes
# specification of parameter dependencies.  In some cases the inclusion
# of a particular parameter is dependent on the state of another,
# usually BOOLEAN, parameter.
#
#
# Key Glossary:
#
# CLASSIFIER - marks the start of a classifier entry
#
# CLASSIFIER_END - marks the end of a classifier entry
#
# WEKA_CLASSIFIER_CLASS - is the fully qualified (includes Java
# packages) classifier class name.
#
# PERMITS_COST_SENS - indicates if cost sensitive is permitted, value
# is Y or N
#
# PERMITS_BAGGING - indicates if bagging is permitted, value is Y or N
#
# PARAMETER_NAME - a descriptive parameter name, also marks the start
# of a parameter entry
#
# PARAMETER_WEKA_TAG - the corresponding Weka option tag for the
# parameter
#
# PARAMETER_DEFAULT_VALUE - a default value for the parameter
#
# PARAMETER_TYPE - several options <INT>=integer, <NUMERICAL>=float,
# <BOOLEAN>=boolean [TRUE | FALSE]
#
```

```
# PARAMETER_CONSTRAINT - value constraint options <NONE>, <POS>,
# <BOUNDED>
#
# PARAMETER_BOUND_UPPER (or LOWER) - <NONE> or numerical if constraint
# is <BOUNDED>
#
# PARAMETER_DEPEND_TAG_AND_TRIGGER_VALUE - if the presence of this tag
# is dependent on the state of another variable, specify the other
# variable and a trigger value delimited by a :.
# Example: PARAMETER_DEPEND_TAG_AND_TRIGGER_VALUE:-R:TRUE
#
# PARAMETER_END - marks the end of a parameter
#
#
#
CLASSIFIER
CLASSIFIER_NAME:Random Forest
WEKA_CLASSIFIER_CLASS:weka.classifiers.trees.RandomForest
PERMITS_COST_SENS:Y
PERMITS_BAGGING:Y
#
PARAMETER_NAME:Iterations
PARAMETER_WEKA_TAG:-I
PARAMETER_DEFAULT_VALUE:10
PARAMETER_TYPE:INT
PARAMETER_CONSTRAINT:POS
PARAMETER_BOUND_UPPER:NONE
PARAMETER_BOUND_LOWER:NONE
PARAMETER_END
#
PARAMETER_NAME:Number of Random Features
PARAMETER_WEKA_TAG:-K
PARAMETER_DEFAULT_VALUE:5
PARAMETER_TYPE:INT
PARAMETER_CONSTRAINT:POS
PARAMETER_BOUND_UPPER:NONE
PARAMETER_BOUND_LOWER:NONE
PARAMETER_END
#
PARAMETER_NAME:Random Seed
PARAMETER_WEKA_TAG:-S
PARAMETER_DEFAULT_VALUE:1
PARAMETER_TYPE:INT
PARAMETER_CONSTRAINT:NONE
PARAMETER_BOUND_UPPER:NONE
PARAMETER_BOUND_LOWER:NONE
PARAMETER_END
CLASSIFIER_END
#
```

Appendix VI. APEX Digestion Schemes – Defining Custom Schemes

The *apex_config* folder contains a file that contains a file, *enzyme_rules.config*, that defines digestion schemes in APEX. The tab delimited text file file can be edited to define new schemes or to adjust the rules of existing schemes. Below is the default state of the enzyme_rules.config file as of version 1.1.

LABEL_NAME	CLEAVE_TARGETS	STOPPER_RES	CLEAVE_TERMINAL
Tyrpsin Digest	KR	P	C
Chymotrypsin Digest	YWFL	P	C
Trypsin>Chymotrypsin	KR>YWFL	P>P	C>C
TrypsinChymotrypsin	KRYWFL	P	C
Trypsin+Chymotrypsin	KR+YWFL	P+P	C+C

The LABEL_NAME field provides a descriptive title for the digestion option. CLEAVE_TARGETS are the amino acids that are targets for the digestion scheme. STOPPER_RES refers to amino acid residues that cause a digestion miss when associated with the cleavage terminal on the cleavage target. CLEAVE_TERMINAL specifies whether cleavage should be on the C or the N terminal. **NOTE: APEX version 1.1 only supports C terminal cleavage rules. Future releases will include N terminal options.**

There are several special characters within the digestion scheme designations. The greater than symbol '>' indicates that the digestion scheme is a two stage, sometimes referred to as sequential or serial, digestion. This is where one enzyme is applied followed by a second enzyme. The '+' symbol refers to a case where the sample is split into two aliquots, digested with two different enzymes, then the digestion peptides are combined and analyzed. We sometimes refer to this as parallel digestion. While parallel digestion is an option, we are not aware of a general need for this at this time.

The file can be edited in Excel® and the file can be output as tab delimited text. It is recommended that users back up their enzyme_rule.config file prior to editing.

Contributions and Acknowledgements

The following list includes some of the key contributors to the underlying APEX technology and the development of this software tool. The list is roughly in chronological order of contribution, from development of the technique, though software prototyping and validation, and on to the design and implementation of this tool. This list can not fully describe the many helpful exchanges and interactions that made the initial idea of this tool a reality. The authors of this tool are sincerely grateful to all who participated, both inside the PFGRC and J. Craig Venter Institute and those at the University of Texas at Austin.

Edward Marcotte, Christine Vogel, and the Marcotte Lab	Center for Systems and Synthetic Biology, Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, 2500 Speedway, University of Texas, Austin TX 78712, U.S.A.	Developed the underlying computational technique, advised regarding parameter options, interface design, and functional requirements.
Alan Rodrigues	Pathogen Functional Genomics Resource Center, J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, U.S.A **(PFGRC/JCVI)**	Researched the APEX technique, prototyped the various computational steps in Perl, spearheaded the PFGRC's evaluation of the technique.
Srilatha Kuntumalla	PFGRC/JCVI	Investigated the technique, supported development of the Perl prototypes, performed proteomic studies to validate the computational technique and both the Perl and this Java implementation, technical bridge between software developers and proteomics/MS realm.
Rong Wang, Huang Shih-Ting, Rembert Piper	PFGRC/JCVI	APEX tool design input, technical support contacts for developers.
Erik Ferlanti,	PFGRC/JCVI	APEX tool design input, technical support for setting up TPP components on the JCVI network,
John Braisted	PFGRC/JCVI	APEX Quantitative Proteomics Tool author, software design and architect. Implemented the computational, program control, and interface of the APEX Tool. Authored the manual and help

		page system.
Alex Saeed, Vasily Sharov, Jianwei Li, Wei Liang, Chun Hua Wan, Miguel Covarrubias, Lynn Stevens	Bioinformatics/Analysis Group, PFGRC/JCVI	Helpful discussions on tool design.

Supporting Software

The authors of the APEX Tool would like to acknowledge three supporting software projects that help to support some of the APEX processing tasks. The classification support in APEX comes from the Weka set of Java machine learning / data mining tools. Weka is a well established set of tools that is widely used in data mining applications (see Appendix IV). The graphs displayed following the z-score test (Utilities and Analysis process panel) are rendered using the JFreeChart graph package. Z-score p-values are generated with support from the JSci Java scientific computational package. The protXML format files that serve as MS input to the APEX tool are processed via the Trans-Proteomic Pipeline (TPP) from the Proteome Center at the Institute for Systems Biology. The quality of these packages is a testament to the ideals of open source software.

References

Futcher B., G.I. Latter, P. Monardo, C.S. McLaughlin, J.I. Garrels. A Sampling of the Yeast Proteome. Mol. Cell. Biol., 19:7357-7368, 1999.

* Lu P., C. Vogel, R. Wang, X. Yao, E. M. Marcotte. Absolute Protein Expression Profiling Estimates the Relative Contributions of Transcriptional and Translational Regulation. Nature Biotech., 25(1):117-124, 2007.

Neidhardt, F.C., H.E. Umbarger. *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology, edn. 2, vol.1 (eds. Niedhardt, F.C. et al.) 13-16 (ASM Press, Washington, DC, 1996)

Mallick P., M. Schirle, S.S. Chen, M.R. Flory, H. Lee, D. Martin, J. Ranish, B. Raught, R. Schmitt, T. Werner, B. Kuster, R. Aebersold. Computational Prediction of Proteotypic Peptides for Quantitative Proteomics. Nature Biotech., 25(1):125-131, 2007.

Witten I.H. and E. Frank "Data Mining: Practical machine learning tools and techniques", 2nd Edition, Morgan Kaufmann, San Francisco, 2005.

Keller A, Eng J, Zhang N, Li X, Aebersold R: A uniform proteomics MS/MS analysis platform utilizing open XML file formats. Mol. Syst. Biol. 2005, 1-17. [http://tools.proteomecenter.org/TPP.php]

* This reference is to the original paper that describes the APEX technique.