Supplemental Text 1: Other Existing Tools for SRM Method Creation and Analysis

Proprietary software:
- Agilent MassHunter Workstation
- Applied Biosystems MRMPilot
- Applied Biosystems MultiQuant
- Thermo-Fisher PinPoint
- Waters TargetLynx
- Single Organism Mr. M (Sherwood *et al.*, 2009)

Freely available software:
- MaRiMba (Sherwood *et al.*, 2009) – Transition list creation
- MRMaid (Mead *et al.*, 2008) – Transition list creation
- MRMer (Martin *et al.*, 2008) – Result data viewing and peak integration
- TIQAM (Lange *et al.*, 2008) – Transition list creation

Supplemental Text 2: Skyline Supported File Formats

Transition list output formats:
- Agilent
- Applied Biosystems
- Thermo Fisher Scientific
- Waters

Mass spectrometry data import formats:
- Agilent
- Applied Biosystems (with ProteinPilot 3.0 Trial Edition installed)
  https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=601680&tab=DetailInfo
- Thermo Fisher Scientific
- Waters (with MassLynx 4.1 installed)
- mzXML (Pedrioli *et al.*, 2004)
- mzML (Deutsch, 2008)

Publicly distributed spectral library formats:
- BiblioSpec (Frewen *et al.*, 2006)
  http://proteome.gs.washington.edu/software/bibliospec/documentation/libs.html
- Global Proteome Machine (GPM), X! Hunter (Craig *et al.*, 2006)
  ftp://ftp.thegpm.org/projects/xhunter/libs/
- National Institute of Standards and Technology (NIST) (Toner, 2008)
  http://peptide.nist.gov/
- SpectraST (Lam *et al.*, 2007)
  http://www.peptideatlas.org/speclib/

Peptide search engine result formats for building spectral libraries:
- Mascot (Perkins *et al.*, 1999)
- IDPicker (Ma *et al.*, 2009)
- Trans Proteomic Pipeline (Keller *et al.*, 2005)
  pepXML and mzXML (Pedrioli *et al.*, 2004)
- X! Tandem (Craig *et al.*, 2004)

References

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Craig,R. *et al.* (2006) Using Annotated Peptide Mass Spectrum Libraries for Protein Identification. *Journal of Proteome Research* **5** (8), 1843-1849

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Frewen,B.E. *et al.* (2006) Analysis of peptide MS/MS spectra from large-scale proteomics experiments using spectrum libraries. *Anal. Chem.*, **78** (16):5678-84

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Martin,D *et al.* (2008) MRMer, an Interactive Open Source and Cross-platform System for Data Extraction and Visualization of Multiple Reaction Monitoring Experiments. *Mol. Cell. Protoemics* **7**:2270-2278

Mead,J *et al.* (2008) MRMaid: the web-based tool for designing multiple reaction monitoring (MRM) transitions. *Mol. Cell. Proteomics* **8**:696-705

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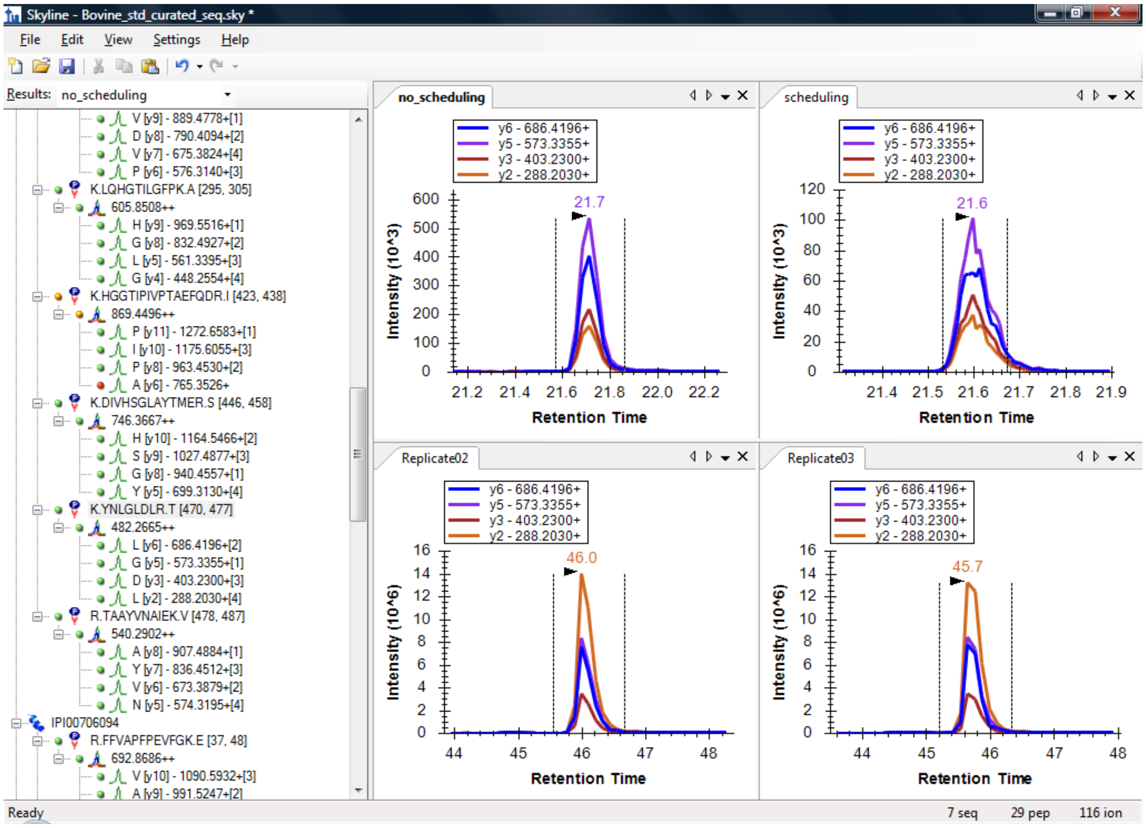
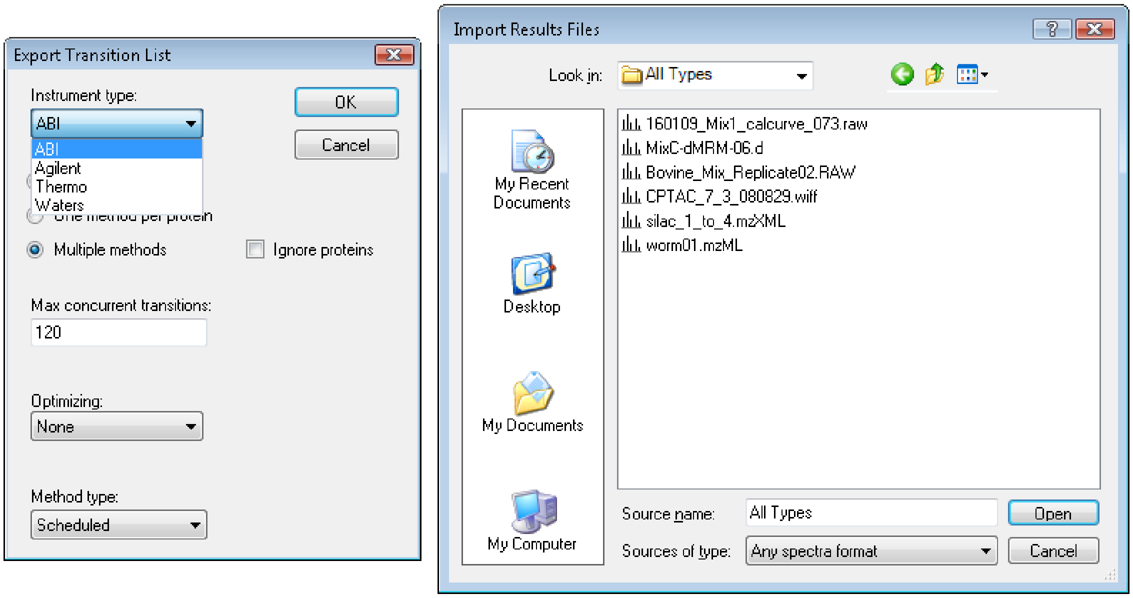
Toner,B. (2008) NIST Team Building Peptide Spectral Library To Improve Mass-Spec Proteomics Analysis. *Bioinform*



Supplemental Figure 1. Vendor neutral data sharing support in Skyline. This support is illustrated using data acquired for a single set of peptides and transitions on an Applied Biosystems 4000 Q Trap in the top 2 panes, with data acquired on a Thermo-Fisher Scientific TSQ-Quantum Ultra in the bottom 2 panes. In this example, the investigator can clearly see the change in ion ratios between the two instruments.



Instrument type: ABI

● Multiple methods

☐ Ignore proteins

Max concurrent transitions: 120

Optimizing: None

Method type: Scheduled

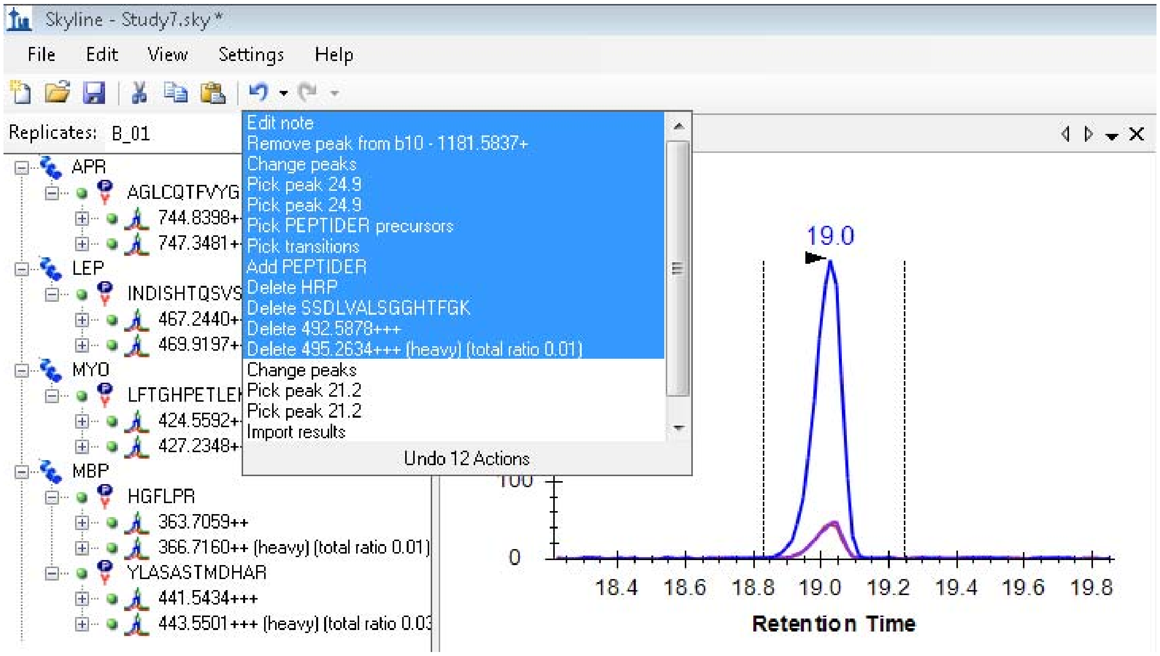
Look in: All Types

160109_Mix1_calcurve_073.raw
MixC-dMRM-06.d
Bovine_Mix_Replicate02.RAW
CPTAC_7_3_080829.wiff
silac_1_to_4.mzXML
worm01.mzML

Source name: All Types

Sources of type: Any spectra format

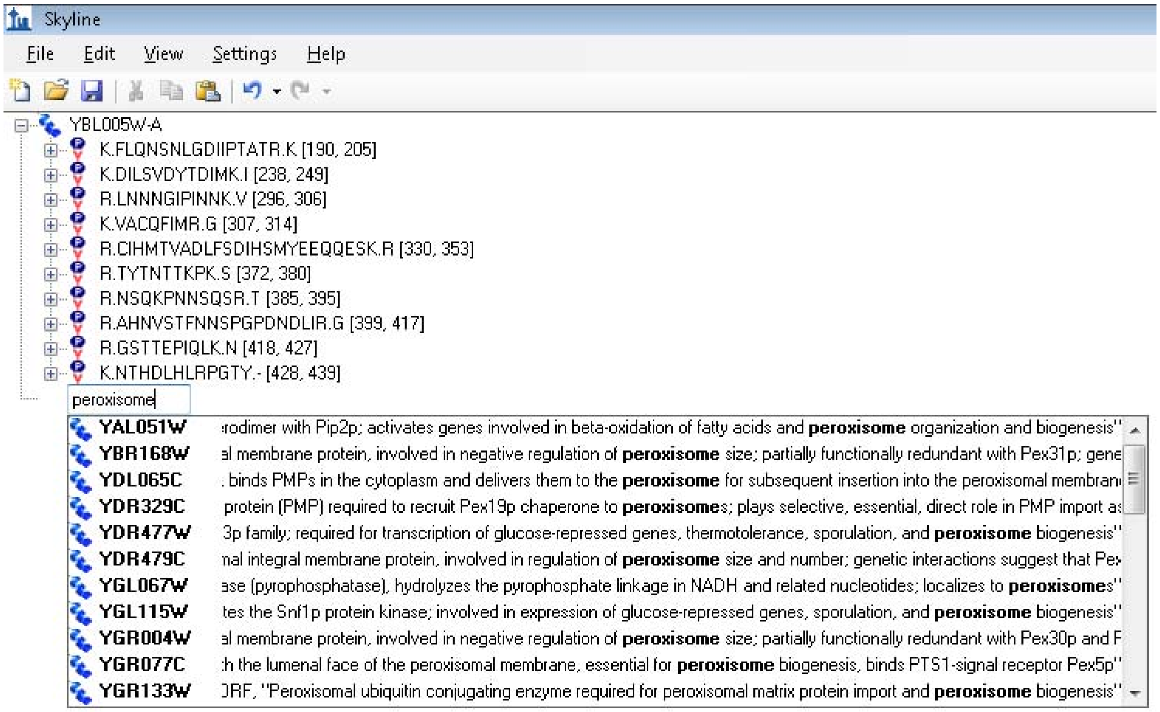
Supplemental Figure 2. The Export Transition List and Import Results Files dialogs in Skyline. These dialogs show the full range of SRM instrument support options available only in Skyline. Here the Export Transition List dialog shows support for creating transition lists ready for use on Agilent, Applied Biosystems, Thermo-Fisher Scientific and Waters instrument. Additionally, the Import Results Files dialog shows support for importing the native files from these instruments as well as the portable mzML and mzXML formats.



Edit note
Remove peak from b10 - 1181.5837+
Change peaks
Pick peak 24.9
Pick peak 24.9
Pick PEPTIDER precursors
Pick transitions
Add PEPTIDER
Delete HRP
Delete SSDLVALSGGHTFGK
Delete 492.5878+++
Delete 495.2634+++ (heavy) (total ratio 0.01)
Change peaks
Pick peak 21.2
Pick peak 21.2
Import results

APR
AGLCQTFVYG
744.8398+
747.3481+
LEP
INDISHTQSVS
467.2440+
469.9197+
MYO
LFTGHPETLE
424.5592+
427.2348+
MBP
HGFLPR
363.7059++
366.7160++ (heavy) (total ratio 0.01)
YLASASTMDHAR
441.5434+++
443.5501+++ (heavy) (total ratio 0.03

Supplemental Figure 3. Skyline shows the full history of actions performed on this document in its undo list. This full history enables the document to be restored to any state in the editing session at a single mouse click.

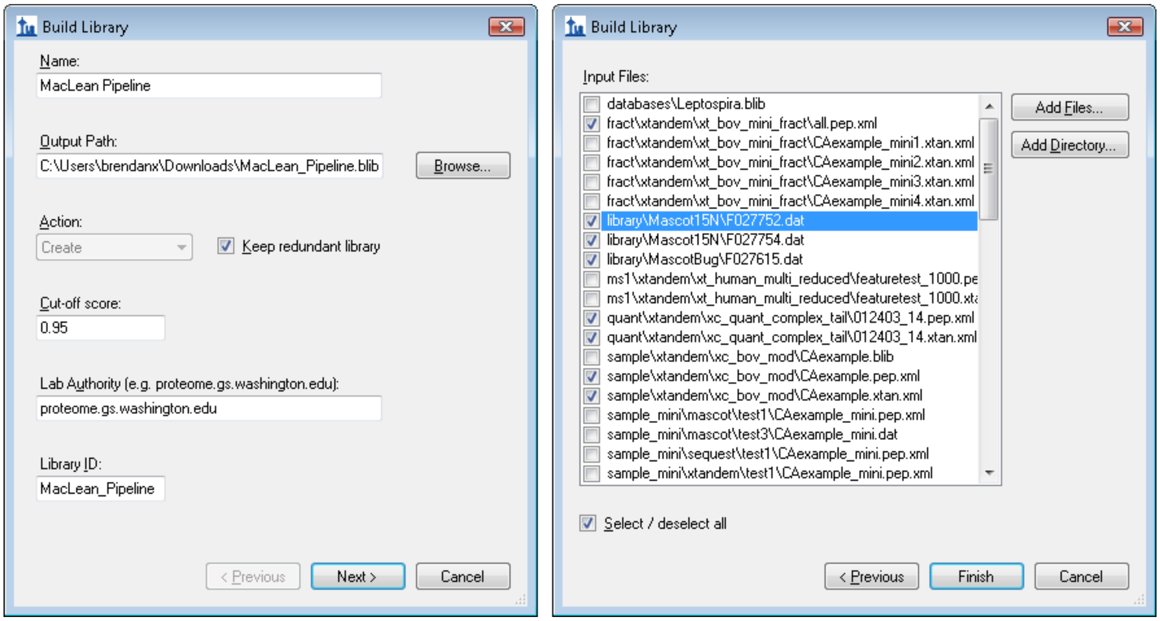


YBL005W-A
K.FLQNSNLGDIIPTATR.K [190, 205]
K.DILSVDYTDIMK.I [238, 249]
R.LNNNGIPINNK.V [296, 306]
K.VACQFIMR.G [307, 314]
R.CIHMTVADLFSDIHSMYEEQQESK.R [330, 353]
R.TYTNTTKPK.S [372, 380]
R.NSQKPNNSQSR.T [385, 395]
R.AHNVSTFNNSPGPDNDLIR.G [399, 417]
R.GSTTEPIQLK.N [418, 427]
K.NTHDLHLRPGTY.- [428, 439]

peroxisome

YAL051W	rodimer with Pip2p; activates genes involved in beta-oxidation of fatty acids and **peroxisome** organization and biogenesis"
YBR168W	al membrane protein, involved in negative regulation of **peroxisome** size; partially functionally redundant with Pex31p; gene
YDL065C	. binds PMPs in the cytoplasm and delivers them to the **peroxisome** for subsequent insertion into the peroxisomal membran
YDR329C	protein (PMP) required to recruit Pex19p chaperone to **peroxisomes**; plays selective, essential, direct role in PMP import a
YDR477W	3p family; required for transcription of glucose-repressed genes, thermotolerance, sporulation, and **peroxisome** biogenesis"
YDR479C	nal integral membrane protein, involved in regulation of **peroxisome** size and number; genetic interactions suggest that Pe
YGL067W	ase (pyrophosphatase), hydrolyzes the pyrophosphate linkage in NADH and related nucleotides; localizes to **peroxisomes**"
YGL115W	tes the Snf1p protein kinase; involved in expression of glucose-repressed genes, sporulation, and **peroxisome** biogenesis"
YGR004W	al membrane protein, involved in negative regulation of **peroxisome** size; partially functionally redundant with Pex30p and F
YGR077C	h the lumenal face of the peroxisomal membrane, essential for **peroxisome** biogenesis, binds PTS1-signal receptor Pex5p"
YGR133W	DRF, "Peroxisomal ubiquitin conjugating enzyme required for peroxisomal matrix protein import and **peroxisome** biogenesis"

Supplemental Figure 4. Screenshot showing the auto-completion capability in Skyline. As an example, when the user types the word "peroxisome" any of yeast proteins from the Background Proteome containing that word in the description can be added to the document by simply selecting it form a list. Skyline also offers auto-completion against the Background Proteome for peptide sequences and protein accession numbers.



Name: MacLean Pipeline

Output Path: C:\Users\brendanx\Downloads\MacLean_Pipeline.blib

Action: Create

☑ Keep redundant library

Cut-off score: 0.95

Lab Authority (e.g. proteome.gs.washington.edu): proteome.gs.washington.edu

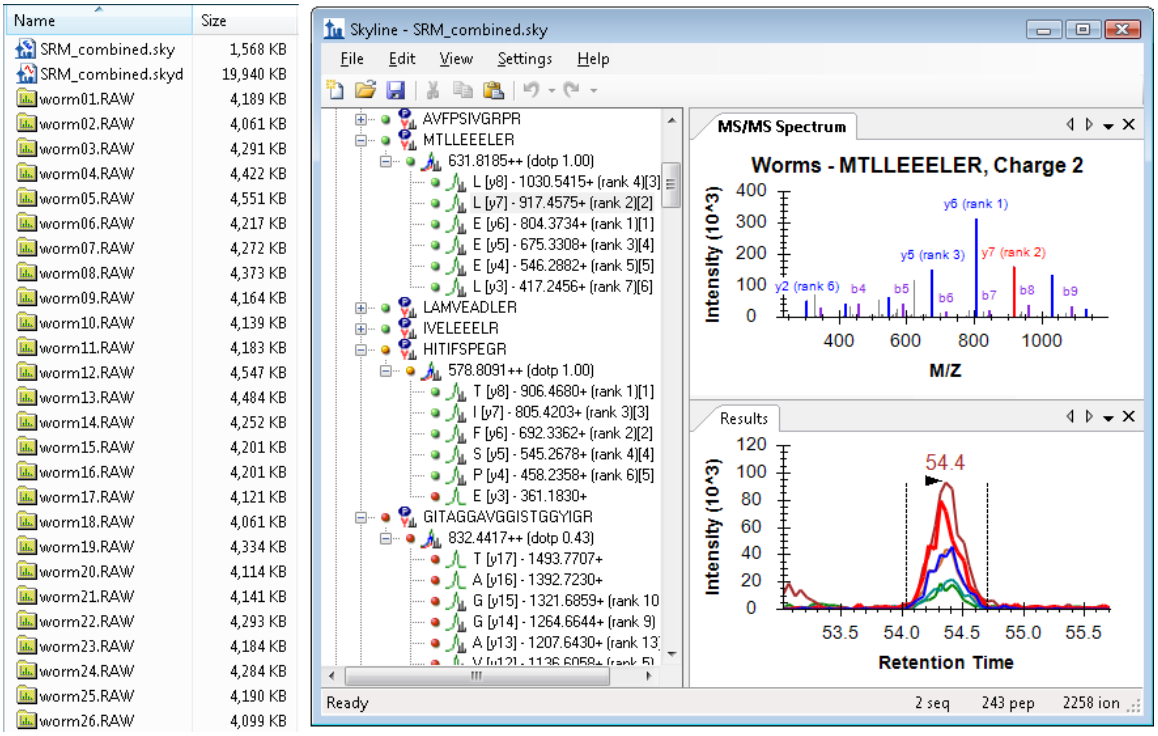
Library ID: MacLean_Pipeline

Input Files:

- ☐ databases\Leptospira.blib
- ☑ fract\xtandem\xt_bov_mini_fract\all.pep.xml
- ☐ fract\xtandem\xt_bov_mini_fract\CAexample_mini1.xtan.xml
- ☐ fract\xtandem\xt_bov_mini_fract\CAexample_mini2.xtan.xml
- ☐ fract\xtandem\xt_bov_mini_fract\CAexample_mini3.xtan.xml
- ☐ fract\xtandem\xt_bov_mini_fract\CAexample_mini4.xtan.xml
- ☑ library\Mascot15N\F027752.dat
- ☑ library\Mascot15N\F027754.dat
- ☑ library\MascotBug\F027615.dat
- ☐ ms1\xtandem\xt_human_multi_reduced\featuretest_1000.pe
- ☐ ms1\xtandem\xt_human_multi_reduced\featuretest_1000.xt
- ☑ quant\xtandem\xc_quant_complex_tail\012403_14.pep.xml
- ☑ quant\xtandem\xc_quant_complex_tail\012403_14.xtan.xml
- ☐ sample\xtandem\xc_bov_mod\CAexample.blib
- ☑ sample\xtandem\xc_bov_mod\CAexample.pep.xml
- ☑ sample\xtandem\xc_bov_mod\CAexample.xtan.xml
- ☐ sample_mini\mascot\test1\CAexample_mini.pep.xml
- ☐ sample_mini\mascot\test3\CAexample_mini.dat
- ☐ sample_mini\sequest\test1\CAexample_mini.pep.xml
- ☐ sample_mini\xtandem\test1\CAexample_mini.pep.xml

☑ Select / deselect all

Supplemental Figure 5. The Skyline library build wizard simplifies the creation BiblioSpec MS/MS spectral libraries from experimental peptide search results. Support for a variety of formats is shown with a complete list supplied in Supplemental Text 1.

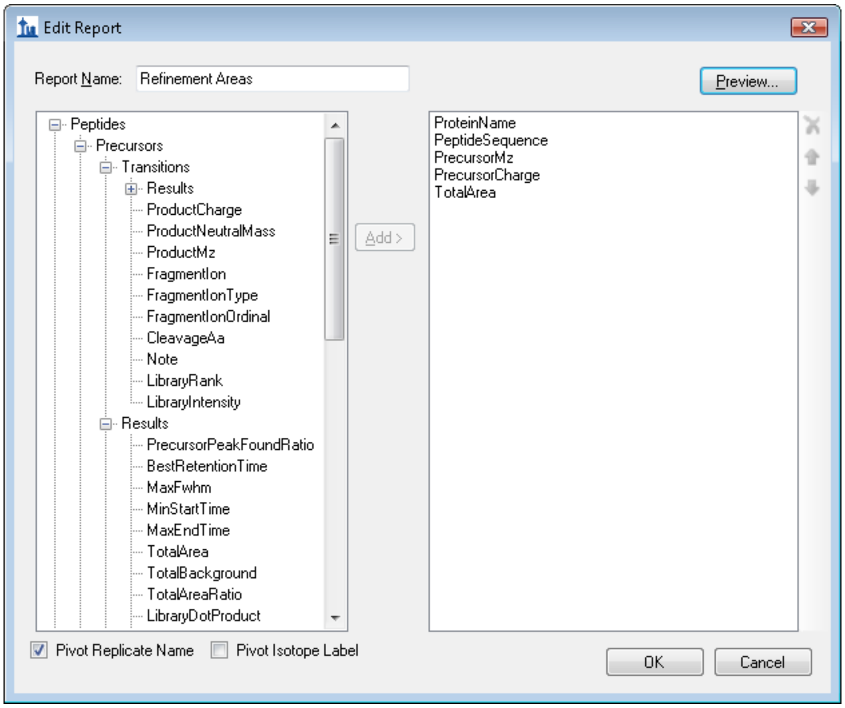


Name	Size
SRM_combined.sky	1,568 KB
SRM_combined.skyd	19,940 KB
worm01.RAW	4,189 KB
worm02.RAW	4,061 KB
worm03.RAW	4,291 KB
worm04.RAW	4,422 KB
worm05.RAW	4,551 KB
worm06.RAW	4,217 KB
worm07.RAW	4,272 KB
worm08.RAW	4,373 KB
worm09.RAW	4,164 KB
worm10.RAW	4,139 KB
worm11.RAW	4,183 KB
worm12.RAW	4,547 KB
worm13.RAW	4,484 KB
worm14.RAW	4,252 KB
worm15.RAW	4,201 KB
worm16.RAW	4,201 KB
worm17.RAW	4,121 KB
worm18.RAW	4,061 KB
worm19.RAW	4,334 KB
worm20.RAW	4,114 KB
worm21.RAW	4,141 KB
worm22.RAW	4,293 KB
worm23.RAW	4,184 KB
worm24.RAW	4,284 KB
worm25.RAW	4,190 KB
worm26.RAW	4,099 KB

- AVFPSIVGRPR
- MTLLEEELER
  - 631.8185++ (dotp 1.00)
    - L [y8] - 1030.5415+ (rank 4)[3]
    - L [y7] - 917.4575+ (rank 2)[2]
    - E [y6] - 804.3734+ (rank 1)[1]
    - E [y5] - 675.3308+ (rank 3)[4]
    - E [y4] - 546.2882+ (rank 5)[5]
    - L [y3] - 417.2456+ (rank 7)[6]
- LAMVEADLER
- IVELEEELR
- HITIFSPEGR
  - 578.8091++ (dotp 1.00)
    - T [y8] - 906.4680+ (rank 1)[1]
    - I [y7] - 805.4203+ (rank 3)[3]
    - F [y6] - 692.3362+ (rank 2)[2]
    - S [y5] - 545.2678+ (rank 4)[4]
    - P [y4] - 458.2358+ (rank 6)[5]
    - E [y3] - 361.1830+
- GITAGGAVGGISTGGYIGR
  - 832.4417++ (dotp 0.43)
    - T [y17] - 1493.7707+
    - A [y16] - 1392.7230+
    - G [y15] - 1321.6859+ (rank 10
    - G [y14] - 1264.6644+ (rank 9)
    - A [y13] - 1207.6430+ (rank 13

Ready 2 seq 243 pep 2258 ion

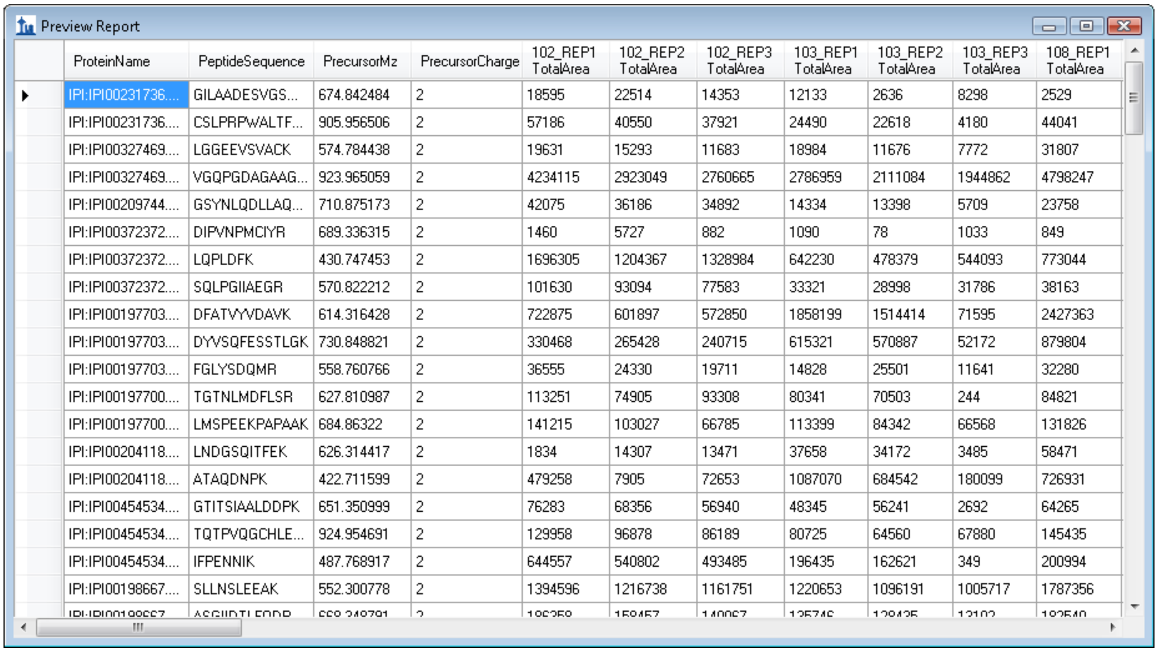
Supplemental Figure 6. The Skyline high-performance data file can handle very large experiments without loss in performance. Experiments requiring over 50 individual native instrument files can be stored efficiently in a single file, often at a fraction of the size of the instrument native files. This figure shows 26 of the 55 instrument files from a TSQ-Quantum Ultra required to measure the original 2908 transitions in this document. The resulting SRM_combined.skyd Skyline data file, requiring 1/10th the disk space of the original RAW files, is shown above. Once the import is complete, this file can be easily shared with collaborators and opened in a fraction of a second without the native instrument files.



Report Name: Refinement Areas

ProteinName
PeptideSequence
PrecursorMz
PrecursorCharge
TotalArea

☑ Pivot Replicate Name ☐ Pivot Isotope Label



ProteinName	PeptideSequence	PrecursorMz	PrecursorCharge	102_REP1 TotalArea	102_REP2 TotalArea	102_REP3 TotalArea	103_REP1 TotalArea	103_REP2 TotalArea	103_REP3 TotalArea	108_REP1 TotalArea
IPI:IPI00231736....	GILAADESVGS...	674.842484	2	18595	22514	14353	12133	2636	8298	2529
IPI:IPI00231736....	CSLPRPWALTF...	905.956506	2	57186	40550	37921	24490	22618	4180	44041
IPI:IPI00327469....	LGGEEVSVACK	574.784438	2	19631	15293	11683	18984	11676	7772	31807
IPI:IPI00327469....	VGQPGDAGAAG...	923.965059	2	4234115	2923049	2760665	2786959	2111084	1944862	4798247
IPI:IPI00209744....	GSYNLQDLLAQ...	710.875173	2	42075	36186	34892	14334	13398	5709	23758
IPI:IPI00372372....	DIPVNPMCIYR	689.336315	2	1460	5727	882	1090	78	1033	849
IPI:IPI00372372....	LQPLDFK	430.747453	2	1696305	1204367	1328984	642230	478379	544093	773044
IPI:IPI00372372....	SQLPGIIAEGR	570.822212	2	101630	93094	77583	33321	28998	31786	38163
IPI:IPI00197703....	DFATVYVDAVK	614.316428	2	722875	601897	572850	1858199	1514414	71595	2427363
IPI:IPI00197703....	DYVSQFESSTLGK	730.848821	2	330468	265428	240715	615321	570887	52172	879804
IPI:IPI00197703....	FGLYSDQMR	558.760766	2	36555	24330	19711	14828	25501	11641	32280
IPI:IPI00197700....	TGTNLMDFLSR	627.810987	2	113251	74905	93308	80341	70503	244	84821
IPI:IPI00197700....	LMSPEEKPAPAAK	684.86322	2	141215	103027	66785	113399	84342	66568	131826
IPI:IPI00204118....	LNDGSQITFEK	626.314417	2	1834	14307	13471	37658	34172	3485	58471
IPI:IPI00204118....	ATAQDNPK	422.711599	2	479258	7905	72653	1087070	684542	180099	726931
IPI:IPI00454534....	GTITSIAALDDPK	651.350999	2	76283	68356	56940	48345	56241	2692	64265
IPI:IPI00454534....	TQTPVQGCHLE...	924.954691	2	129958	96878	86189	80725	64560	67880	145435
IPI:IPI00454534....	IFPENNIK	487.768917	2	644557	540802	493485	196435	162621	349	200994
IPI:IPI00198667....	SLLNSLEEAK	552.300778	2	1394596	1216738	1161751	1220653	1096191	1005717	1787356

Supplemental Figure 7. The Skyline custom report designer helps users quickly export data into tabular formats compatible with Excel and R.. The report shown supplies total peptide area for all peptides measured pivoted on the replicate name. This format greatly simplifies using Excel to perform statistical tests for significant change in peptide abundance between replicates from cases and controls.