PEAKS® Online 11

HIGH-THROUGHPUT, MULTI-USER, PROTEOMICS
LC-MS/MS ANALYSIS SOFTWARE

ACCELERATE DISCOVERY PROTEOMICS WITH HIGH THROUGHPUT DATA ANALYSIS

ACCURATE AND SENSITIVE IDENTIFICATION FOR BOTH DDA AND DIA ANALYSIS

ROBUST LABEL-FREE AND LABELLED QUANTIFICATION SUPPORT

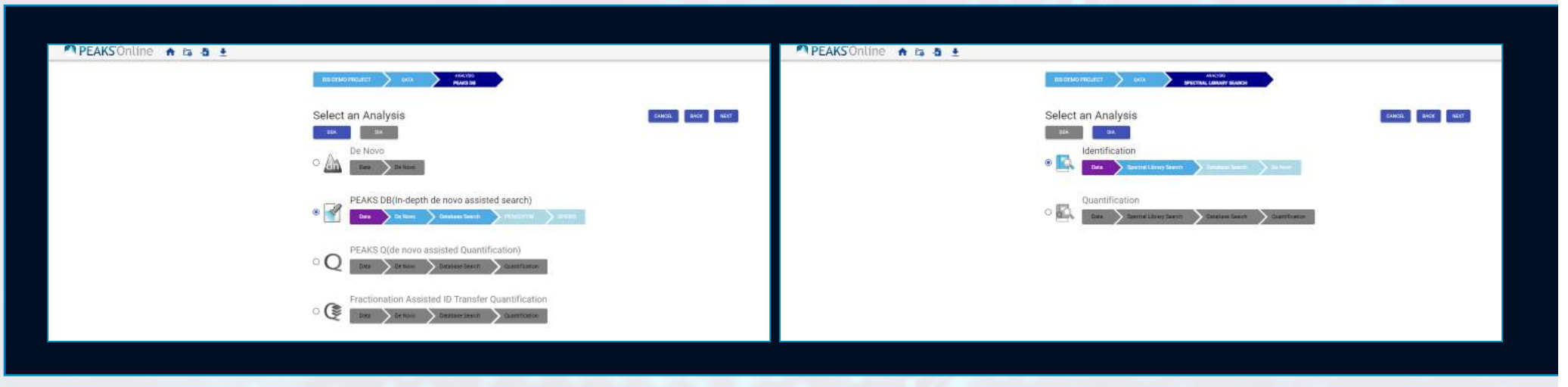
NEW DEEP LEARNING ENABLED DIA WORKFLOW AND DEEPNOVO PEPTIDOME ANALYSIS

BIOINFORMATICS SOLUTIONS INC.

PEAKS® is a specialized tool that offers unrivalled peptide identification rates driven by deep learning technology and integrated with library search, direct database search and *de novo* sequencing.

PEAKS® Online 11 supports data-dependent and data-independent acquisition analyses (DDA and DIA respectively) and ion mobility mass spectrometry (IMS-MS). As a vendor-neutral computing platform, it is capable of directly loading raw mass spectrometry data and standard data formats. Deploy the PEAKS® workflows to identify the presence of peptides and proteins in your project for 1) DDA data analysis including *de novo* sequencing, PEAKS DB (database search) identification,, PEAKS PTM (post translational modification) analysis, SPIDER homology search and PEAKS DeepNovo Peptidome (specialized workflow for peptidomics data) and 2) DIA data analysis including spectral library search, direct database searching and *de novo* sequencing.

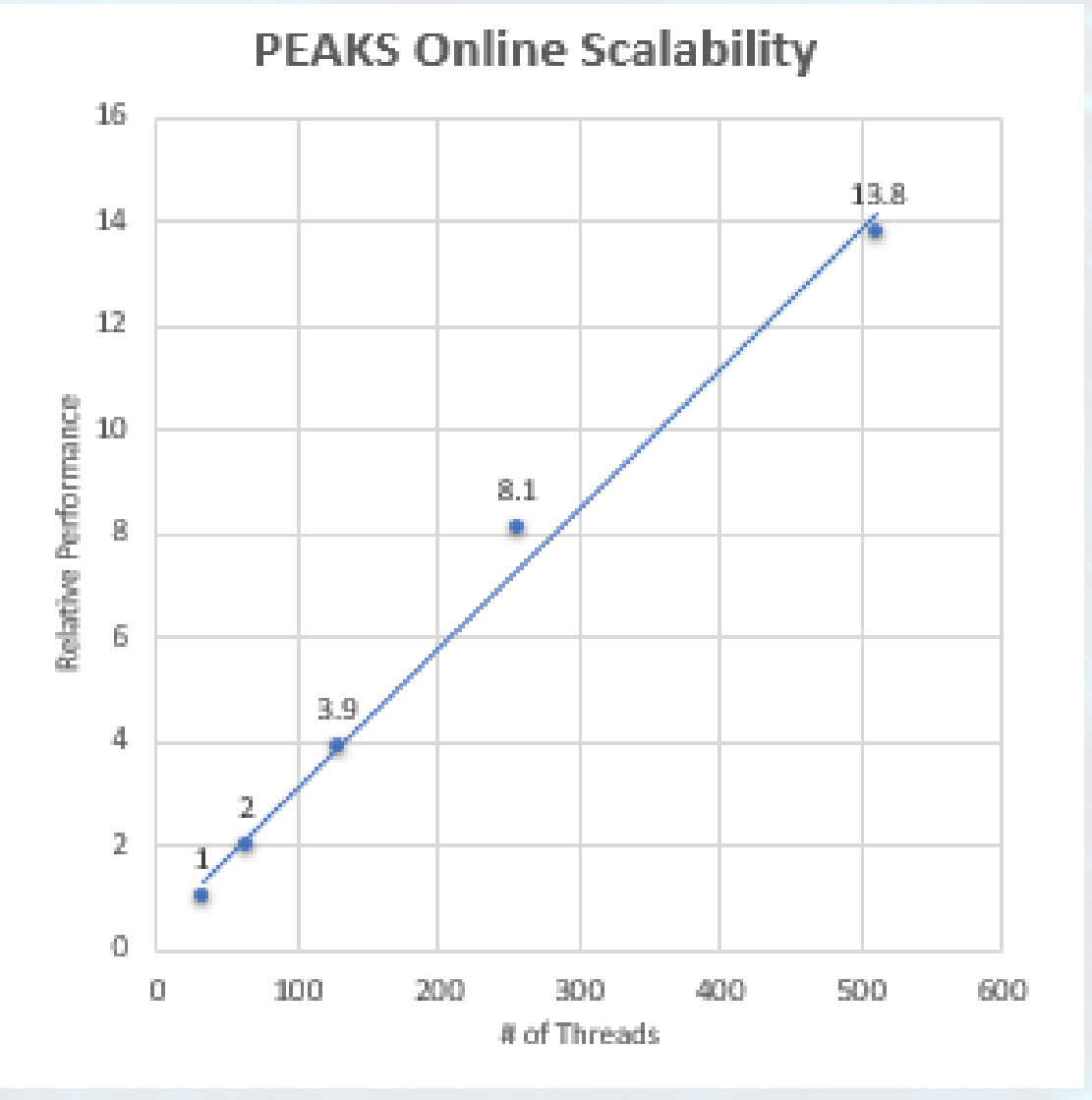
Quantification analysis by labeling and label-free quantification (LFQ) can also be performed using the PEAKS Q addon module. Intuitive result visualization tools are provided at every stage of analysis and results can be exported.



Use PEAKS® Online 11 to take advantage of powerful and shared computing resources to perform LC-MS/MS protein & peptide identification and quantification analyses. The restructured platform allows large datasets to be processed efficiently by multiple users at the same time; with the ability to run on any cluster, multi-CPU machine, or cloud server.

PEAKS SERVER-BASED SOLUTION KEY FEATURES

Re-running analyses has never been so easy. With PEAKS® Online, the algorithm will determine the quickest way to reanalyze your results and use existing results, if applicable, instead of running the whole dataset again.



672 data files, 2016 hrs MS run time analyzed in less than 18 hrs

Data monitoring for acquisition machine allows real-time processing of data as it comes off the mass spectrometer.

Allow concurrent access from multiple users to process multiple projects in parallel.

High performance and advanced algorithms to provide a complete, vendor-neutral solution for discovery proteomics, including protein/peptide *de novo* sequencing, identification and quantification.

Centralized configuration and monitoring system to easily maintain, prioritize and share all PEAKS® Online data analyses.

Advanced System Architecture

Built on top of the latest technologies to fully utilize the computing power of your hardware to provide:

High throughput solution: Allows concurrent access from multiple users to support paralelism at project and data level.

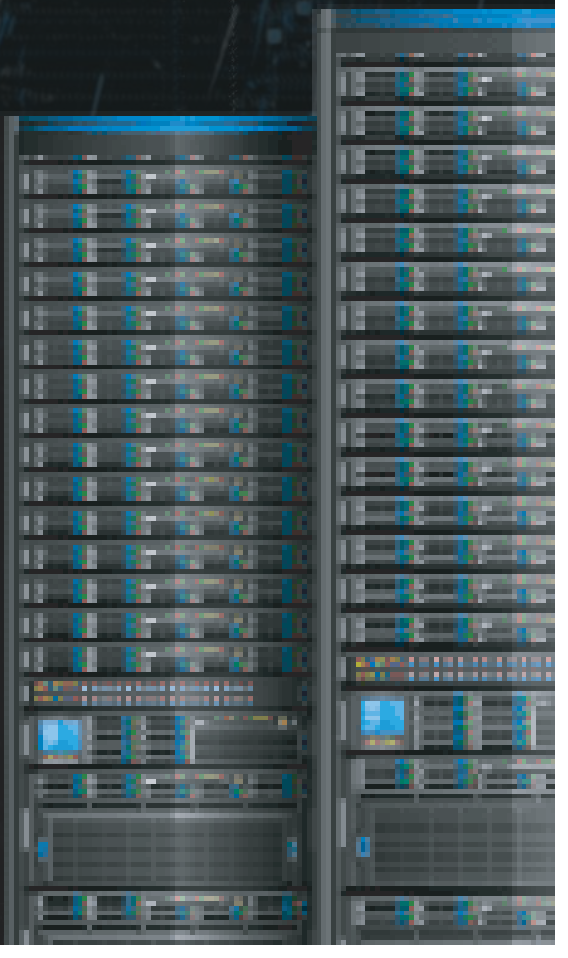
Distributed database: Yields higher I/O performance and better fault tolerance.

Ready to scale: Vertically and horizontally, add new worker node(s) or database.

Cross-platform deployment: Deploy the server on any Windows or Linux systems.

Dual interfaces: The command line interface offers the abilty to automate data analysis workflows and result exporting while the web interface provides a graphical user interface to visually configure workflows and easily assess results in detail.

True automation with PEAKS® daemon: Automated Instrument Link seamlessly connects your acquisition to your analysis in one easy to use workflow.



Don’t get left in the dark!
Work together as a cohesive research group

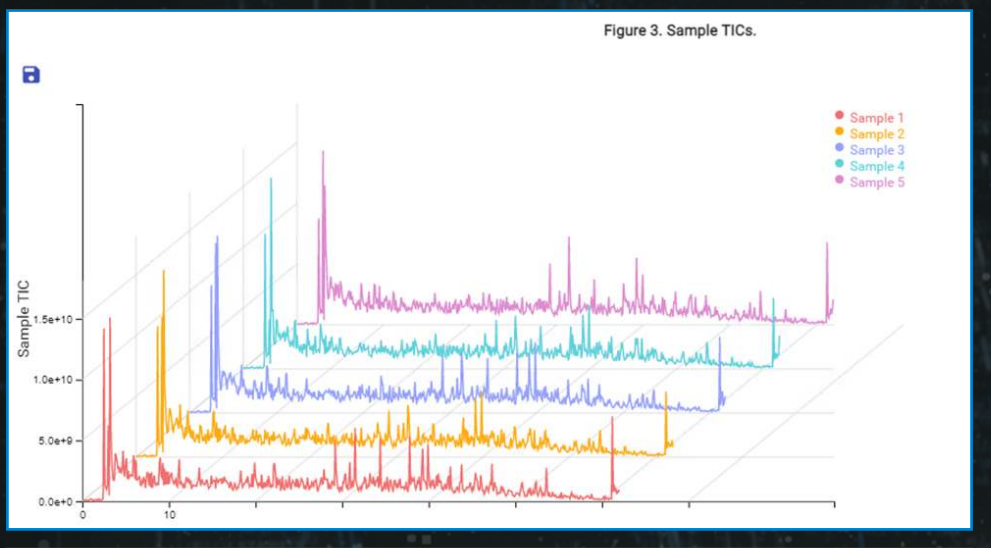
PEAKS® Online means high-throughput data processing on a shared resource. This server-designed proteomics software is fully parallelized with the ability to run on a cluster of multi-CPU machine or cloud server.

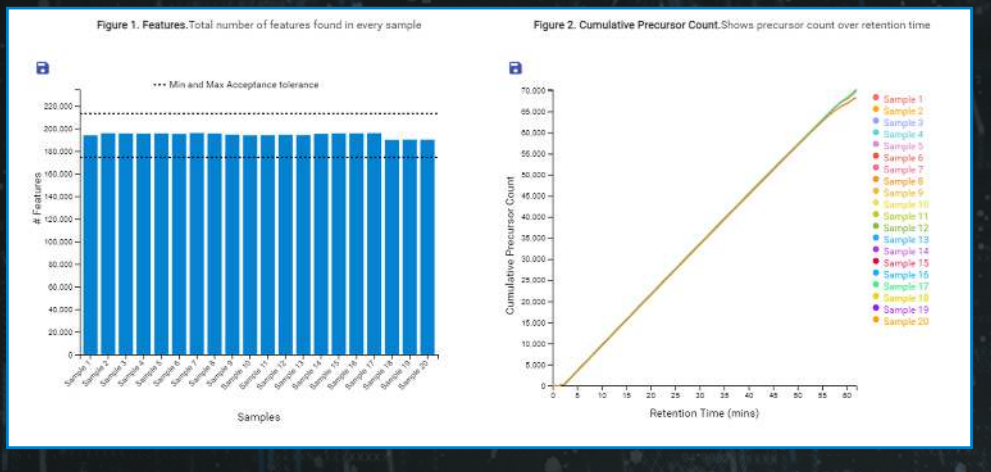
Users are able to run the same proven algorithms included in the PEAKS Studio solution, efficiently and on a larger scale.

By using a web interface client, users can send/retrieve data to/from the server and view the results, on any operating system, in an intuitive manner.

Take complete control from raw data to report

Align your team’s efforts with administrative controls to standardise workflows, databases, PTMs, quantification methods, project sharing and Quality Control (QC) analysis.





With the new Quality Control (QC) analysis in PEAKS® Online 11 users can assess statistical information of the raw data and/or results and gain beneficial insight into the attributes of the LC-MS acquisition. This automated tool is designed for both, DDA and DIA data and will supply the elements to determine the quality of the data and evaluates the setup of the experiment.

Ultra sensitive peptide identification rates driven by deep learning technology and integrated with library search, direct database search and *de novo* sequencing

Generally speaking, there are three common ways to interpret tandem mass spectrometry data: database search, *de novo* sequencing and spectral library search:

SPECTRAL LIBRARY SEARCH:
Given a spectrum and spectral library, find a peptide in the spectral library that has the best match with the spectrum.

DIRECT DATABASE SEARCH:
Given a spectrum and protein sequence database, construct an in-silico spectral library using spectrum and iRT prediction models to find a peptide in the spectral library that has the best match with the spectrum.

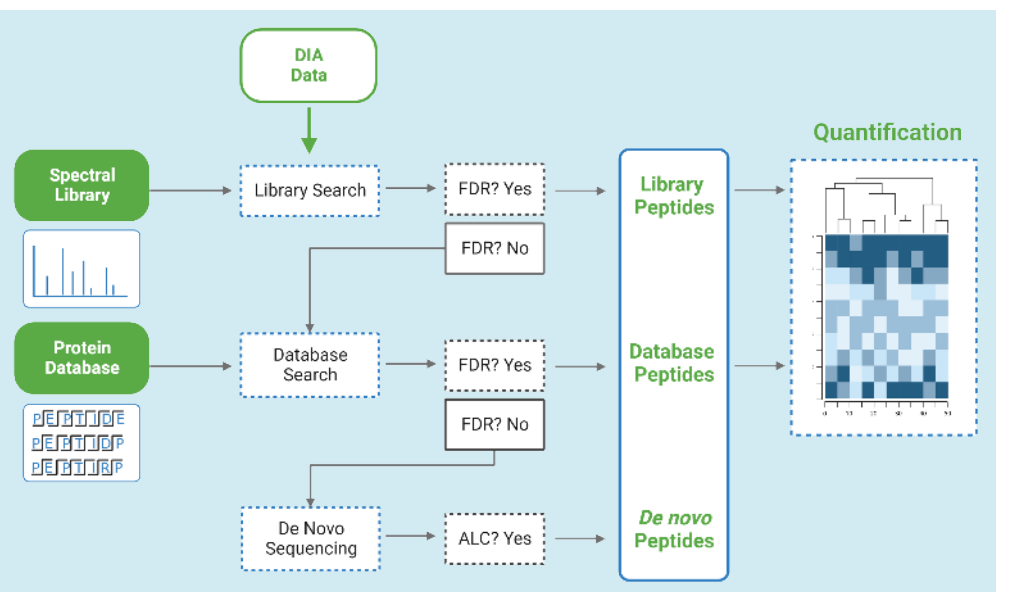
***DE NOVO* SEQUENCING:**
Given a spectrum, find a peptide that has the best match with the spectrum.

1. When the peptides are believed to be in a protein sequence database, then a database search approach is preferred.

2. When studying a particular proteome, a peptide spectral library for the targeted biological system being studied can be used to focus your analysis.

3. However, when such a sequence database or spectral library is unavailable, *de novo* sequencing is needed to derive the peptide sequence directly from the spectrum.

PEAKS® offers a robust solution for DIA data analysis. It incorporates three methods of peptide identification: spectral library search, direct database search, and de novo sequencing. The search is performed using an expanding search space. First, a library search is performed against a library of previously identified spectra. By predicting the false discovery rate, peptides that pass the filter are saved. MS/MS spectra that don't match a peptide within the false discovery rate threshold are brought forward to a direct database search. Confident database matches are added to the result. Then, using the same FDR approach, unmatched spectra from the database search are analysed using de novo sequencing.



Deep learning advances accuracy and sensitivity of data analysis

PEAKS® Online uses advancements in deep learning-based spectrum prediction models to perform both DDA and DIA database search by predicting the retention time and spectra in silico for each plausible peptide. In addition, PEAKS furthers the use of deep learning to perform *de novo* sequencing for both DDA and DIA data, which could help identify polypeptides from out-of-frame ORFs for example.

By integrating spectral library search, database search and *de novo* sequencing into a single workflow, PEAKS offers accurate and comprehensive analysis on all types of data. Benchmarking data shows the exceeding peptide identification rates.

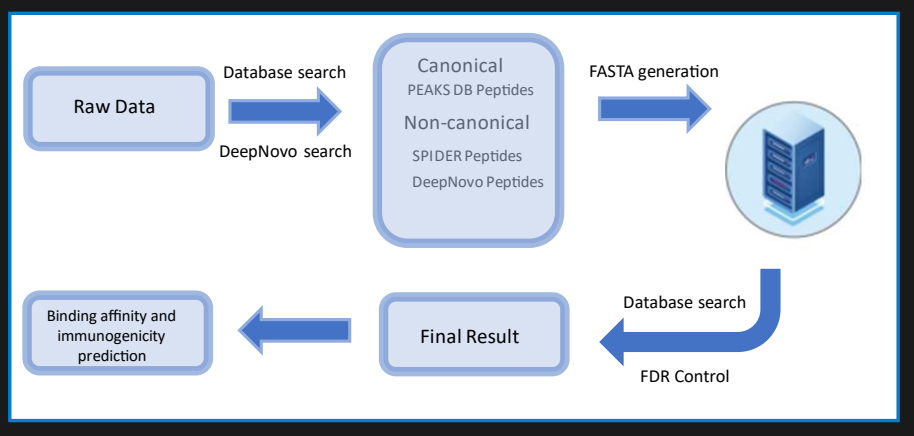
To overcome the boundaries of discovery, PEAKS® introduces the first *de novo* sequencing approach for DIA to provide truly unbiased results

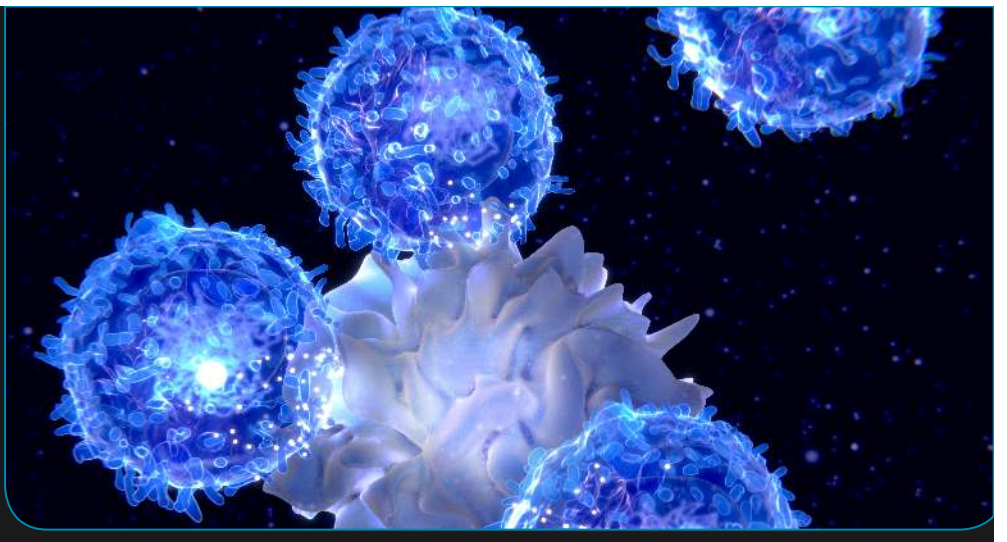
PEAKS® *DeepNovo* Peptidome

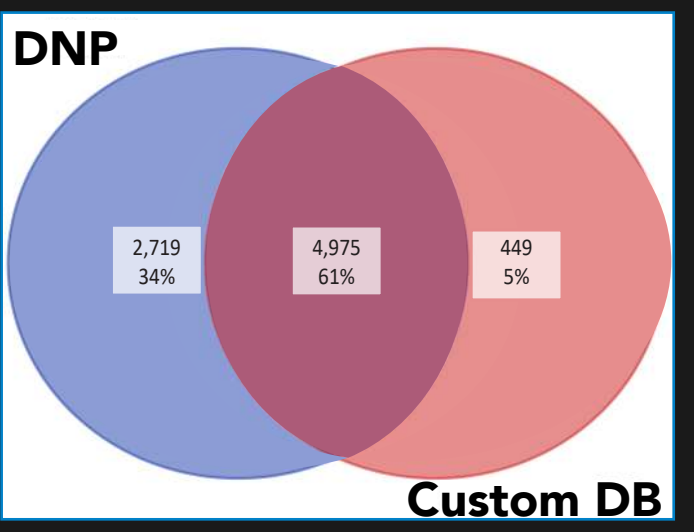
WORKFLOW

This newly developed solution is a specialized workflow for peptidomics data that combines database searching, *de novo* sequencing, and identification of mutated peptides By training DeepNovo deep learning model using peptidomics datasets the sensitivity and accuracy of peptide identification can significantly be improved. Furthermore, *de novo* peptides (non canonical) are combined with database peptides (canonical) for more accurate estimation of false discovery rate.

The final output of peptides are categorized as Database, DeepNovo or Homologs (mutated peptides) and can be directly exported for binding affinity and immunogenicity predictions.

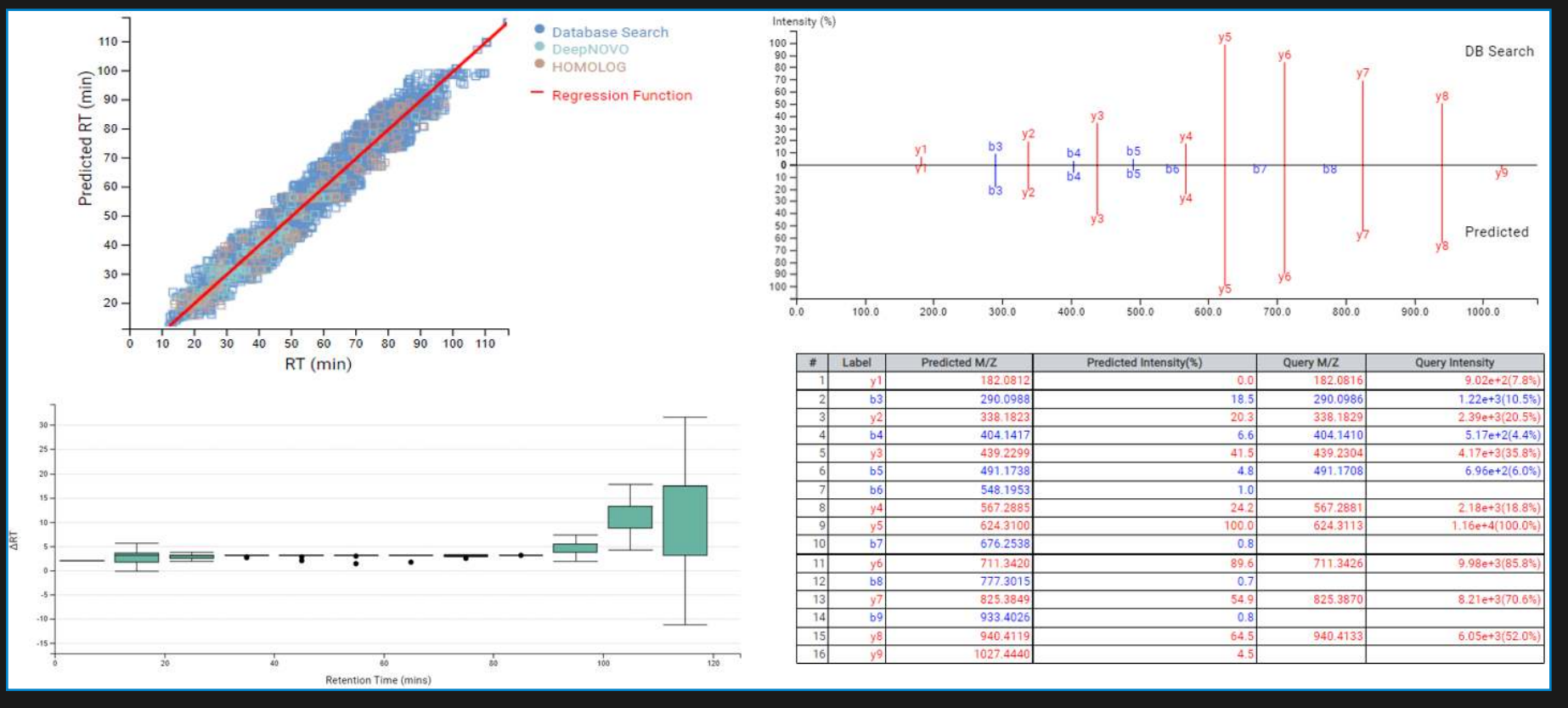






Advanced solution for Immopeptidomics

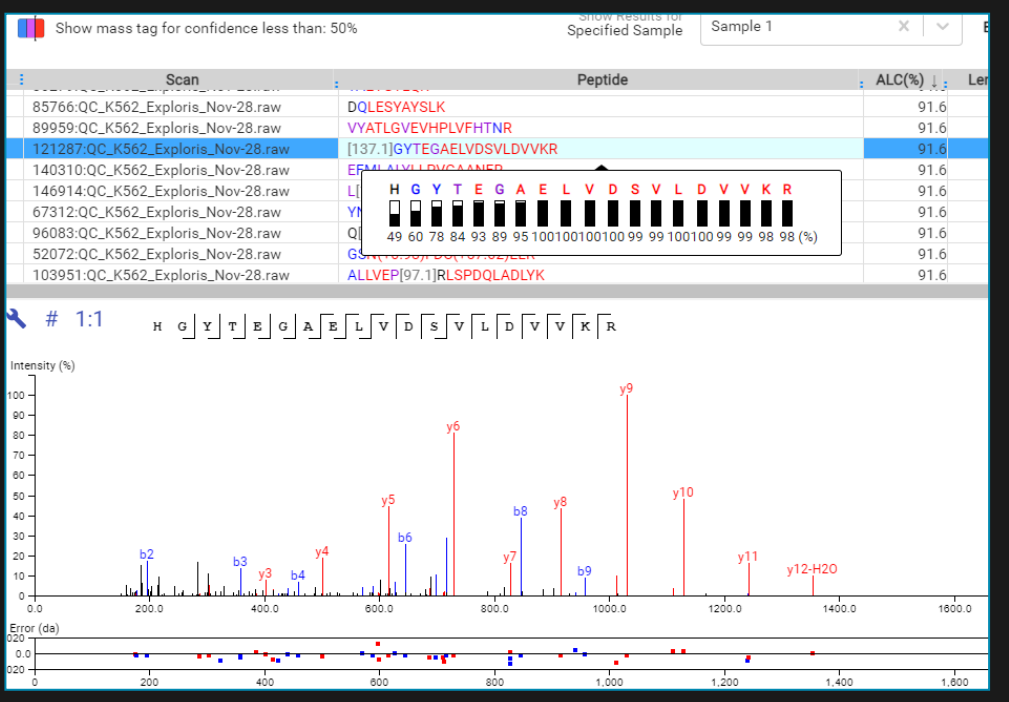
The peptides can be derived from non coding regions of the genome, aberrantly expressed transcripts, splicing of proteasomal products, or may contain mutations. From a re-analysis of a dataset recently published by Apavaloaei et al. (2022), DeepNovo Peptidome (DNP) workflow uncovers the majority of peptides (>90%) without the requirement for custom database generation from RNA seq data.



#	Label	Predicted M/Z	Predicted Intensity(%)	Query M/Z	Query Intensity
1	y1	182.0812	0.0	182.0816	9.02e+2(7.8%)
2	b3	290.0988	18.5	290.0986	1.22e+3(10.5%)
3	y2	338.1823	20.3	338.1829	2.39e+3(20.5%)
4	b4	404.1417	6.6	404.1410	5.17e+2(4.4%)
5	y3	439.2299	41.5	439.2304	4.17e+3(35.8%)
6	b5	491.1738	4.8	491.1708	6.96e+2(6.0%)
7	b6	548.1953	1.0		
8	y4	567.2885	24.2	567.2881	2.18e+3(18.8%)
9	y5	624.3100	100.0	624.3113	1.16e+4(100.0%)
10	b7	676.2538	0.8		
11	y6	711.3420	89.6	711.3426	9.98e+3(85.8%)
12	b8	777.3015	0.7		
13	y7	825.3849	54.9	825.3870	8.21e+3(70.6%)
14	b9	933.4026	0.8		
15	y8	940.4119	64.5	940.4133	6.05e+3(52.0%)
16	y9	1027.4440	4.5		

PEAKS® *de novo* peptide sequencing is well-recognized worldwide and the base of all PEAKS analyses

The innovative PEAKS® *de novo* sequencing algorithm accurately constructs a peptide sequence without the use of a database. These sequences are then used to enhance the PEAKS DB, PEAKS PTM, and SPIDER analyses to improve both, accuracy and sensitivity.



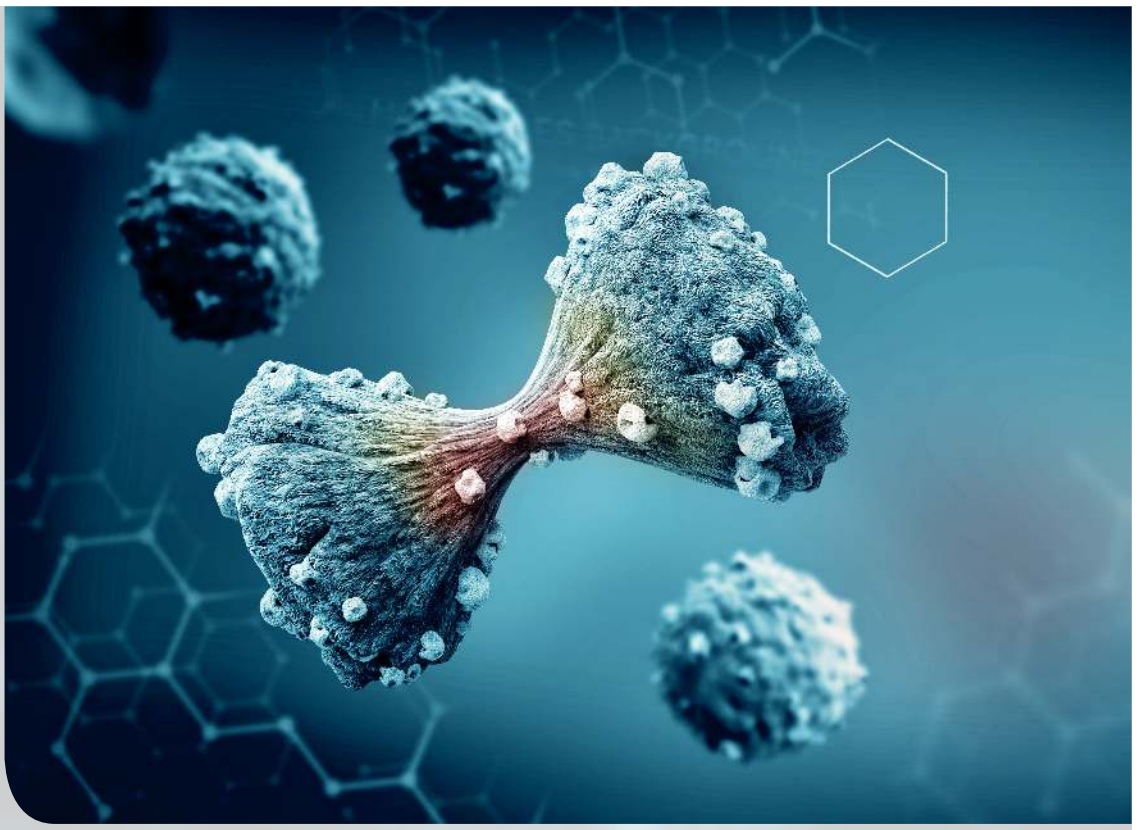
Examine accuracy at the amino acid level

Local confidence scores are assigned for each amino acid. You can easily separate confident amino acid assignments from false positives.

Find novel peptides not recorded in protein database

De novo results from scans missed in protein databases are summarized in ëde novo onlyí results. Partial protein matches or *de novo* tags are also given and can be viewed directly.

PEAKS® enhances the separation of true/false hits by integrating *de novo* sequencing into a database search workflow. This unique approach identifies more peptides and proteins with increased confidence.

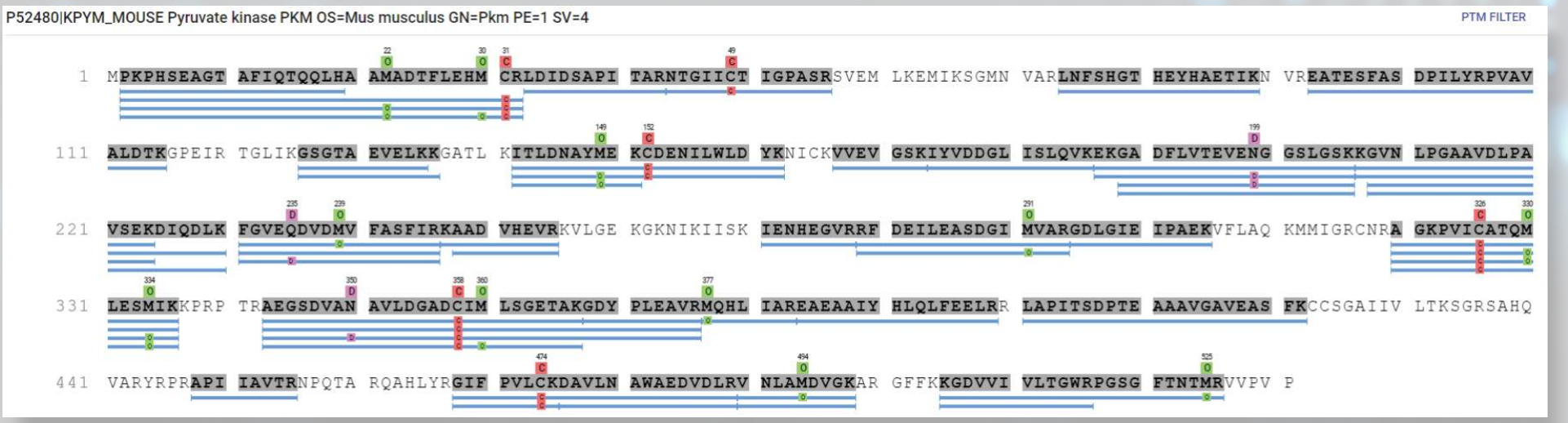


Protein coverage heatmap for quick and easy comparison across multiple samples

Easily compare between multiple samples in a project using the PEAKS® Online protein coverage heatmap. The increasing colour intensity indicates a higher abundance of supporting spectra within the corresponding sample.

No need to sacrifice details when analyzing large datasets

Interested in the protein coverage in a particular sample? PEAKS® Online allows users to select an individual sample to view the detailed coverage information, just as in PEAKS® Studio.



Quantification provides greater insight into proteomic mysteries. Researchers need a software tool to support them as they press further in to the understanding of life sciences.

PEAKS® is equipped with not only a powerful identification algorithm, but also embraces paralleled quantification capabilities to perform:

Label-free quantification:

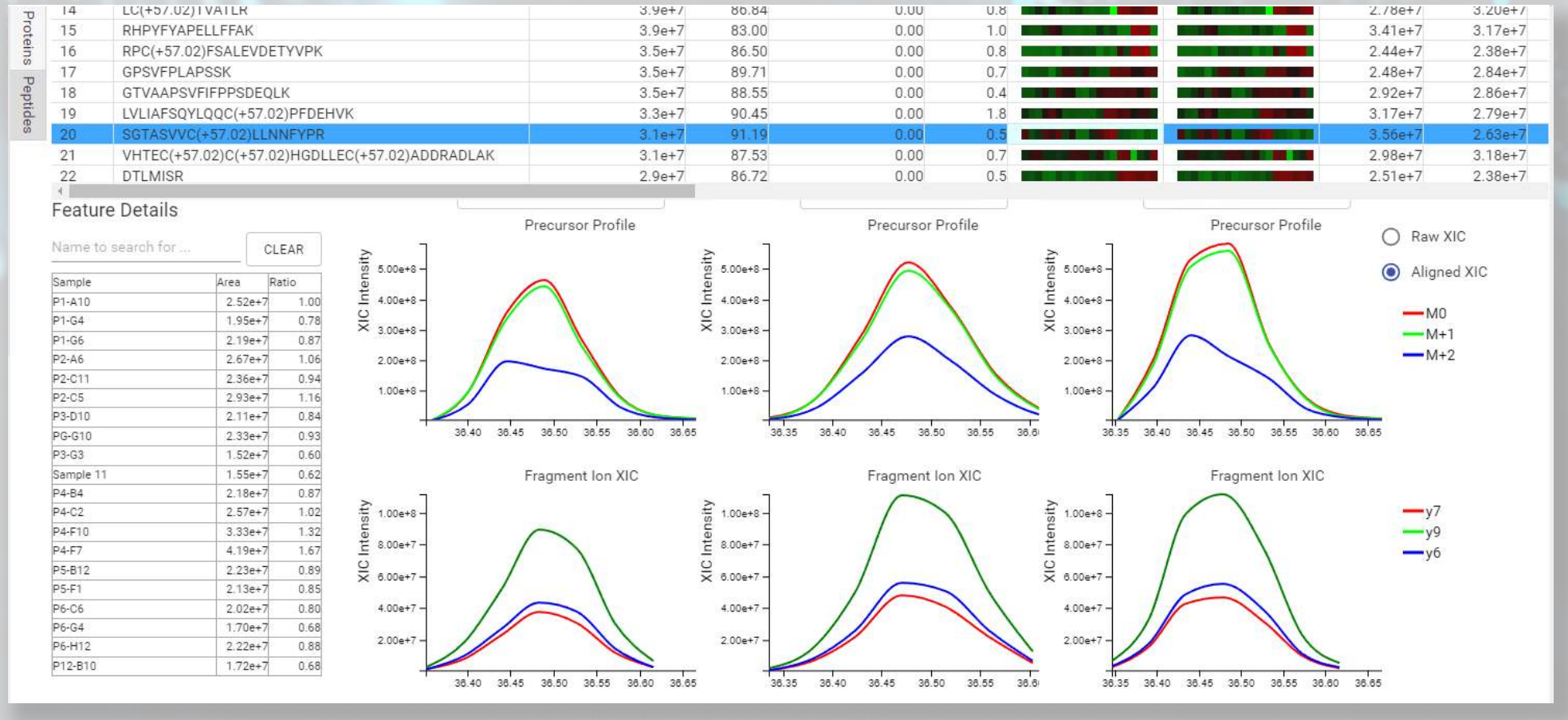
The ability to quantify the levels of proteins present in the samples by label-free quantification (LFQ) offers an efficient, cost-effective workflow to further understand the biological significance. PEAKS Qís LFQ function provides researchers with the option to calculate protein abundance either by using the well-known Top-3 peptides method or by using all unique supporting peptides. Researchers can then thoroughly investigate differences in peptide/protein abundance between samples while confident in high-level, accurate results.

Precursor ion quantification:

Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC) is a powerful and popular approach for mass spectrometry (MS)-based quantitative proteomics. PEAKS Qís SILAC quantification enables unsurpassed sensitivity of peptide feature detection through a novel peptide feature detection algorithm to find peptide feature pairs.Researchers can take advantage of the intuitive interface showing paired features at first glance and minimize the biases from missing values.

Reporter ion quantification:

Isobaric tags (ex. TMT/iTRAQ) have identical masses and chemical properties that allow heavy and light isotopologues to co-elute. The tags are then cleaved from the peptide by collision-induced dissociation during MS/MS, which is used for quantification. For large-scale, protein quantification studies, researchers can use PEAKS Q to expand the sample size with reference channels to enhance the accuracy of quantification.



NEW PEAKS® Online

Deep learning technology

The brand new DIA workflow and DeepNovo Peptidome Analysis use deep learning technology to improve identification accuracy and sensitivity by using algorithms to predict retention time, fragment ion intensity, mass-to-charge ratios and ion mobility.

Distributed System for High performance Computing

PEAKS® Online uses the latest distributed computing technology to achieve high-throughput performance with established PEAKS® workflows for multiple users on a network. Accelerate your discovery proteomics research.

Assess essential attributes of the raw data & results

The new automated Quality Control (QC) analysis tool is designed for both, DDA and DIA data and will supply the elements to determine the quality of the data and evaluates the setup of the experiment.

Bioinformatics Solutions, Inc.
140 Columbia St. W., Suite 202
Waterloo, Ontario N2L 3K8
Canada
Tel: (519) 885-8288
Fax: (519) 885-9075
sales@bioinfor.com
www.bioinfor.com