NISTMSQC - Software for Monitoring LC-MS Performance

Mass Spectrometry Data Center
Chemical and Biochemical Reference Data Division
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United States Department of Commerce

under interagency agreement with the

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Clinical Proteomic Technologies for Cancer

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About: This document is intended to provide instructions on how to run the NISTMSQC software on your computer. The metrics have been described and benchmark values have been reported in two publications (19837981, 19858499). If you have questions about the metrics, please refer to these documents first.

For more background information, you may also wish to visit the following URLs:

http://www.nist.gov/cstl/chemical_properties/data/perfmetproteomics.cfm
http://proteomics.cancer.gov/

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Description:

Important Note on Data Formats: Use of this version is limited to analysis of data acquired on Thermo Scientific© ion trap and hybrid mass spectrometers (e.g., LTQ, LTQ-Orbitrap, FT-LTQ), generating MS/MS spectra by CID, and stored in .RAW format. Future releases may allow comparison of data files generated on mass spectrometers produced by other manufacturers provided sources of accessible data formats can be identified.

This program was written to systematically evaluate analytical performance of a common discovery-based proteomics platform by monitoring selected output from a liquid chromatography -mass spectrometry (LC-MS) system. The software was developed to help researchers identify sources of variations due to analytical problems. It is hypothesized that if analytical variations can be minimized using defined mixtures, variations due to biological differences in complex samples can be more confidently identified.

NISTMSQC expects that the MS/MS spectra are produced from analysis of a tryptic digest of a protein mixture. However, future releases will allow any set of MS/MS data files, such as those from analysis of metabolites or other small molecules and can be identified by a spectral library search, to be used. Typically, data files might come from routine analysis of a QC standard over time but may be from different instruments in the same or different laboratories. Analysis is carried out by calculating and comparing a defined set of data metrics from one or more set of MS data files. The use of this program does not require any new data acquisition; it has been designed to be run post-data acquisition and is therefore suitable for examining older data files for historical purposes. However, best practice for this software would be iterations of controlled runs of a sample, followed data analysis, and correction of any problems identified by fluctuations in key metrics.

This software contains many component software applications which are controlled by a single Perl program. The software is intended to be run as a "pipeline." That is, processing starts from RAW mass spectrometry data files and is passed through many programs, where output from the previous application is required by the next. It is therefore important that the program be allowed to run in its entirety. Progress of the pipeline is reported by the software and a reasonable attempt has been made to catch common errors and provide suitable remedies.

System Requirements:

Microsoft© Windows™
XCalibur™ (preferably the same version used on the data acquisition computer)
Perl (as can be freely downloaded from http://www.activestate.com/activeperl/)

Spectral Libraries and Sequence Databases

One or more search library/database packs. These should be relevant to the sample you are using. For example, if your QC sample is S. cerevesiae (i.e., yeast), you will need to extract the yeast.zip archive in the 'libs' directory of your installation. Library packs for some common laboratory samples can be downloaded from here,

ftp://chemdata.nist.gov/download/paul/collab/NISTMSQC/libs/

Quick Start:

1) This software is intended to be run from the Windows console. To open a console window, click on Start->Run and type `cmd`.

2) Use the command `cd` to change to the **NISTMSQC\scripts** directory created by extracting the zip archive. For example,

```
cd C:\NISTMSQC\scripts
```

3) The control program is called `run_NISTMSQC_pipeline.pl`, and it can be run with a set of command-line arguments (described in detail below) like the following example:

```
run_NISTMSQC_pipeline.pl --in_dir "C:\directory_with_RAW_files" --out_dir "C:\NISTMSQC\out_dirs\test1" --out_file "C:\NISTMSQC\reports\test1.txt" --instrument_type ORBI --library yeast
```

This command will process the RAW files in "`C:\directory_with_RAW_files`" and produce the report file "`C:\NISTMSQC\out_files\test1.txt`". `--in_dir,` and `--out_dir` can point to any local or networked drive. See below for more on command-line arguments and options.

Useful tips: Typing the `up arrow` from the console will bring up the last command issued. This allows editing of a command-line instead of re-typing. Additionally, the tab key can be used to auto-complete directory paths.

Included Software Components and Versions (version 1.0):

Program	Category	Order in NISTMSQC	Version	Date	Source	Reference (PubMed)
run_NISTMSQC_pipeline	Control	N/A	0.99	11/13/09	NIST	19837981
ReAdW4Mascot2	Converter	1	1.2	20091027a	NIST	19837981
MSPepSearch	Search Engine	2*	0.9	11/17/2009	NIST	none
ProMS	MS1 analysis	3 (optl.)		12/23/09	NIST	
SpectraST	Search Engine	2*	3.1	20090908	ISB	17295354
OMSSA	Search Engine	2*	2.1.7	9/9/09	NCBI	15473683
nistms_metrics	Calc. Metrics	3		11/16/09	NIST	19837981
merge_pep_results	Calc. Metrics	4		9/26/08	NIST	19837981
ParseMetrics	Output Parser	5		11/13/09	NIST	none

* Indicates only one can be selected to run at a time.

Description of Arguments (required):

--in_dir Full path to a directory containing Thermo .RAW files or previously generated MGF+MS1 files. If you wish to use previously generated MGF+MS1 files, these MUST have been produced by a recent version of ReAdW4Mascot2. More than one set of files can be processed by specifying multiple `--in_dir` arguments, as in the example:

```
--in_dir "C:\NISTMSQC\runs1 --in_dir "C:\NISTMSQC\runs2"
```

In this example, "`C:\NISTMSQC\runs2`" contains a second, optional set of files. However, as few as 1 file can be analyzed.

--out_dir Full path to directory where all intermediate output files are to be written. If this directory does not exist, NISTMSQC will attempt to create it for you. NISTMSQC will write output files from *all* input directories to this path. Example:

```
--out_dir "C:\NISTMSQC\out\runs1"
```

--out_file Full path to file where NISTMSQC summary is to be written. This tab-delimited, text file contains all of the calculated metrics values for all runs and series. Example:

```
--out_file "C:\NISTMSQC\metrics\runs1.txt"
```

If this file name has been previously used, NISTMSQC will "increment" the file. For example, instead of overwriting `runs1.txt`, the file `runs1__1.txt` will be created instead.

--library Name of library file located in the 'lib' directory of your installation. (Note: this is NOT a full path) `MSPepSearch`, `OMSSA`, `SpectraST` and FASTA libraries should all have the same root file name. So, if custom libraries are generated, files should be named according to this convention. Example:

```
--library yeast --library sigmaups1
```

Multiple libraries (`--library`) can ONLY be used with MSPepSearch (default).

--instrument_type (allowable values: `LCQ`, `LTQ`, `LXQ`, `ORBI` and `FT`) Used to specify the instrument model used to generate the data. If ORBI or FT is specified, precursor tolerance will be reduced and monoisotopic precursor masses will be used. Additionally, `ReAdW4Mascot2` will attempt to correct any precursor mass miscalls made by XCalibur™ by re-evaluating the MS1 isotopic envelope for the sampled ion.

```
--instrument_type ORBI
```

Command-line Options (NOT required):

--search_engine (allowable values: `MSPepSearch` [default], `OMSSA` or `SpectraST`) Used to specify the search engine identifying the MS/MS spectra. `OMSSA` is a sequence search engine and will only search BLAST-formatted databases. These are provided in the bundles found on the FTP site (above). The other two search engines require MS/MS mass spectral libraries. Library requirements are specific to the search engine option used and will be adjusted automatically by NISTMSQC. Example:

```
--search_engine OMSSA
```

In the above option, peptides will be identified using `OMSSA` searching a BLAST-formatted database.

--fasta Full path to a fasta file. If not specified, NISTMSQC will automatically look for a fasta file in the 'libs' directory of the name specified using `--library`. FASTA files are ONLY used during protein mapping by `nistms_metrics` (i.e., if `--mode full` has been set) and are NOT required for general

use. Additionally, if `MSPepSearch` was used to search multiple libraries AND `--mode full` has been set, you will need to create a FASTA file containing sequences found in all libraries specified.

```
--fasta "C:\NISTMSQC\libs\maltose_binding_protein.fasta"
```

In the above example, the protein sequence(s) found in the indicated file will be to calculate protein-level metrics.

--sort_by (allowable values: `date` [default] and `name`) Used to order the runs as columns in the final output file. Sometimes when files are copied their date stamps change in unpredictable ways. Therefore, if names of files give a better ordering ("ASCII-betically") within a series, `name` should be specified.

```
--sort_by name
```

--overwrite_all If specified, all processing on RAW files will be repeated and any output files will be overwritten. Useful if converter has been updated, for example.

```
--overwrite_all
```

--overwrite_searches If specified, searches will re-run and older output files (TSV or pepXML) will be overwritten. Peak list converter (i.e., `ReAdW4Mascot2`) will not re-run.

```
--overwrite_searches
```

--no_peptide If specified, only a spectral library search engine is allowed and FASTA option is ignored. Useful if searching a library of metabolite MS/MS spectra.

```
--no_peptide
```

--mcp_summary If specified, a parsed report of only the metrics described in the MCP publication (Rudnick *et al*, 19837981) will be generated as a CSV file that can be opened with a spreadsheet application like Microsoft© Excel™. The field headers also contain the metric *codes* (e.g., C2-A) referenced in the publication. This file is suitable for rapid generation of plots.

```
--mcp_summary
```

--mode (allowable values are `lite` [default] and `full`) if `full` is specified, additional peptide and protein-level analysis will be performed. **NOTE:** Information generated in the output by specifying this option is experimental and no documentation is available.

```
--mode full
```

--pro_ms If specified, results from ProMS, a NIST-developed MS1 data analysis program, will be used instead of values calculated by ReAdW4Mascot2. ProMS requires MS1 profile spectra mzXML format, so these (large) files will additionally be generated during data conversions. In our hands, ProMS's XIC methods give more consistent results especially for high-resolution MS1 data.

--pro_ms

--log_file If specified, output from the pipeline will be specified to a log file. These files can be found in the same location as --out_file with the extension .LOG.

```
--log_file
```

--ini_tag Name of tag in scripts\ms.ini file, e.g, [test]. This option allows manual editing of a section of the ms.ini file. This may be used to re-run an analysis after changing the ordering of files or by grouping them as series. To group a set of runs by series, insert SERIES between FILE links. In order to use this option, edit the value between the braces in ms.ini and save the file. NOTE: Editing this file incorrectly may cause problems; use caution when editing the file. Additionally, no checking of other arguments in the ms.ini section is currently done or are tags checked for duplication. If the specified tag appears more than once, the first one will be used.

```
--ini_tag yeast_dilutions
```

--help Descriptions of arguments and options are printed to the screen, then NISTMSQC exits.

```
--help
```

Example of output

Note on Output/Metric Fields: Fields not described in Rudnick *et al* are continuously under development and appear for evaluation purposes. Questions concerning these output fields can be sent via email to the technical contacts listed at the beginning of this document.

The following output was generated by NISTMSQC from the analysis 5 technical replicates of an *E. coli* lysate and is given as an example:

Files Analyzed (5)					
1	C:\projects\NISTMSQC3\out_dirs\ecoli\sh_072808p_E_coli.RAW.MGF.TSV				
2	C:\projects\NISTMSQC3\out_dirs\ecoli\sh_072808p_E_coli_080730160920.RAW.MGF.TSV				
3	C:\projects\NISTMSQC3\out_dirs\ecoli\sh_072808p_E_coli_080730063917.RAW.MGF.TSV				
4	C:\projects\NISTMSQC3\out_dirs\ecoli\sh_072808p_E_coli_080729113858.RAW.MGF.TSV				
5	C:\projects\NISTMSQC3\out_dirs\ecoli\sh_072808p_E_coli_080729210909.RAW.MGF.TSV				
Run Number	1	2	3	4	5
Spectrum Counts					
MS2 Scans	16766	17342	17158	17301	17352
MS1 Scans/Full	5329	5295	5395	5357	5297
MS1 Scans/Other	0	0	0	0	0
First and Last MS1 RT (min)					
First MS1	15	15	15	15	15
Last MS1	184	183.99	183.99	184	184
Tryptic Peptide Counts					
Peptides	3023	3037	3122	3021	3059
Ions	3518	3540	3692	3540	3568
Identifications	3704	3752	3902	3745	3775
Abundance Pct	98.99	99.14	99.32	98.9	99.39
Abundance/1000	9206028	11682686	12057072	14164751	13852958
Ions/Peptide	1.16	1.17	1.18	1.17	1.17
IDs/Peptide	1.23	1.24	1.25	1.24	1.23
Peptide Counts					
Peptides	3041	3055	3146	3045	3082
Ions	3539	3561	3719	3566	3594
Identifications	3726	3775	3931	3774	3805
Semi/Tryp Peps	0.006	0.006	0.008	0.008	0.008
Semi/Tryp Cnts	0.006	0.006	0.007	0.008	0.008

Semi/Tryp Abund	0.01	0.009	0.007	0.011	0.006
Miss/Tryp Peps	0.269	0.255	0.258	0.273	0.27
Miss/Tryp Cnts	0.287	0.275	0.28	0.298	0.288
Miss/Tryp Abund	0.288	0.257	0.266	0.3	0.278
Net Oversample	1.23	1.24	1.26	1.25	1.24
Ions/Peptide	1.16	1.17	1.18	1.17	1.17
IDs/Peptide	1.23	1.24	1.25	1.24	1.23
Middle Peptide Retention Time Period (min)					
Half Period	32.62	33.94	34.3	35.29	34.62
Start Time	48.55	46.85	47.6	46.9	46.79
Mid Time	62.38	61.42	61.76	62.55	61.95
Qratio Time	1.36	1.33	1.42	1.25	1.28
MS2 scans	4840	5084	5131	5323	5207
MS1 Scans	633	652	664	692	676
Pep ID Rate	46.62	45.01	45.86	43.14	44.52
ID Rate	55.22	54.68	55.86	51.91	52.78
ID Efficiency	0.37	0.37	0.37	0.34	0.35
MS1 During Middle (and Early) Peptide Retention Period					
S/N Median	99.3	116	117.5	108.3	108.5
TIC Median/1000	164104	222292	224154	285832	265984
Npeaks Median	960	987	985	1008	1000
Scan-to-Scan	1.059	1.061	1.058	1.06	1.061
S2S-3Q/Med	1.695	1.664	1.677	1.718	1.706
S2S-1Qrt/Med	0.817	0.835	1.144	0.914	0.85
S2S-2Qrt/Med	0.997	1.014	1.108	1.028	1.037
S2S-3Qrt/Med	1.02	0.923	0.935	0.881	0.993
S2S-4Qrt/Med	0.918	0.849	1.042	1.012	0.841
ESI Off Middle	0	0	0	0	0
ESI Off Early	0	0	0	0	0
Max MS1 Jump	1.36	1.41	1.82	1.41	1.4
Max MS1 Fall	1.4	1.28	1.83	1.37	1.35
MS1 Jumps >10x	0	0	0	0	0
MS1 Falls >10x	0	0	0	0	0
MS1 Total Ion Current For Different RT Periods					
1st Quart ID	0.13	0.12	0.12	0.14	0.12
Middle ID	0.22	0.24	0.23	0.28	0.25
Last ID Quart	0.67	0.66	0.63	0.72	0.63
To End of Run	0.33	0.34	0.37	0.28	0.37

Total Ion Current For IDs at Peak Maxima					
Med TIC ID/1000	140438	189625	188495	241630	227245
InterQ TIC	2.32	1.89	1.91	2.29	2
Mid InterQ TIC	2.32	1.89	1.91	2.29	2
Precursor m/z for IDs					
Median	612.59	622.82	625.28	649.34	643.34
Half Width	289.59	280.78	288.99	296.63	292.79
Quart Ratio	1.614	1.586	1.604	1.601	1.596
Precursor Min	303.2	303.2	303.7	300.7	304.2
Precursor Max	1465.7	1656.4	1484.2	1655.9	1655.9
Med @ Q1 TIC	749.4	717.4	731.7	754.1	738.7
Med @ Q4 TIC	486.2	497.8	501.7	497.8	500.3
Med @ Q1 RT	478.7	481.8	481.8	487.3	487.7
Med @ Q4 RT	825.7	811.2	826.4	846.4	841.4
Med Charge +1	797.4	787.9	802.8	800.4	802.4
Med Charge +2	605.8	616.3	616.3	632.3	625.8
Med Charge +3	631.3	642.7	645	675	671.3
Med Charge +4	640.3	617.8	653.1	677.1	664.3
Number of Ions vs Charge					
Charge +1	9	9	17	10	17
Charge +2	2252	2388	2444	2340	2378
Charge +3	1079	992	1071	1020	1026
Charge +4	198	171	186	195	172
Charge +5	1	1	1	1	1
Averages vs RT for IDed Peptides					
Length Q1	9.62	9.59	9.52	9.62	9.53
Length Q4	20.14	19.57	19.9	20.53	20.31
Charge Q1	2.2	2.16	2.16	2.16	2.14
Charge Q4	2.66	2.6	2.6	2.65	2.64
Precursor m/z - Peptide Ion m/z (+2 Charge Only, Reject >0.45 m/z)					
Spectra	2168	2271	2302	2221	2247
Median	-0.0011	-0.0004	0	-0.0005	-0.0004
Mean Absolute	0.0063	0.0083	0.0097	0.0069	0.0071
ppm Median	-2.02	-0.75	0	-0.95	-0.64
ppm InterQ	3.36	3.39	3.44	3.49	3.42

Ion IDs by Charge State (Relative to +2)					
+2 Ion Count	2252	2388	2444	2340	2378
Charge +1	0.004	0.004	0.007	0.004	0.007
Charge +2	1	1	1	1	1
Charge +3	0.479	0.415	0.438	0.436	0.431
Charge +4	0.088	0.072	0.076	0.083	0.072
Average Peptide Lengths for Different Charge States					
Charge +1	7	7.67	7.53	7.4	7.41
Charge +2	11.75	11.83	11.84	12.05	11.97
Charge +3	17.07	17.14	17.25	17.93	17.85
Charge +4	22.74	22.3	23.04	23.89	23.38
Average Peptide Lengths For Charge 2 for Different Numbers of Mobile Protons					
NAA,Ch=2,MP=-1	12.62	11.85	12.19	11.9	11.91
NAA,Ch=2,MP=0	11.8	11.68	11.77	11.85	11.73
NAA,Ch=2,MP=1	11.71	11.88	11.85	12.13	12.06
NAA,Ch=2,MP=2	19	20.67	20	21	18.67
Numbers of Ion Ids at Different Charges with 1 Mobile Proton					
Ch=1 MP=1	2	2	2	1	2
Ch=2 MP=1	1633	1667	1717	1606	1629
Ch=3 MP=1	713	662	723	673	691
Ch=4 MP=1	119	119	120	122	109
Percent of IDs at Different Charges and Mobile Protons Relative to IDs with 1 Mobile Proton					
Ch=1 MP=-1	50	50	100	300	150
Ch=1 MP=0	300	300	650	600	600
Ch=1 MP=1	100	100	100	100	100
Ch=2 MP=-1	2.8	3.2	3.1	3.9	4.1
Ch=2 MP=0	35	39.8	39	41.4	41.4
Ch=2 MP=1	100	100	100	100	100
Ch=3 MP=-1	4.5	3	3	3.6	3.9
Ch=3 MP=0	39.4	39	37.3	41	38.4
Ch=3 MP=1	100	100	100	100	100
Precursor m/z - Monoisotope Exact m/z					
More Than 100	1391	1559	1616	1582	1618
Betw 100.0-50.0	1124	1157	1168	1162	1136
Betw 50.0-25.0	761	651	739	666	667
Betw 25.0-12.5	246	183	182	149	163

Betw 12.5-6.3	16	11	14	7	10
Betw 6.3-3.1	1	0	0	0	0
Betw 3.1-1.6	0	0	0	0	0
Betw 1.6-0.8	0	0	0	0	0
Top Half	22	2	4	13	10
Next Half (2)	90	63	53	81	102
Next Half (3)	335	208	185	277	338
Next Half (4)	756	596	532	723	772
Next Half (5)	1135	984	1036	1094	1153
Next Half (6)	853	1101	1167	979	872
Next Half (7)	315	502	600	358	309
Next Half (8)	0	0	0	0	0
MS2 ID Spectra					
NPeaks Median	427	454	458	485	474
NPeaks InterQ	1.54	1.5	1.49	1.55	1.51
S/N Median	79.33	87.12	86.38	89.08	89.78
S/N InterQ	3.14	3.2	3.14	3.13	3.19
ID Score Median	0.55	0.554	0.55	0.552	0.554
ID Score InterQ	0.117	0.12	0.119	0.122	0.124
IDSc Med Q1Msmx	0.561	0.571	0.567	0.575	0.571
MS1 ID Max					
Median	1202701	1477312	1479548	1821886	1772069
Half Width	2174033	2750052	2661805	3400904	3181242
Quart Ratio	5.15	5	4.97	5.15	4.94
Median MidRT	1516479	1828956	1803880	2407164	2156945
75/25 MidRT	4.3	4.5	4.4	4.3	4.4
95/5 MidRT	34.5	36	33.4	35.3	34.2
75/25 Pctile	5.2	5	5	5.1	4.9
95/5 Pctile	62.7	63.7	62.8	68.6	64.4
Fraction of MS2 Identified at Different MS1max Quartiles					
ID Fract Q1	0.491	0.48	0.484	0.453	0.474
ID fract Q2	0.38	0.381	0.377	0.346	0.368
ID Fract Q3	0.316	0.319	0.331	0.308	0.302
ID Fract Q4	0.295	0.272	0.299	0.259	0.27
MS1 ID Abund at MS2 Acquisition					
Median	555319	673623	693291	871913	833839
Half Width	796075	920701	956005	1181498	1086006

75/25 Pctile	3.9	3.5	3.7	3.6	3.5
95/5 Pctile	32	30.8	30.2	31.6	31.1
MS2 ID Abund Reported					
Median	15731	16961	16706	19577	18539
Half Width	15044	18065	17818	18401	19024
75/25 Pctile	2.5	2.7	2.7	2.4	2.6
95/5 Pctile	10.4	12	11.7	12.2	11.9
Max Peak Width for IDs (sec)					
Median Value	75.3	74.04	74.46	75.3	74.64
Third Quart	93.78	93	94.02	93.78	93.9
Last Decile	119.4	120.66	121.8	122.1	121.98
Peak Width at Half Height for IDs					
Median Value	19.86	19.8	20.1	19.86	19.68
Med Top Quart	20.46	20.58	20.58	20.52	20.58
Med Top 16th	20.76	21.18	20.64	21.12	20.7
Med Top 100	21.3	20.88	21.06	22.44	20.88
Median Disper	6.12	7.02	7.02	6.9	6.9
Med Quart Disp	5.04	5.82	5.46	5.46	5.76
Med 16th Disp	5.22	5.1	5.22	6	5.28
Med 100 Disp	4.98	5.64	6.36	7.56	5.52
3Quart Value	23.4	23.76	24.06	23.7	23.7
9Dec Value	29.46	30.72	30.42	30.96	31.32
MS1 Interscan/s	3.09	3.12	3.1	3.06	3.07
MS1 Scan/FWHM	6.42	6.34	6.49	6.49	6.41
IDs Used	3508	3526	3687	3518	3558
Peak Widths at Half Max over RT deciles for IDs					
First Decile	16.08	14.58	15.66	14.94	15.24
Median Value	20.34	19.98	19.62	19.62	18.78
Last Decile	21.3	21.9	22.02	22.26	22.38
Nearby Resampling of IDs - Oversampling Details					
Repeated IDs	187	214	212	208	211
Med RT Diff/s	62.46	63.6	63.18	63.24	63
1Q RT Diff/s	60.06	60.84	60.54	60.72	60.24
1Dec RT Diff/s	11.1	31.56	20.22	10.08	10.44
Median dm/z	0	0.01	0	0	0.01
Quart dm/z	0	0	0	0	0

Wide RT Differences for IDs (> 4 min)					
Peptides	27	30	28	30	22
Spectra	84	112	105	104	91
Fraction of Repeat Peptide IDs with Divergent RT (RT vs RT-best ID) - Chromatographic 'Bleed'					
- 4 min	0.0027	0.0034	0.0031	0.0042	0.0026
+ 4 min	0.0051	0.0109	0.0071	0.0064	0.0058
Early and Late RT Oversampling (Spectrum IDs/Unique Peptide IDs) - Chromatographic: Flow Through/Bleed					
First Decile	0.978	0.979	0.97	0.99	1.011
Last Decile	1.153	1.252	1.202	1.237	1.215
Peptide Ion IDs by > 3 Spectra (Hi) vs 1-3 Spectra (Lo) - Extreme Oversampling					
Pep Ions (Hi)	7	8	9	10	10
Ratio Hi/Lo	0.002	0.002	0.002	0.003	0.003
Spec Cnts (Hi)	32	56	55	50	53
Ratio Hi/Lo	0.009	0.015	0.014	0.013	0.014
Spec/Pep (Hi)	4.571	7	6.111	5	5.3
Spec Cnt Excess	0.003	0.008	0.007	0.005	0.006
Ratios of Peptide Ions IDed by Different Numbers of Spectra - Oversampling Measure					
Once/Twice	28.24	26.18	26.96	28.42	26
Twice/Thrice	5.71	7.22	7.76	5	7.33
Single Spectrum Peptide Ion Identifications - Oversampling Measure					
Peptide Ions	3389	3403	3559	3410	3432
Fract >1 Ions	0.04	0.04	0.04	0.04	0.04
1 vs >1 PepIon	22.74	21.68	22.38	22	21.32
1 vs >1 Spec	10.12	9.2	9.62	9.42	9.25
MS1max/MS1sampled Abundance Ratio IDs - Inefficient Sampling					
Median All IDs	1.78	1.79	1.78	1.74	1.81
3Q All IDs	3.22	3.3	3.3	3.2	3.31
9Dec All IDs	6.73	7.03	6.9	6.87	6.73
Med Top 100	11.56	9.39	10.61	8.6	11.2
Med Top Dec	6.03	6.03	6.19	5.46	6
Med Top Quart	3.75	3.91	3.81	3.93	3.92
Med Bottom 1/2	1.43	1.39	1.38	1.36	1.41
RT(MS1max)-RT(MS2) for IDs (sec)					

Med Diff Abs	9	9	9	9	9
Median Diff	7	6	6	7	7
First Quart	0	-1	-1	0	0
Third Quart	12	11	11	11	11
Ion Injection Times for IDs (ms)					
MS1 Median	49	38	39	29	30
MS1 Maximum	144	131	158	111	118
MS2 Median	100	100	100	100	100
MS2 Maximun	100	100	100	100	100
MS2 Fract Max	0.873	0.82	0.815	0.721	0.76
Relative Fraction of Peptides in Retention Decile Matching a Peptide in Other Runs					
All Deciles	0.658	0.655	0.636	0.657	0.649
First Decile	1.095	1.129	1.159	1.185	1.168
Last Decile	0.059	0.126	0	0	0
Comp to First	0	0.658	0.658	0.658	0.658
Comp to Last	0.649	0.649	0.649	0.649	0
Relative Uniqueness of Peptides in Decile Found Anywhere in Other Runs					
First Decile	1.1	1.13	1.16	1.19	1.17
Last Decile	0.06	0.13	0	0	0
Differences in Elution Rank (Percent) of Matching Peptides in Other Runs					
Average Diff	0.998	0.914	0.987	0.973	0.947
Median Diff	0.295	0.293	0.288	0.278	0.269
Comp to First	0	0.325	0.327	0.26	0.269
Comp to Last	0.231	0.322	0.293	0.232	0
Median Ratios of MS1 Intensities of Matching Peptides in Other Runs					
Median Diff	1.39	1.359	1.345	1.354	1.339
Median*2 Diff	1.84	1.789	1.778	1.775	1.743
Comp to First	0	1.423	1.401	1.357	1.379
Comp to Last	1.379	1.341	1.32	1.315	0
Comp to First*2	0	1.918	1.875	1.772	1.796
Comp to Last*2	1.796	1.754	1.724	1.696	0
Uncorrected and RT Corrected Relative Intensities of Matching Peptides in Other Runs					
Uncor rel First	0	0	0	0	0
Uncor rel Last	0	0	0	0	0
Corr rel First	0	0	0	0	0

Corr rel Last	0	0	0	0	0
Magnitude of RT Correction of Intensities of Matching Peptides in Other Runs					
Comp to First	0	0	0	0	0
Comp to Last	0	0	0	0	0
Top Ion Abundance Measures					
Top 10% Abund	20	20	22	22	22
Top 25% Abund	89	85	92	91	94
Top 50% Abund	359	354	376	368	371
Fractab Top	0.009	0.009	0.009	0.01	0.008
Fractab Top 10	0.062	0.064	0.058	0.057	0.057
Fractab Top 100	0.268	0.274	0.262	0.263	0.259
End Series=1					
End Runseries Results					

Other Details:

Search engine thresholds for peptide/compound identification

The implemented values are empirical approximations of a 1% false discovery rate threshold on the peptide spectrum match (PSM) level. These are approximate values and should NOT be considered exact. An analysis of decoy matches or other statistical evaluation should be performed as needed if a more precise, dataset-specific threshold is desired. In the current version, threshold values cannot be adjusted without modifying source code. This may become an option for each search engine in a later release.

Search Engine	Score	Value
MSPepSerach	Score	450
OMSSA	E-value	0.1
SpectraST	Fval	0.45

OMSSA Searches

OMSSA searches are run using the iterative searching option `-is <threshold as indicated above>`. This option performs a semitryptic search of spectra scoring poorly or not at in a preliminary fully tryptic search. In the semitryptic iteration, only protein sequences with at least one fully tryptic matching peptide with an E-value scoring better than `threshold` are considered as candidates.

Metrics Reporting All Zeroes

Currently, the following two metrics report no values:

```
Median Ratios of MS1 Intensities of Matching Peptides in Other Runs
Magnitude of RT Correction of Intensities of Matching Peptides in Other Runs
```

Values for these inter-run metrics will be added in a future release.

Commercial implementations of NISTMSQC or its components

MassQC - Proteome Software, Inc. (http://www.massqc.com/)

SpecMSMS - BioProximity (http://specmsms.com/)

Footnote:

Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.