QCorr Users' Manual

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QCorr is software to identify the molecular weight of a peptide ion directly from its corresponding tandem mass spectrum using a cross correlation function. This approach has proven successful for a wide variety of spectra of differing charge states and overall quality.

1 Introduction

Tandem mass spectra are typically acquired either from known precursor ions or after an exploratory MS scan using a relatively small (i.e., 1-5 *m/z*) isolation window. This approach provides a high degree of flexibility (i.e., data-dependent acquisition) as well as precursor molecular weight and charge state information. Depending on the type of mass spectrometer employed, high resolution and accurate mass measurements can also be obtained that can be useful for identification and quantification purposes. In accordance with this strategy, most database search algorithms utilized for peptide identification from tandem mass spectra [1-3] require the molecular weight of the precursor ion be provided as well as the mass accuracy of the measurement. This information helps to restrict the search to only those potential peptides within the mass range of interest.

However, it is often difficult to accurately measure the molecular weight of the precursor ion especially using the relatively low resolution approaches that are most

common in high throughput proteomic analyses where isotopic peaks with charge >2 are difficult to resolve. In addition, good quality tandem mass spectra can often be acquired and identified from signals that are barely detectable in normal MS mode operation where the MS mode typically suffers from chemical noise. An alternative to directly measuring and determining peptide molecular weights has been explored by researchers developing *de novo* sequencing approaches [4]. Along these lines, we have developed an approach to identify the molecular weight of a peptide ion directly from its corresponding tandem mass spectrum using a cross correlation algorithm.

2 Installation and Execution

To install QCorr, download the latest release of the program (the URL for doing should be in an email from The Scripps Research Institute). The Zip file should include a .jar file (QCorr.jar), a test ms2 file (test.ms2), and a file titled "QCorr.params". QCorr is written in the java programming language and requires java to be installed on the machine where it will be run.

For windows installations: extract the Zip into the directory C:\QCorr. Copy QCorr.bat to the C:\Winnt or C:\Windows directory. This batch file will let you start QCorr from whatever directory you might be in because the directory where Windows is installed is almost always on the operating system path.

To set up for a run of QCorr, you should collect the following into a directory on your machine:

• Spectra

Your spectra should be in the MS2 file format. See

http://fields.scripps.edu/sequest/unified/MS2Format.html

• QCorr.params

This configuration file determines the options used by QCorr. For more information on this file, see section 3.

Next, open a command-line window (sometimes called MS-DOS Prompt and sometimes called Command Prompt). Switch to the drive and directory in which QCorr is to run. Run the program with this invocation:

QCorr ms2_file_goes_here

Or

QCorr *.ms2 (to run on all ms2's in the directory)

The software should start processing spectra. It's worth noting at this point that the performance of QCorr depends on the parameters chosen in the QCorr.params. Recommended settings are in section 3.

The program will start with the first spectrum in the MS2 file and process each spectrum in the file in turn. As the software completes its analysis of each spectrum, it appends the calculated molecular weight along with the cross correlation score to a results file (original-filename_Precursors.txt). It reports the number of spectra processed so far and then continues to the next.

3 Configuration

The QCorr.params file is used to configure QCorr. The first part of QCorr.params configures the way in which QCorr functions. These options include:

• InjWindow: Size of the isolation window used in MS/MS data collection. We typically add 1 to the actual value to account for peptides isolated near the edge of the isolation window.

• MassAccuracy: Mass accuracy to be used in the cross correlation calculation. The typical value for ion trap MSMS spectra is 0.1 and for Orbitrap MS/MS spectra is 0.01. Note that decreasing this value increases run time.

• Normalize: Whether or not to normalize the intensities of the MS/MS spectrum? Default = false.

• FilterPeaks: Boolean to use only the top X, where X is defined in the "PeakThreshold" field, number of fragment ion peaks (ranked by intensity)? Default = false.

• PeakThreshold: Threshold that defines the number of peaks to be used in the cross correlation. Default = 200.

The second part of QCorr.params configures the way in which QCorr outputs the results. These options include:

• OutputFile: Boolean that determines if a results file with the name originalfile_Precusors.txt is produced.

• OutputCCArray: Creates a file that shows the cross correlation scores as a function of calculated MW for each spectrum.

(1) Eng, J. K.; McCormack, A. L.; Yates, J. R., III *Journal of the American Society for Mass Spectrometry* **1994**, *5*, 976-989.

(2) Perkins, D. N.; Pappin, D. J. C.; Creasy, D. M.; Cottrell, J. S. *Electrophoresis* **1999**, *20*, 3551-3567.

(3) Sadygov, R. G.; John R. Yates, I. *Analytical Chemistry* **2003**, *75*, 3792-3798.

(4) Dancik, V.; Addona, T. A.; Clauser, K. R.; Vath, J. E.; Pezner, P. A. *Journal of Computational Biology* **1999**, *6*, 327-342.