Pepitome: evaluating improved spectral library search for identification complementarity and quality assessment

Supplemental File 1

Search engine configurations

MyriMatch for LTQ

PrecursorMzTolerance= 1.25
FragmentMzTolerance = 0.5
DuplicateSpectra = true
UseChargeStateFromMS = false
NumChargeStates = 3
UseSmartPlusThreeModel = true
TicCutoffPercentage = 0.98
DigestionRules = "trypsin"
NumMinTerminiCleavages = 1
UseAvgMassOfSequences = true
MinCandidateLength = 5
DynamicMods = "M ^ 15.9949 (Q * -17.026 ($ 42.015 C @ 57.021"
MaxDynamicMods = 3

MyriMatch for LTQ-Orbitrap (same as "MyriMatch for LTQ" with the following parameters changed)

PrecursorMzTolerance= 10
PrecursorMzToleranceUnits = ppm
AdjustPrecursorMass = true
MinPrecursorAdjustment = -1.008665
MaxPrecursorAdjustment = 1.008665
PrecursorAdjustmentStep = 1.008665
NumSearchBestAdjustments = 3
UseChargeStateFromMS = true
NumChargeStates = 4
UseAvgMassOfSequences = false

MyriMatch for Q-TOF (same as "MyriMatch for LTQ-Orbitrap" with the following parameters changed)

PrecursorMzTolerance= 20
PrecursorMzToleranceUnits = ppm
FragmentMzTolerance = 75
FragmentMzToleranceUnits = ppm

Pepitome for LTQ

PrecursorMzToleranceRule = "avg"
MonoPrecursorMzTolerance = "10 ppm"
AvgPrecursorMzTolerance = "1.5 mz"
FragmentMzTolerance = "0.5 mz"
SpectrumListFilters = "peakPicking true 2-;chargeStatePredictor false 3 2 0.9"
RecalculateLibPepMasses = false
CleanLibSpectra = true
LibTicCuttoffPercentage = 0.98f
LibMaxPeakCount = 100
CleavageRules = "trypsin"
MaxMissedCleavages = 2
MinTerminiCleavages = 1
MinPeptideLength = 5
MaxResultRank = 2
FASTARefreshResults = false

Pepitome for LTQ-Orbitrap (same as "Pepitome LTQ" with the following parameters changed)

PrecursorMzToleranceRule = "auto"
SpectrumListFilters = "peakPicking true 2-;chargeStatePredictor false 4 2 0.9"
RecalculateLibPepMasses = true
MonoisotopeAdjustmentSet = "[-1,2]"

Pepitome for Q-TOF (same as "Pepitome LTQ-Orbitrap" with the following parameters changed)

MonoPrecursorMzTolerance = "0.1 mz"
FragmentMzTolerance = "0.1 mz"
RecalculateLibPepMasses = false

SpectraST parameters for LTQ

indexRetrievalMzTolerance = 1.25
indexRetrievalUseAverage = true
expectedCysteineMod = CAM
outputExtension = pep.xml
ignoreSpectraWithUnmodCysteine = false
ignoreChargeOneLibSpectra = true
ignoreAbnormalSpectra = false
hitListTopHitFvalThreshold = 0.0
hitListLowerHitsFvalThreshold = 0.45
hitListShowHomologs = true
hitListOnlyTopHit = false
filterMinPeakCount = 10
filterMaxPeaksUsed = 150

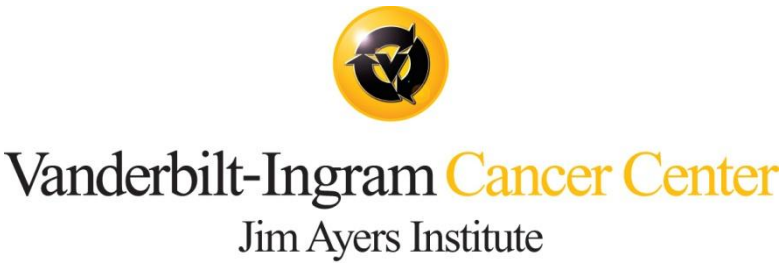
SpectraST parameters for LTQ-Orbitrap (same as "SpectraST LTQ" with the following parameters changed)

indexRetrievalMzTolerance = 0.007
indexRetrievalUseAverage = false

SpectraST parameters for QTOF (same as "SpectraST LTQ-Orbitrap" with the following parameters changed)

indexRetrievalMzTolerance = 0.1

Supplemental File 2



Standard Operating Procedure

Title: Instrument BSA Acceptance Checks

SOP Number: 3xx

Revision: 0

Date: July 17, 2008

Prepared By: Kent Shaddox

1.0 Overview

1.1 This SOP is written to describe the steps taken to ensure that the BSA solution analyzed meets acceptable criteria for coverage and/or mass axis shift.

1.2 The following features are checked for ensuring data quality: clean chromatography, consistent base peak chromatogram, presence of contaminants, high BSA coverage, and high mass accuracy of selected BSA peptides (for LTQ-Orbitrap instruments). Failure to produce BSA runs confirming to the protocol triggers instrument maintenance.

2.0 Reagents and Materials

2.1 BSA Solution - 2mg/mL in a 0.9% aqueous NaCl solution containing sodium azide; Pierce Protein Research Products – Cat #23209.

1x and 10x solutions of BSA are prepared and analyzed as follows:

2.1.1 To 100µL BSA stock solution (2mg/ml), add 660µL of 100mM Ammonium Bicarbonate.

2.1.2 Add 100µL of 45mM DTT resuspended in 1mL 100mM Ammonium Bicarbonate (reduction) and incubate at 50°C for 20 minutes.

2.1.3 Add 100µL of 100mM IAM resuspended in 1mL 100mM Ammonium Bicarbonate (alkylation) and place in the dark at room temperature for 20 minutes.

2.1.4 Add 40µL trypsin (0.1µg/µL) and incubate overnight at 37°C.

2.1.5 To 30µL of this solution add 970µL 0.1% formic acid. This makes a 6.26µg/mL solution of BSA, referred to as 10x BSA.

2.1.6 Dilute 20uL of 10x BSA (section 4.1.5) with 180uL 0.1% formic acid for a final concentration of 0.626µg/mL solution of BSA, referred to as 1x BSA

3.0 Apparatus

3.1 Thermo LTQ and LTQ-XL Mass Spectrometer

3.2 Thermo LTQ-Orbitrap Mass Spectrometer

3.3 Eksigent nano LC 1D and 1D-Plus

4.0 Procedure

4.1 BSA solution is analyzed prior to any sample set and after every ten samples. In addition to checking the percent coverage and/or mass axis shift, the chromatography of the BSA solution should be checked to ensure the instrument performance is acceptable.

4.2 After instrument acquisition is complete, select the appropriate raw data file for the BSA standard in the Xcalibur\Data\current directory on the instrument. Copy this file and paste it into the MSRC-BSA1\lcq folder.

4.3 The raw data file needs to be converted to from a raw file to a data file by running the "Create DTA" program. Open "Create DTA" and enter the Sample ID, which identifies the BSA solution run (1x or 10x), the instrument it was analyzed on, the month it was analyzed and the last four number of the raw data file (i.e. 10xBSAOrbi2JULY1234).

4.4 Enter the analyst initials and Sample Run Name (same as Sample ID), then select the correct raw file from the drop down menu. Enter operator's initials, "Create DTA" and wait for processing to finish.

4.5 When finished, the program will ask what you want to do next. From the drop down menu, select Home" and "Go", which will bring up the Proteomics Browser Suite.

4.6 Select "Turbo Sequest", be sure the correct data file is pulled up in the directory, enter operator initials and "Run Sequest".

4.7 When finished, the program will ask what you want to do now. Select "View Status" and "Go". During this step, the refresh icon can be used to check the status of Sequest. When the file has been processed, it will disappear from the Sequest query list.

4.8 Next, go to CHIPS website and enter your user name and password. An instrument list appears on the screen. Select the instrument and press "edit". The files will be grouped by month, so select the appropriate month and press "edit". Scroll down to the bottom of the page and choose "Add Runs" from multiple Sequest directories. The Sequest directories pop up. Choose the desired file. Note: More than one file can be chosen for processing at this point.

4.9 Once the files have been selected, select the filter to be used for processing the file. Select "Amy's 1-3 extra" from the menu and click "Add with filter". When this step is complete, it will say that it has successfully added the directory and show the file name(s). Select "Continue".

4.10 To check the percent BSA coverage, scroll down to find the appropriate data file and look for the result for "Albumin (Bos Taurus)" in the list.

4.10.1 For the LTQ and LTQ-XL instruments, the 1x BSA is checked for percent coverage. Percent coverage should be around 60%. If the percent coverage is less than 55%, instrument maintenance should be performed and the BSA rerun to ensure percent coverage is acceptable. Response on the 1x BSA should be around 1E6 and around 1E7 on the 10x BSA.

4.10.2 For the Orbitrap instruments, the 10x BSA is checked for percent coverage. Percent coverage should be between 60-70% at the beginning of a sample set. The percent coverage and chromatography should be monitored throughout the run. If either is unacceptable, perform instrument maintenance. Response on the 10x BSA should be around 1E8.

4.11 To check for mass axis shift on the Orbitrap instruments, open up the chromatogram and click on the thumbnail in the lower right window. This allows the user to highlight the area of the chromatogram of interest, which elutes from approximately 20 to 60 minutes.

4.12 Next, click on the thumbnail in the lower left hand window. From the toolbar at the top of the screen, select "Display", then "Mass options" and enter 5 for the number of decimals in the set mass precision section.

4.13 Open the Excel spreadsheet used to calculate the mass axis shift called "Daily ppm check list". Enter the name of the file being checked. Six masses are checked for mass axis shift:

Monoisotopic Mass	z	Peptide Sequence
473.902742	3	SLHTLFGDELCK
547.317432	3	KVPQVSTPTLVEVSR
582.31897	2	LVNELTEFAK
653.3617	2	HLVDEPQNLIK
722.324655	2	YICDNQDTISSK
820.472509	2	KVPQVSTPTLVEVSR

4.14 Enter the experimental mass found for each mass. Excel calculates the mass axis shift by dividing the difference of the monoisotropic mass and experimental mass by the experimental mass and multiplying by 1000000. Note: To zoom into a specific peak, integrate the area of the chromatogram under the peak. To return to the original chromatogram, use the "Zoom Reset" icon in the top toolbar.

4.14.1 At the beginning of a run sequence, values should be no larger than $\pm$2 ppm. Values should be monitored throughout the run (after every ten samples). The calculated mass axis shift may fluctuate during the run (i.e. to as high as $\pm$10 ppm), but as long as BSA percent coverage and mass axis shift are acceptable the sequence can keep running. Note: If the initial mass axis shift is greater than $\pm$2 ppm, recalibrate the instrument before any sample analysis.

4.15 Label the accepted BSA runs as "high quality" and rejected BSA runs as "low quality".

5.0 Additional Notes

5.1 Prepared BSA solution is generally good for about two weeks. New solution should be prepared sooner if degradation of the solution is evident.