

Thermo Scientific Pinpoint Software (beta)

Frequently Asked Questions

23-April-2009

The following questions and answers may prove useful to users of the Pinpoint software. If you have additional questions, would like additional information about the topics below, or would like to provide input about the Pinpoint software, please contact the Pinpoint support team by clicking the *Contact the Pinpoint team* link on the Pinpoint software Web page or emailing pinpoint@thermofisher.com.

Q: How many peptides per protein must be identified to determine whether the protein is up- or down-regulated?

A: There is no absolute answer. In general, the more peptides identified, the greater the confidence in the expression ratios found. Carr et al. have published a paper suggesting 3 peptides per protein. Ideally, you would select peptides to cover the greatest possible portion of the total sequence.

Q: Why use product ion rank correlation coefficients and probability value scoring?

A: Product ion rank correlation provides the best metric of similarity for matching previously acquired ion trap CID spectra with SRM triple quadrupole spectra. While ion trap and triple quadrupole instruments use different fragmentation and detection technologies, their respective product ion ranks should still be similar since the activation energy applied is quite similar in the two instruments. The algorithm used in Pinpoint correlates product ion rank and assigns greater weight to high-abundance ions. Previous experiments have shown that at least five product ions are necessary to yield meaningful coefficients and probability values.

A key variable is the Q2 collision energy setting in the triple quadrupole mass spectrometer. With larger peptides, if the collision energy is set too high it can result in secondary fragmentation leading to artificially high ranking of y3, y4, and/or y5 product ions and skewed scores. A standard peptide set covering a wide mass range and sequence is being developed to aid in proper adjustment as well as comparison and correlation of different instruments.

Q: Do the product ion rank correlation coefficient and p-values eliminate some matches?

A: Yes, the purpose of determining both p-values and rank correlation coefficients is to eliminate matches that are likely the result of background noise or coeluting isobaric peptides. Assessing the p-value adds confidence that rank correlation coefficient is the result of measuring the target peptide and not a random hit. This is especially important for low-level proteotypic peptides.

Q: Are scheduled (timed) SRM methods supported?

A: Yes. Timed SRM methods are supported by TSQ 2.0 instrument drivers. TSQ 2.0 instrument drivers can be loaded on TSQ Access, Ultra, and Vantage triple quadrupole mass spectrometers. The software supports building timed SRM methods from previously acquired data.

Q: How many spectral libraries can be incorporated into the software and what sources of libraries (file types) are supported?

A: You can load an unlimited number of spectral libraries, but the more spectral libraries you load the longer it will take to process data. Currently supported sources of libraries include:

- Bioworks software (SRF files)
- Proteome Discoverer software
- SIEVE software
- Mascot software
- Bibliospec software (MacCoss lab see University of Washington in Seattle)
- PeptideAtlas
- MRMAAtlas
- CPAS

Q: Will spectral libraries be extended to X! Tandem?

A: Discussions are underway regarding creating interoperability between Pinpoint software and the Global Proteome Machine Organization (GPM) programs/libraries such as X! Tandem. Discussions are also underway regarding establishing interoperability between Pinpoint software and NIST programs/libraries.

Q: How reproducible is fragmentation in triple quadrupole instruments?

A: Initial experiments on multiple TSQ instruments show that peptides tend to have a relatively flat dissociation voltage, yielding consistent product ion ratios. The equation used to predict the dissociation (collision) energy for peptides is extremely reliable (collision energy = parent m/z * 0.035). A standard peptide set covering a wide mass range and sequence is being developed to aid in comparison and correlation of different instruments.

Q: What is the maximum number of transitions that can be use on a TSQ Vantage triple quadrupole mass spectrometer?

A: The answer depends on the degree of scheduling. The narrower the time window around each set of SRM transitions used per peptide, the more SRM transitions can be used. The current maximum number of SRM transitions that can be used in an experiment is over 10,000.