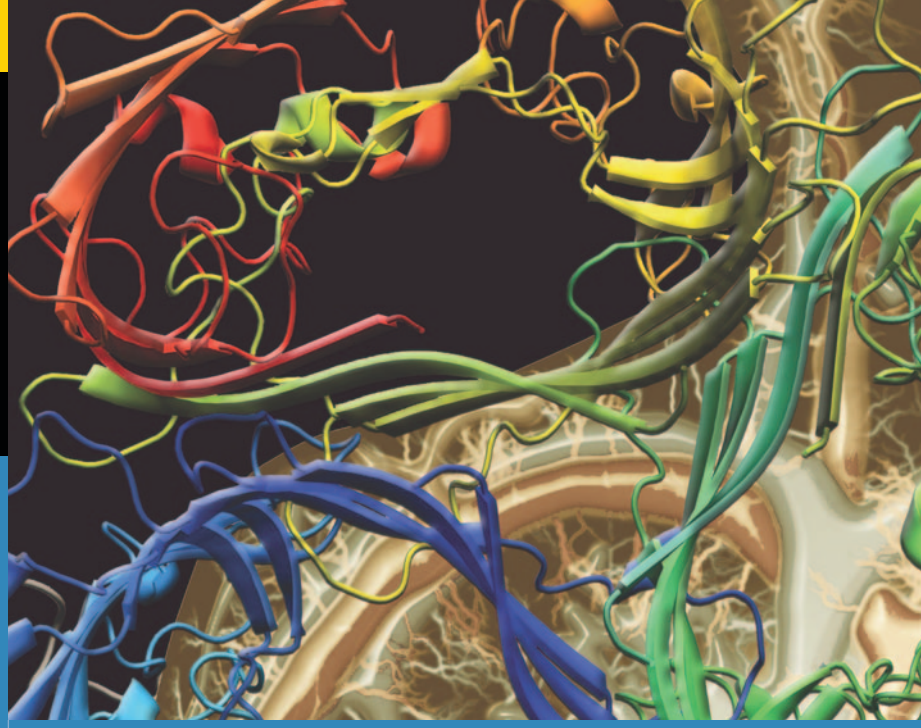
MASS SPECTROMETRY

Thermo Scientific SIEVE Software for Differential Expression Analysis



Automated, label-free, semi-quantitative analysis of proteins, peptides, and metabolites based on comparisons of LC/MS and GC/MS data

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Differential expression analysis for proteomics and metabolomics

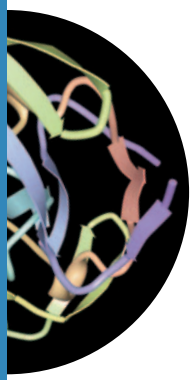
Discovery of protein and metabolite biomarkers is an important step in understanding metabolic pathways and disease mechanisms, and in identifying potential drug and diagnostic targets. Label-free differential expression analysis has quickly proved useful as a fast, cost-efficient, and effective way to find putative biomarkers.

Thermo Scientific SIEVE software provides an easy-to-use automated solution for evaluating the large amounts of LC/MS and GC/MS data generated by label-free sample analyses. It effectively locates compounds with statistically significant abundance differences between sample populations. In addition to standard two-population differential analyses, SIEVE™ software can perform trend analyses to locate compounds that vary with dosage, time, or other trend classifications. SIEVE is a statistically-rigorous data mining tool that has the power to compare hundreds of LC/MS data files at a time, but can also perform a simple comparison of two sample files.

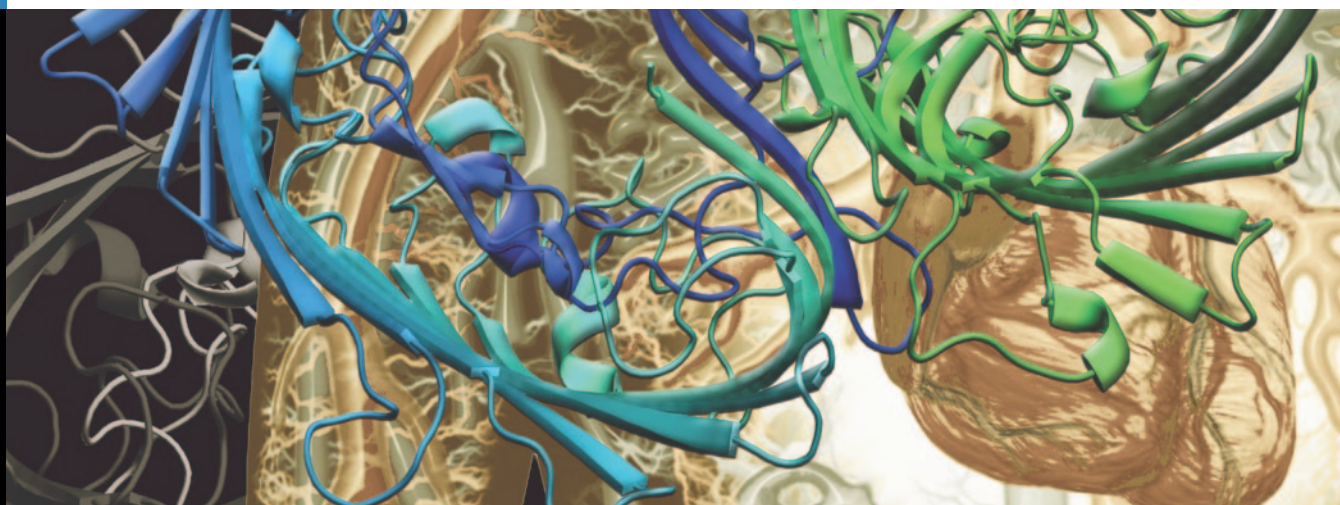
SIEVE software helps identify putative biomarkers rapidly. It pre-filters complex data, greatly reducing the number of compounds that need to be evaluated and significantly decreasing time spent on identification. SEQUEST®, one of the most cited protein search algorithms, searches peptides against public or private databases to identify proteins. And the compound-rich, public-domain ChemSpider™ database search application identifies metabolites in user-selected databases.

Combining SIEVE software with the unique analytical power of Thermo Scientific LC/MS and GC/MS instruments such as the LTQ™ family, LTQ Orbitrap™ family, and Exactive™ systems creates complete solutions for proteomics and metabolomics applications.

SIEVE software...



- Provides the Experiment Definition Wizard which makes it easy to define and automate experiments for both control-versus-treatment and trend analyses
- Uses a new chromatographic alignment algorithm that minimizes the effects of chromatographic variability, thereby making differential analysis more reliable
- Processes label-free data so there is no risk of added variability or limits to trend analyses due to sample labeling
- Uses MS intensities from raw data to find differences without modeling or manipulating peaks, so valuable data is not lost
- Quickly identifies compounds using proven search engines: SEQUEST and ChemSpider
- Supplies results in database format for seamless export and interoperability with applications such as Ingenuity Pathways Analysis (IPA) software that link SIEVE results to known biological pathways



SIEVE software workflow

From experiment definition to differential analysis and identification, SIEVE software provides an easy-to-use, automated workflow.

STEP ONE

Experiment definition

SIEVE software's Experiment Definition Wizard is unlike any other. It uses an intuitive multistep process that makes it remarkably easy to build analyses. It guides you to choose the experiment type: Proteomic or Small Molecule, and Control versus Treatment or Control Compare Trend; the data files to process; the parameters used to perform the differential analysis; and the identification method. It simplifies analyses by eliminating choices not relevant to your experiment. For example, if you define a proteomics experiment, the Experiment Definition Wizard will automatically enable protein identification using SEQUEST.

The Experiment Definition Wizard makes it easy to build differential analysis experiments

STEP TWO

Align chromatograms

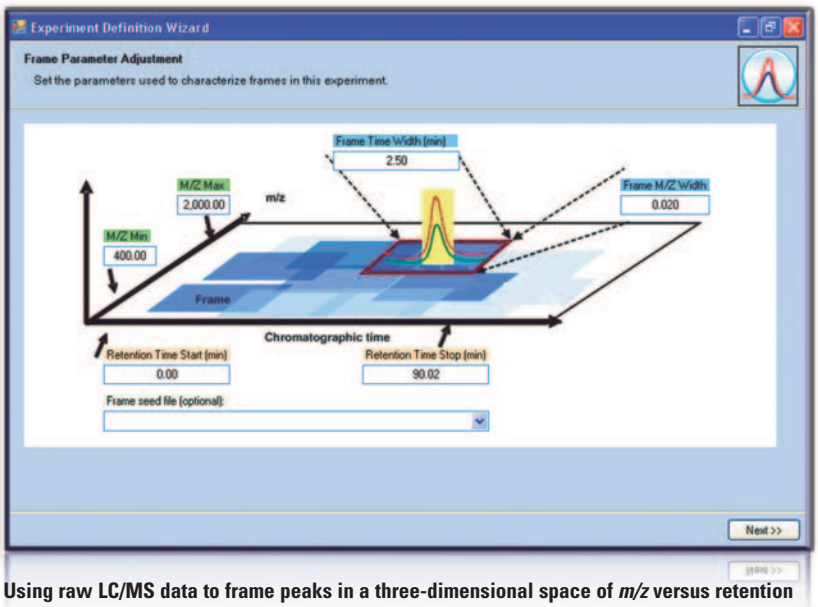
To correct for inherent chromatographic variability that would otherwise make differential analyses unreliable, SIEVE software uses an innovative chromatographic alignment algorithm. Using full-scan data from each data file, it automatically calculates optimal correlations between spectra and then creates a matrix of spectra-to-spectra correlations that are used to align chromatograms. SIEVE software's graphical user interface makes it easy to assess and adjust the alignment. Because it is memory and CPU efficient, the alignment algorithm can process very large sample files in parallel. And because it uses full-scan data, spiked landmarks are not required to correctly align chromatograms.

Chromatographic elution times are the largest source of variability in differential analysis experiments. SIEVE software's proprietary chromatographic alignment algorithm compensates for this inherent variability, making results more reliable than ever before.

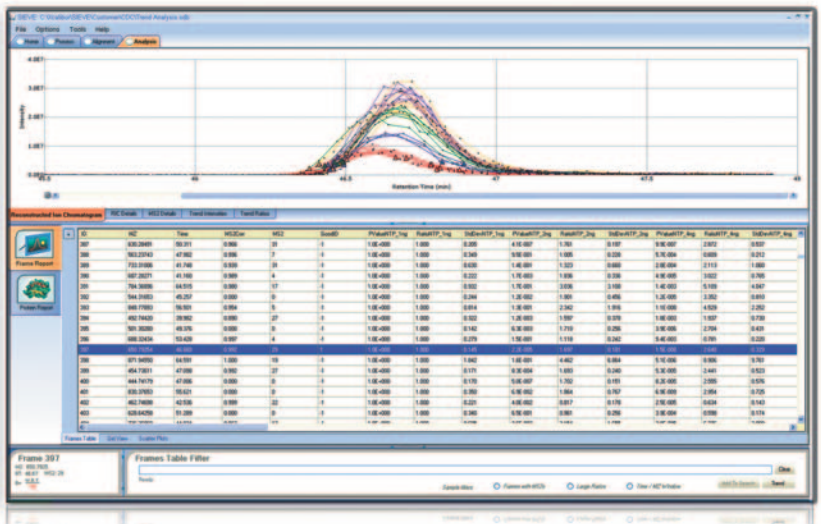
Automatically find differences

SIEVE software uses MS intensities from raw LC/MS or GC/MS data to find abundance differences without the need to manipulate or "model" peaks, a process which arbitrarily simplifies or discards potentially valuable data. Instead, SIEVE software uses a proprietary process – Recursive Base-Peak Framing – to generate a unique "frame" for each group of peaks within a specified *m/z* and retention time range. It collects all peaks above a given threshold from all raw data so no information is lost. Using raw data provides the greatest confidence in qualitative and quantitative results. Other peak-finding approaches are inherently error-prone and interfere with accurate quantitation when applied to the complex data encountered in differential analyses.

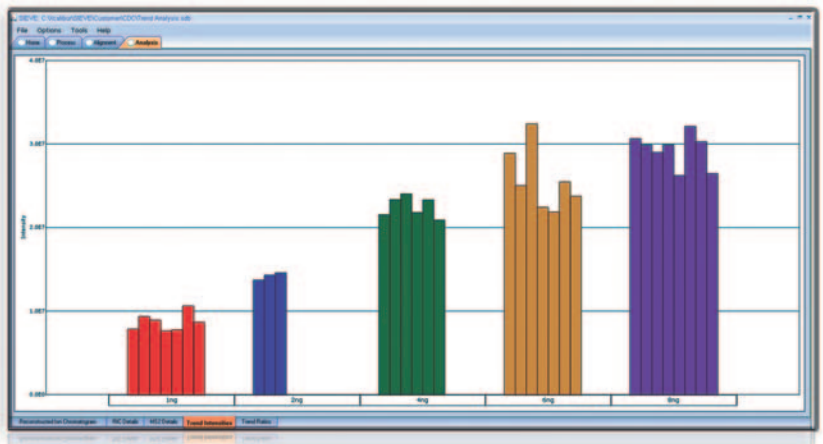
Frame-by-frame, the algorithm determines if there is a statistically significant abundance difference between the control and treated samples. The Frames Report displays key information such as reconstructed ion chromatograms, retention times and *m/z* values in an interactive interface that makes it easy to assess frames. Advanced users have the ability to interrogate the data in much more detail and can assess how the mass spectral data is interpreted. The Frames Report also supplies useful information about the quality of the results: the p-value, ratio, standard deviation for the ratio, number of MS^2 scans, MS/MS correlation, ratio and total ion chromatogram (TIC) normalized ratio. For trend analyses, bar charts displaying changes in intensity versus treatment make trend differences easy to find.



Using raw LC/MS data to frame peaks in a three-dimensional space of *m/z* versus retention time versus peak intensity provides more confidence in results compared to peak-modeling approaches.



The Frames Report makes it easy to review differential analysis results.

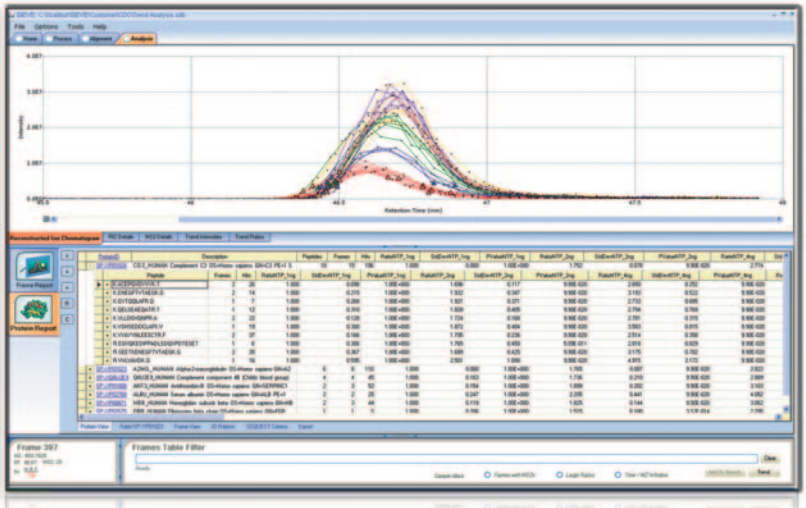


Bar charts showing changes in intensity versus treatment make trend differences easy to observe.

Identify compounds of interest

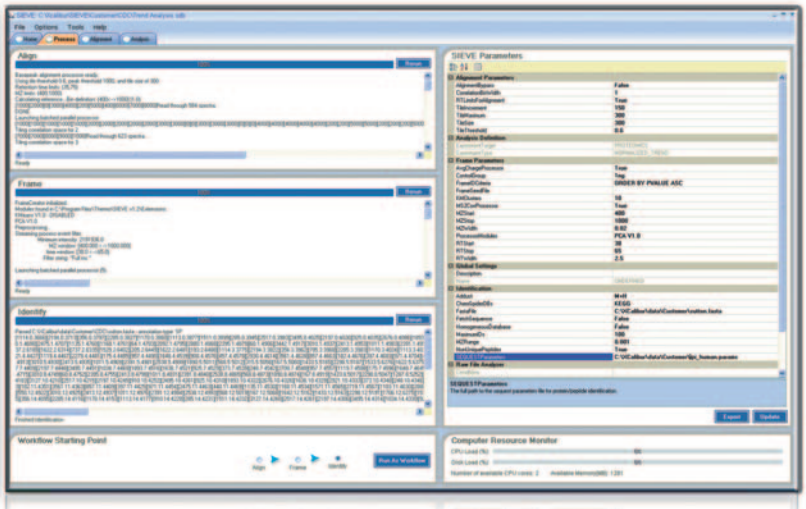
Compounds of interest can be identified using one of two proven tools seamlessly linked within the SIEVE software: SEQUEST and ChemSpider.

Following a SEQUEST search, the SIEVE software generates a Protein Report, a hierarchical representation of the proteins, peptides and SEQUEST identification results. It consolidates qualitative and quantitative results for easy exploration and validation of protein identifications. Protein and peptide ratios and confidence limits are easy to check using Peptide Whisker Plots. "Mouse overs" provide detailed protein and peptide information.

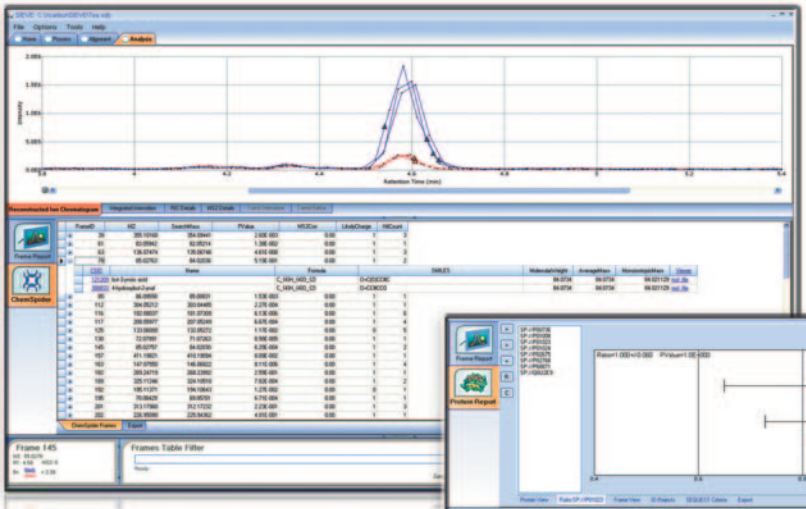


The Protein Report consolidates qualitative and quantitative results and displays them in an easy-to-navigate, hierarchical display.

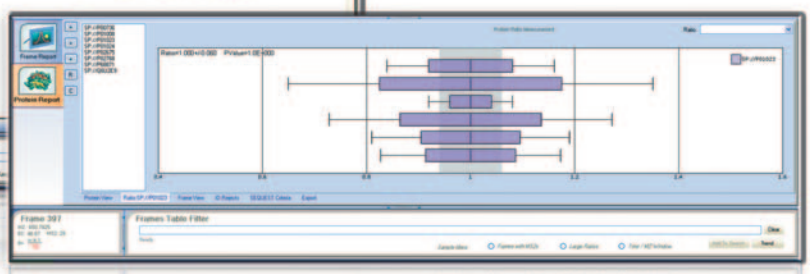
ChemSpider is a public-domain small-molecule database search application that identifies metabolites in user-selected databases using nominal-mass or accurate-mass information. It is the richest single source of structure-based chemistry information available, providing users with access to millions of chemical structures.



SIEVE software is flexible; it can be fully automated for unattended analyses or can perform iterative workflows in which parameters can be adjusted as desired.



The ChemSpider Report makes it easy to review metabolites identified by ChemSpider, a popular public-domain, small-molecule database search application.



Whisker plots of individual peptide measurements provide a quick visual check of the quality of the differential analysis.

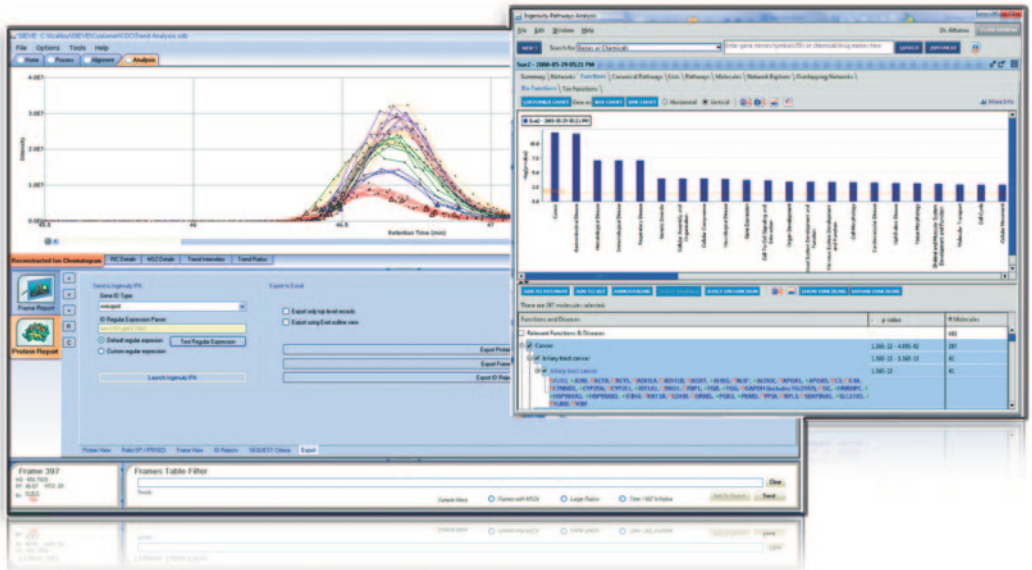
Automated or interactive workflows

SIEVE software can be completely automated and run entire workflows, including chromatographic alignment, finding abundance differences via framing, and identification, without manual intervention.

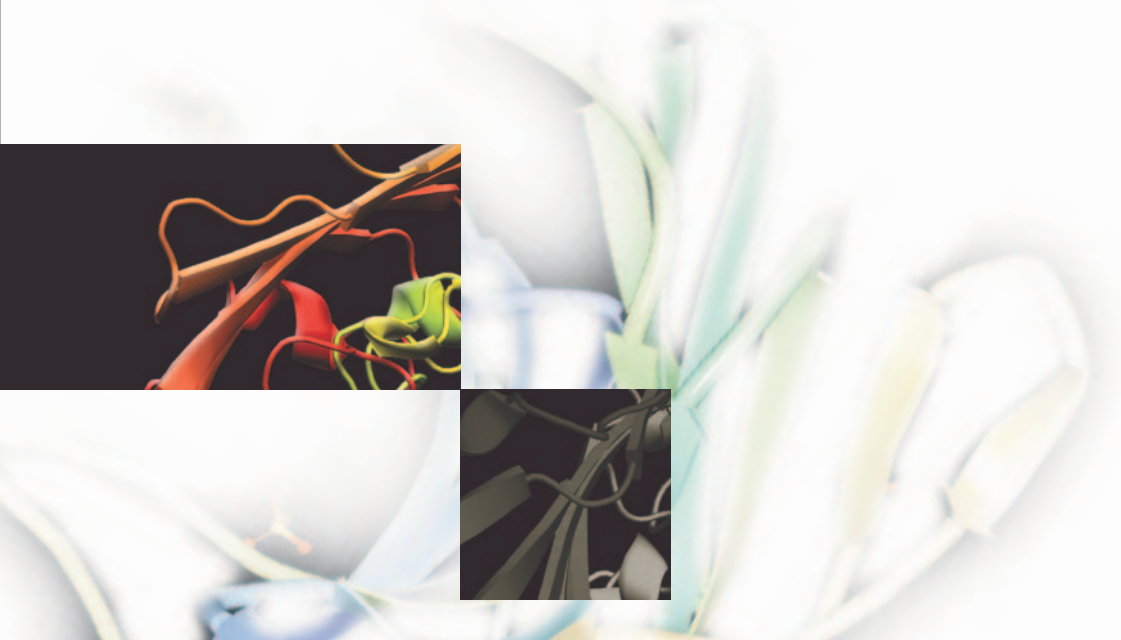
SIEVE software also supports time-saving interactive workflows. As each part of an experiment is completed, the user can review results and adjust parameters in real time. The workflow can be started and stopped at any step without having to go back and redo previous steps. For example, users can rerun identifications using a different database, without having to go back and realign chromatograms or reframe data.

SIEVE software integration and interoperability

Seamless integration and interoperability with spreadsheet, statistical, and systems biology applications help you present results, perform further statistical analyses, and connect compound identifications to biological pathways. For example, SIEVE software can be integrated with Ingenuity Pathways Analysis (IPA), a widely-adopted application that enables researchers to model, analyze and understand complex biological and chemical systems. SIEVE software can also be integrated with Thermo Scientific Pinpoint software, a selected-reaction monitoring (SRM) builder for assay development, and with Thermo Scientific Mass Frontier software for small-molecule spectral interpretation.



Exporting results to Ingenuity Pathways Analysis (IPA) connects peptide assignments with the systems biology underlying the experiments. It enables users to model, analyze and understand complex biological and chemical systems.



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