Multiplexed protein quantification using the isobaric TMT method: Improving reproducibility and protein coverage with PD 2.1

Rosa Viner

HUPO 2015

TMT quantification in PD 2.0 and previous

- Using reporter ion intensity values from MS/MS of MS^3 spectrum
- Calculate ratios for each PSM based on intensity values
- Calculate peptide group and protein ratios using median and variability of PSM ratios
- “Normalization” of protein ratios using median ratio of “Top N” proteins

Changes to TMT quantification in PD 2.1

1) S/N thresholds
2) Protein abundances from summed peptide S/N
3) Scaled abundances
4) New UI for ratio calculation
5) Correction factors for TMT 10-plex
6) Option to include razor peptides for protein quantification

Note that almost of all these changes are also applied to isotope-labeled quantification (e.g. SILAC)

Collaboration with Harvard Medical School for TMT quantification

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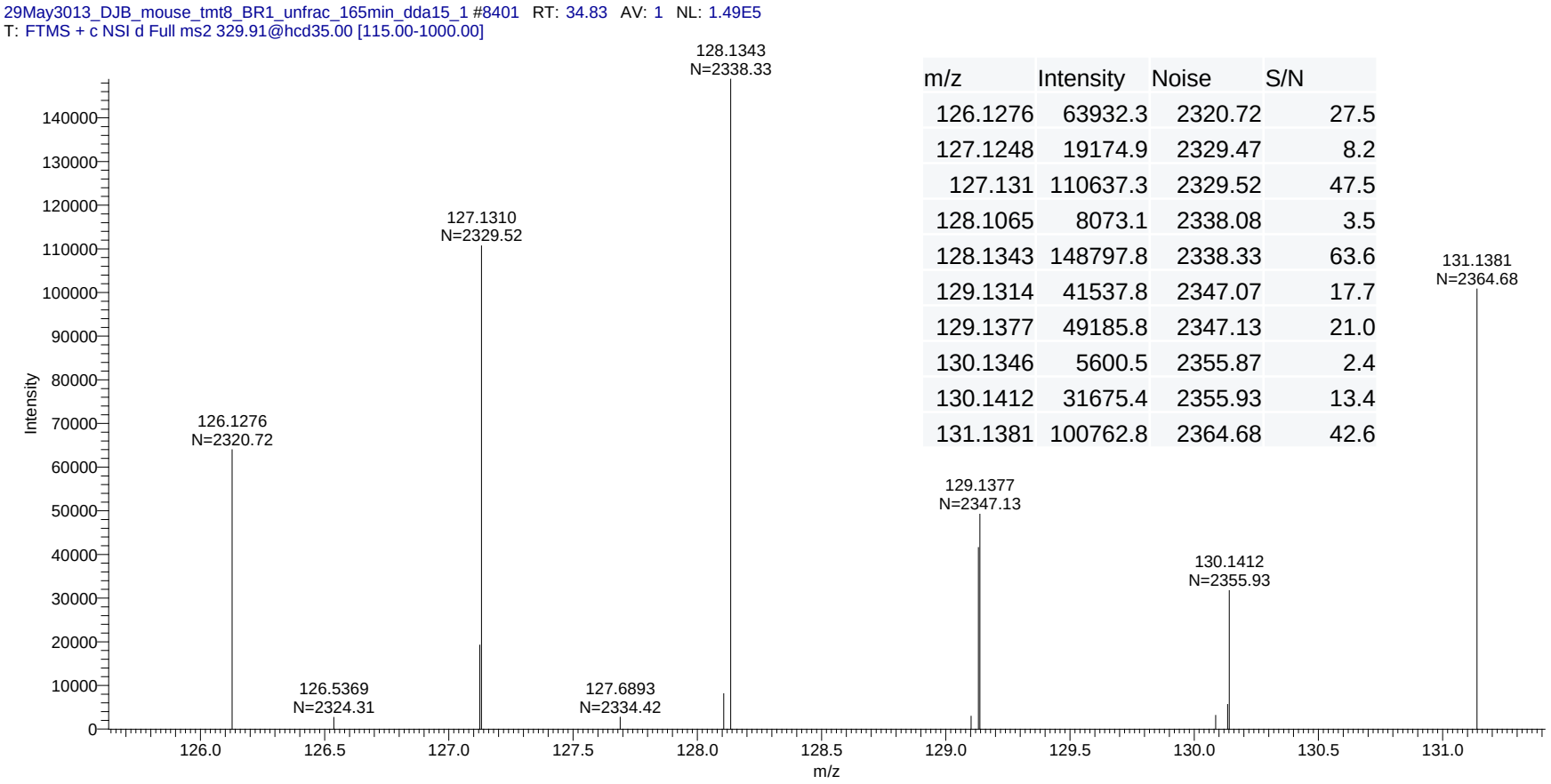
Gygi Method for TMT quantification – Step 1

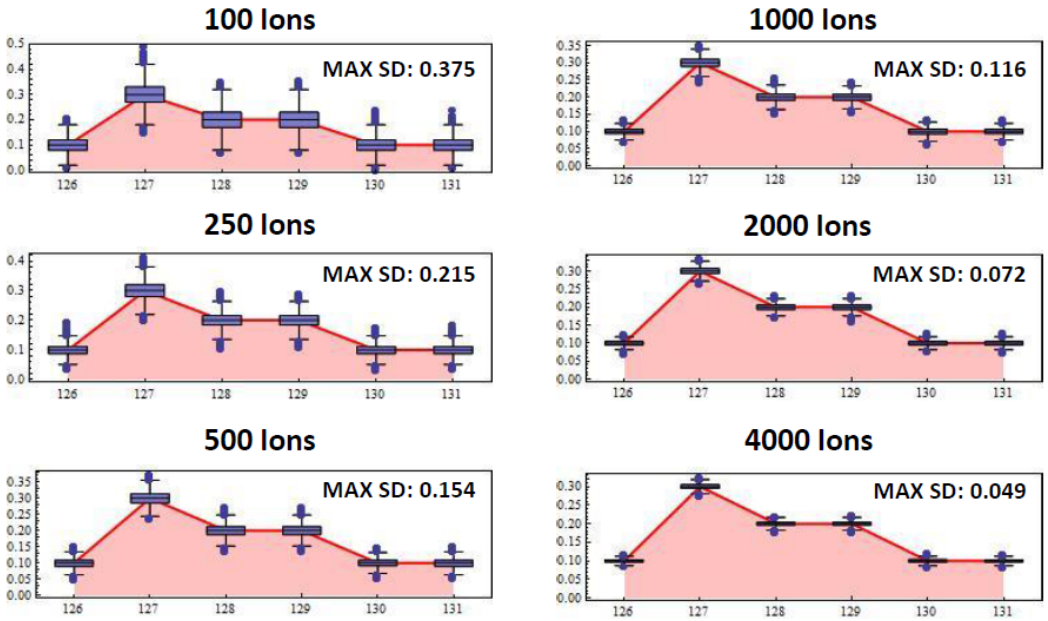
1. Extract S/N values for each reporter ion in the MS/MS data



m/z	Intensity	Noise	S/N
126.1276	63932.3	2320.72	27.5
127.1248	19174.9	2329.47	8.2
127.131	110637.3	2329.52	47.5
128.1065	8073.1	2338.08	3.5
128.1343	148797.8	2338.33	63.6
129.1314	41537.8	2347.07	17.7
129.1377	49185.8	2347.13	21.0
130.1346	5600.5	2355.87	2.4
130.1412	31675.4	2355.93	13.4
131.1381	100762.8	2364.68	42.6

Why S/N?

- S/N is proportional to the number of ions in any Orbitrap detector, while intensity measurements will differ across instruments
- The measurement error is related to the number of detected ions



Distributions determined via 10,000 simulations

S/N thresholds for different resolutions

S/N Thresholds Depend on Resolution and Channel Count

Target S/N Threshold (equivalent to ~400 ions for 6-plex)

Instrument	Conversion Factors for S/N Cutoff	S/N Cutoff for 6-Plex	S/N Cutoff for 8-Plex	S/N Cutoff for 10-Plex
7.5k Velos	0.7	59	NA	NA
15k Elite	1.0	80	NA	NA
30k Elite	1.4	114	152	189
42k Fusion (400 m/z)	2.9	232	309	386

$S/N \propto r^{0.5}$

Constant Mean # Charges per Channel

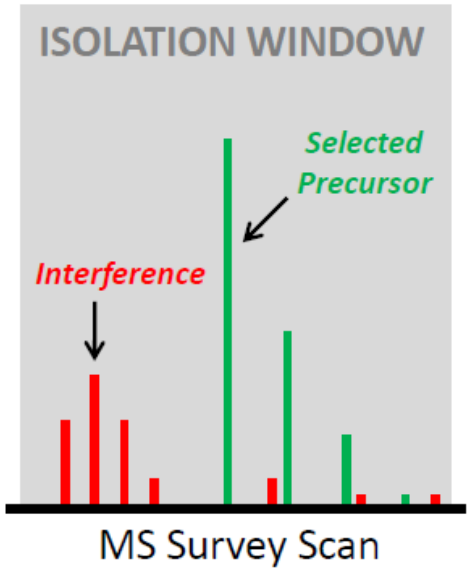
For a high-precision 10-plex TMT experiment, we recommend a minimum summed S/N of ~400 within each MS3 spectrum. This corresponds to less than 10% coefficient of variation between two equally abundant channels.

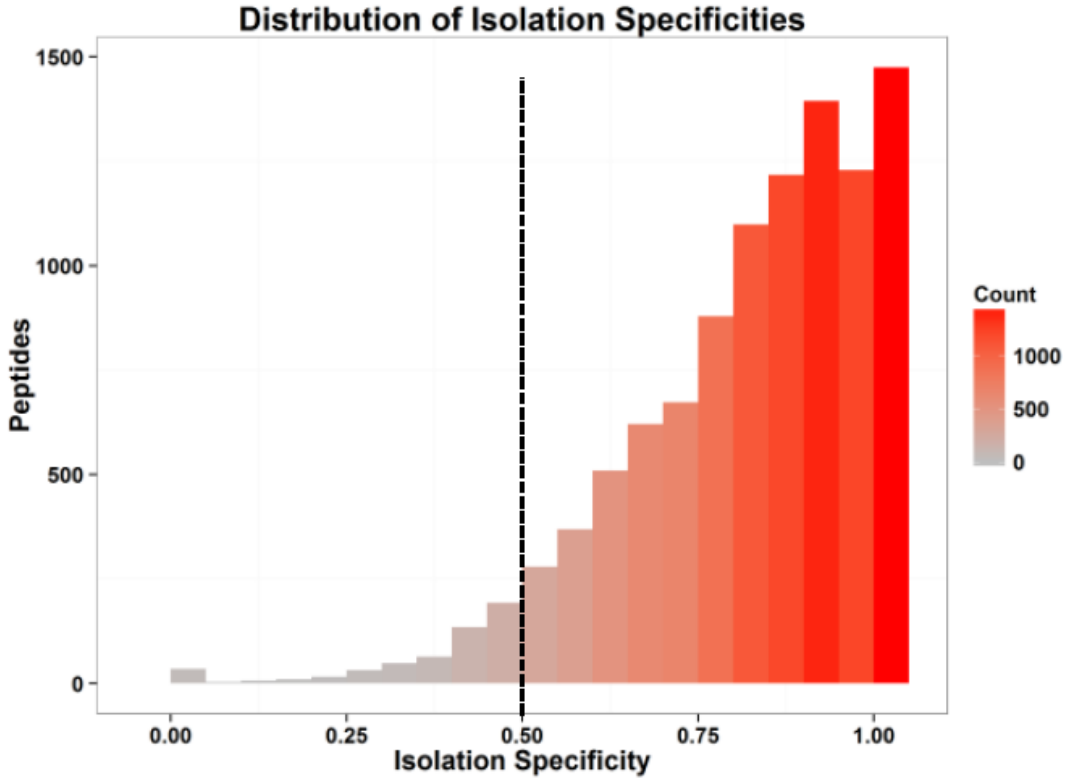
Different cutoffs may be appropriate for different applications, depending on the accuracy and precision required. For more routine applications, we require a minimum summed S/N of ~200

Martin Wuehr
Graeme McAlister

Gygi Method for TMT quantification – step 2

2. Filter peptides with precursor isolation interference





$$\text{Isolation Specificity} = \frac{\text{Target Isotopes}}{\text{Total Intensity}}$$

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Gygi Method for TMT quantification – step 3

3. Sum S/N values for peptides to summed protein S/N

1. Group peptides by protein
2. Filter peptides based on summed S/N and isolation specificity
3. Sum S/N for each reporter ion across all remaining peptides

Sequence	126	127N	127C	128	129N	129C	130	131	Sum S/N	Isolation Spec.
TLATDILMGVLK	103.8	95.6	78.8	122.6	74.5	104.0	87.7	90.7	757.7	0.9558
FQIYFDNCPLLTIPGR	21.9	17.1	14.1	26.1	11.4	18.9	14.3	17.0	140.8	1.000
REVDDLGPEVGDIK	25.2	18.3	16.2	24.7	13.2	17.9	21.3	15.0	151.8	0.6655
FQIYFDNCPLLTIPGR	42.0	37.0	37.1	46.4	31.8	47.0	29.5	36.6	307.4	0.8513
YGVIILDEAHER	231.2	202.4	176.5	265.7	156.1	197.7	239.1	190.9	1659.6	0.4753
LDLGEDYPSGK	135.6	108.9	119.3	135.6	104.3	134.2	120.2	115.0	973.1	0.8195
DRFTDILVR	117.2	109.4	97.8	120.0	82.0	103.3	97.1	98.2	825	0.9332
EAMNDPLLER	60.7	52.0	39.5	59.6	37.5	59.8	55.1	52.4	416.6	0.9313
RGVACTQPR	6.235	8.117	7.819	8.547	10.4	13.8	15.6	9.85	80.366	0.9902
SLMSADNVR	141.8	111.6	124.0	157.4	91.4	135.9	136.3	111.5	1009.9	0.7475
TOTALS:	**601.1**	**514.5**	**496.7**	**641.6**	**421.5**	**584.2**	**525.9**	**504.4**	**4289.7**	---

S/N too low

Too much precursor interference

S/N too low

Sum totals

DHX15 Peptides

Gygi Method for TMT quantification – step 4

4. Normalization based on total protein S/N

Gene Symbol	126	127N	127C	128N	128C	129N	129C	130N	130C	131
SLC12A2	2591	2696	2534	2606	2597	2605	2574	2675	3662	3667
SOX21	0	0	0	0	0	0	0	0	0	0
ZFP91	284	172	232	111	107	98	71	287	0	0
INCENP	2186	2317	2187	2277	2272	2313	2248	2226	2697	2796
PHF20L1	468	435	442	473	442	487	452	445	1169	1093
CDK12	3467	3597	3473	3453	3414	3505	3339	3412	4000	3927
CDK13	765	732	767	829	705	869	828	725	1364	1346
CDK13	200	196	186	183	215	208	207	207	0	0
CDK2	5697	5797	5730	5506	5756	5538	5408	5358	4935	5357
YES1	1162	1158	1149	1155	1187	1176	1123	1164	2275	1976
LCK	4503	4470	4548	4497	4542	4598	4524	4536	4149	4040
BLK	1856	1790	1861	1912	1865	1847	1864	1852	3742	3646
CDK9	1444	1434	1421	1421	1444	1498	1457	1346	1789	1798
LYN	9187	9006	9190	8878	8573	8822	8517	8582	9847	9408
LYN	126	117	114	126	130	130	115	135	208	211
CDK6	4912	4937	4805	4857	4912	4886	4905	4859	3654	3576
CDK1	8564	8607	8582	8221	8824	8353	8468	8048	7740	8164
CDK5	2448	2674	2435	2467	2624	2472	2510	2629	2089	2163

1. Sum TMT signal For each column: 47411 47463 47221 46504 46985 46934 46101 45858 51229 51004

2. Norm. Factors: 0.926 0.927 0.922 0.908 0.917 0.916 0.899 0.895 1.000 0.996

3. Multiply each row by normalization factors to get normalized values.

Gygi Method for TMT quantification – Step 4

4. Normalization using a single selected protein

Gene Symbol	126	127N	127C	128N	128C	129N	129C	130N	130C	131
SLC12A2	2591	2696	2534	2606	2597	2605	2574	2675	3662	3667
SOX21	0	0	0	0	0	0	0	0	0	0
ZFP91	284	172	232	111	107	98	71	287	0	0
INCENP	2186	2317	2187	2277	2272	2313	2248	2226	2697	2796
PHF20L1	468	435	442	473	442	487	452	445	1169	1093
CDK12	3467	3597	3473	3453	3414	3505	3339	3412	4000	3927
CDK13	765	732	767	829	705	869	828	725	1364	1346
CDK13	200	196	186	183	215	208	207	207	0	0
CDK2	5697	5797	5730	5506	5756	5538	5408	5358	4935	5357
YES1	1162	1158	1149	1155	1187	1176	1123	1164	2275	1976
LCK	4503	4470	4548	4497	4542	4598	4524	4536	4149	4040
BLK	1856	1790	1861	1912	1865	1847	1864	1852	3742	3646
CDK9	1444	1434	1421	1421	1444	1498	1457	1346	1789	1798
LYN	9187	9006	9190	8878	8573	8822	8517	8582	9847	9408
LYN	126	117	114	126	130	130	115	135	208	211
CDK6	4912	4937	4805	4857	4912	4886	4905	4859	3654	3576
CDK1	8564	8607	8582	8221	8824	8353	8468	8048	7740	8164
CDK5	2448	2674	2435	2467	2624	2472	2510	2629	2089	2163

1. TMT Signal for Target Protein: 5697 5797 5730 5506 5756 5538 5408 5358 4935 5357

2. Norm. Factors: 0.983 1.000 0.989 0.949 0.993 0.955 0.933 0.924 0.851 0.924

3. Multiply each row by normalization factors to get normalized values.

Gygi Method for TMT quantification – Step 5

5. Scale total intensity across channels to 100%

Value	126	127N	127C	128N	128C	129N	129C	130N	130C	131	SUM
Normalized Intensity	2591	2696	2534	2606	2597	2605	2574	2675	3662	3667	28207
Fraction of Ions (Scaled to 100)	9.19	9.56	8.98	9.24	9.21	9.23	9.13	9.49	12.98	13.00	

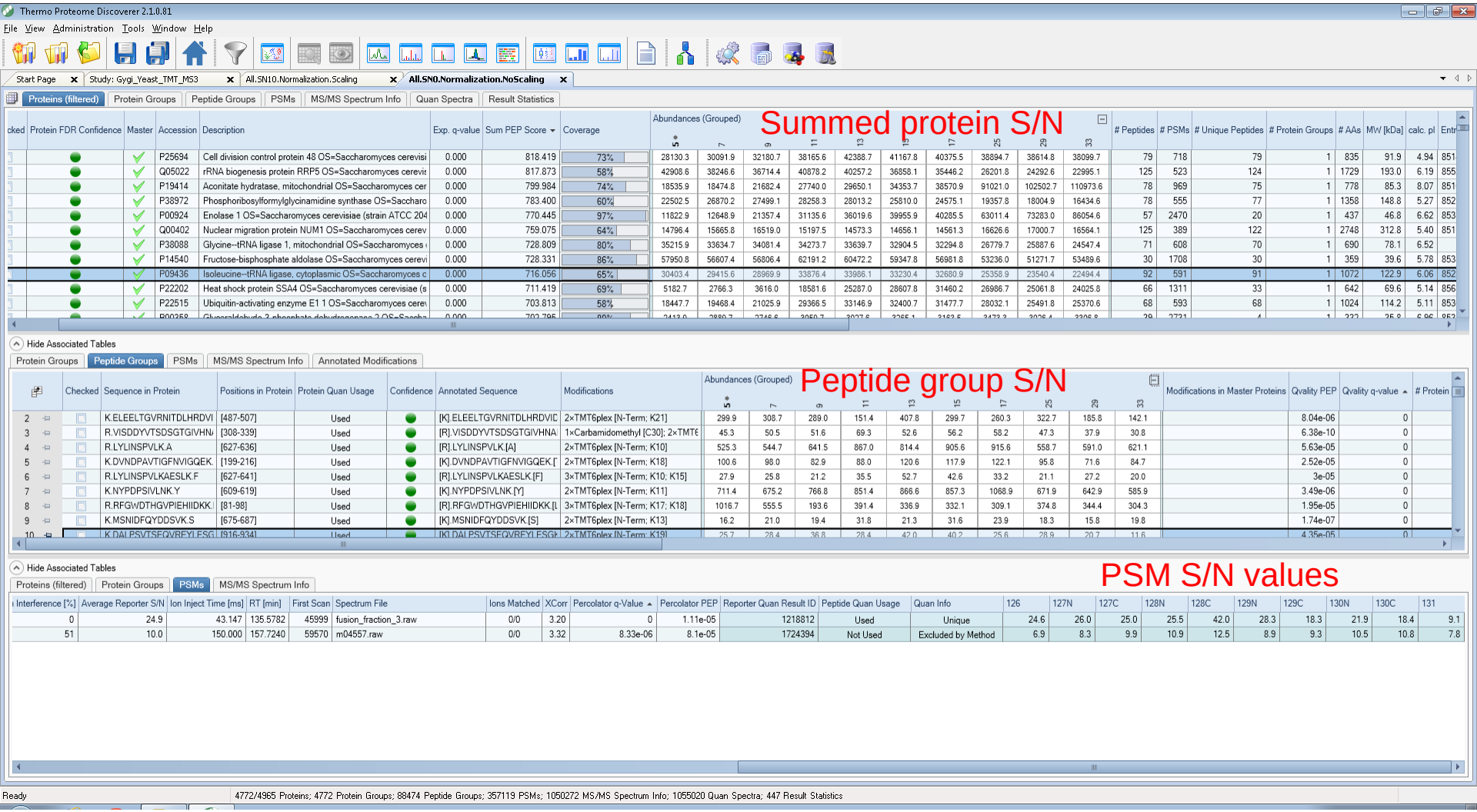
Scaling Procedure:

1. Sum reporter ion intensity across all channels
2. Divide each column by the summed intensity
3. Multiply by 100

Why Bother Rescaling?

1. Corrects for differences in reporter ion abundance due to different numbers of observed peptides
2. Enables better heat map generation
3. Facilitates comparison of quantitative profiles from protein to protein
4. Formats data for downstream analysis (statistics, clustering, PCA, etc)

Summed S/N values displayed in PD 2.1



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Pros/Cons of the summed S/N approach

Pros:

- Higher abundance peptides are weighted more strongly
- Outliers due to low abundance peptides are eliminated
- Summed S/N values are easily profiled
- Summed S/N values are tolerant of missing values
  - Ratios will produce 0 or infinite values for reporter ions with 0 intensity
- Very straightforward

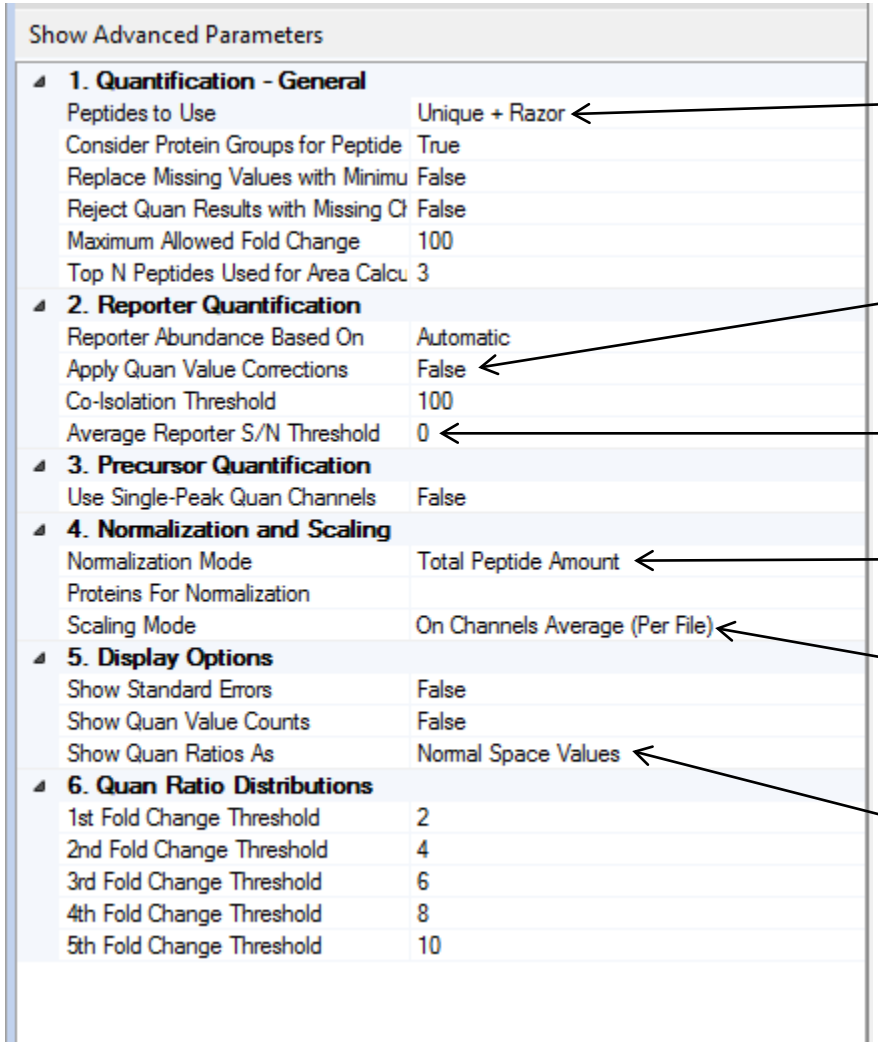
Cons:

- No measure of variability of individual peptide measurements

PD 2.1 TMT quantification

- Slight modifications to the Gygi approach
  - Use “Average S/N” threshold across all channels
    - More easily applied to reporter ion quantification methods with different numbers of quantification channels
    - User enters a single S/N value
  - Normalize on total peptide signal rather than protein signal
    - Summed peptide group abundances across each sample
    - More like a TIC normalization
  - Scale each channel to an average of 100% rather than a total of 100%
    - Easier to see which channels are changing
    - Easier to choose heat map colors
  - Ratios are still calculated
    - User has a choice to inspect the summed S/N values, the scaled abundances, or the ratios for any given peptide or protein

Protein and peptide quantifier node in Consensus workflow (new parameters in PD 2.1)



Razor peptides

Correction factors

Average S/N per channel

New normalization options

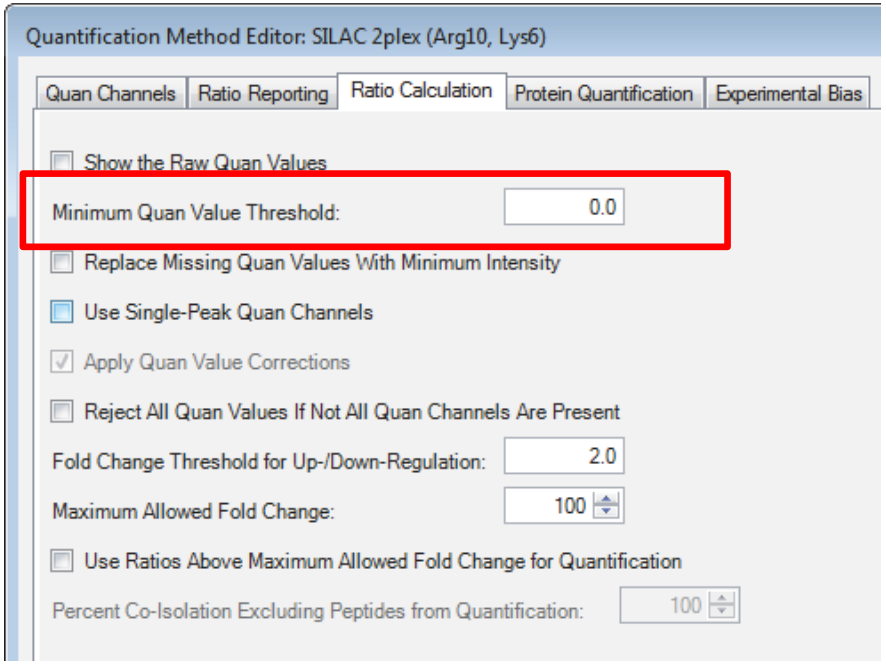
Scaling

Option to display log2 ratios

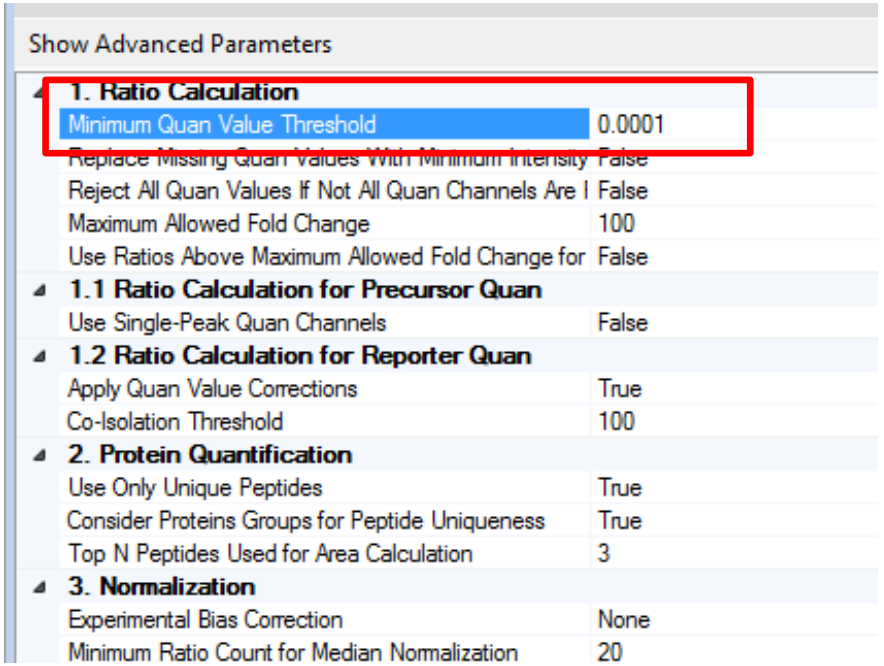
Previous versions of PD – use intensity thresholds

- User can set an intensity threshold for acceptance of a reporter ion in a given PSM for use in peptide group and protein quantification

PD 1.4



PD 2.0



PD 2.1 S/N calculation

- PD 2.1 asks for an **Average** S/N value across all measured report ions for a given method

2. Reporter Quantification	
Reporter Abundance Based On	Automatic
Apply Quan Value Corrections	True
Co-Isolation Threshold	50
Average Reporter S/N Threshold	10

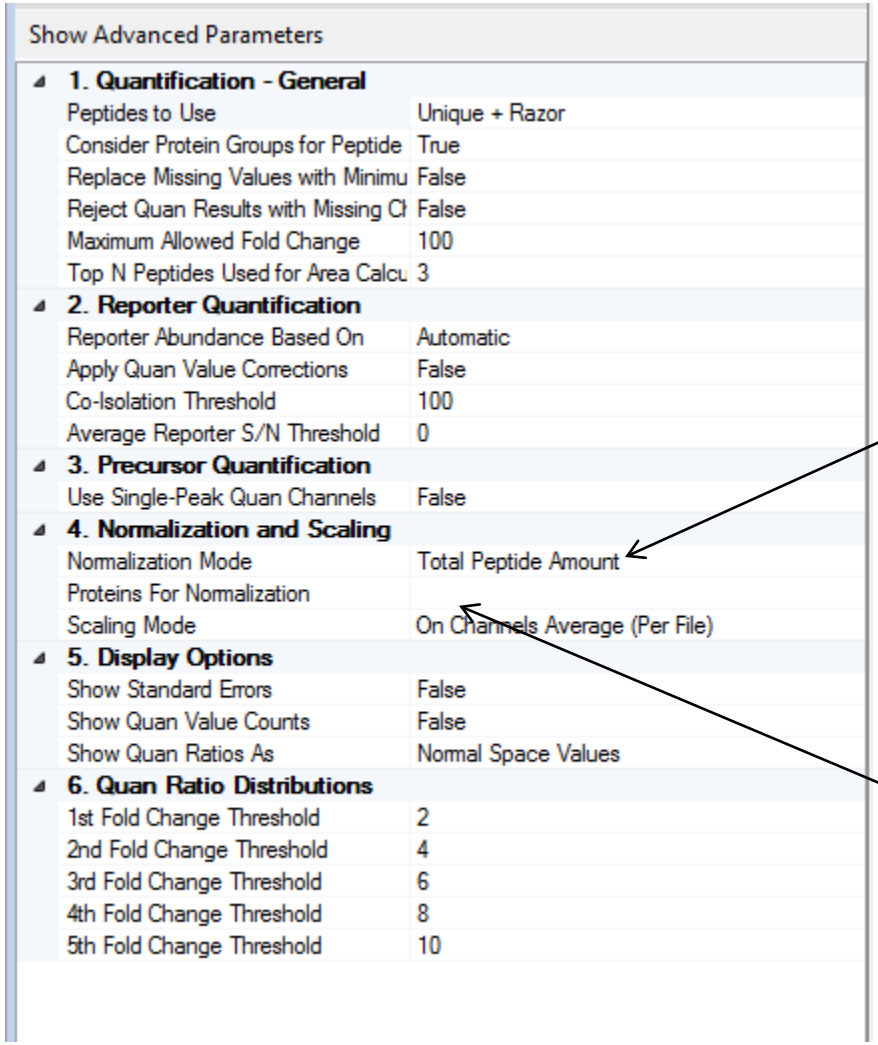
- If the average S/N value is above the user set value, the PSM is included in the peptide and protein quantification:

ter Quan Result ID	Peptide Quan Usage	Quan Info	126	127N	127C	128N	128C	129N	129C	130N	130C	131
		Unique	19.0	23.0	19.6	19.0	17.3	16.7	12.4	17.0	14.0	9.3
1486778	Used	Unique	12.5	19.9	21.7	12.0	17.4	11.0	15.6	17.2	20.5	18.3
1244696	Used	Unique	3.6	10.8	8.8	15.8	11.9	12.5	9.4	7.0	11.1	10.3
1244694	Not Used	Excluded by Method	8.6	10.4	7.0	9.5	8.0	10.0	6.6	11.0	8.5	7.9
1244693	Not Used	Excluded by Method	2.2	1.6	1.5	1.5	3.4	3.4	3.0	5.8		2.3
1384282	Used	Unique	114.2	103.4	52.4	71.4	37.9	16.5	16.5	9.0	44.4	16.8
		Unique	44.3	42.7	35.9	42.2	49.9	33.4	31.6	33.0	35.9	26.3
		Unique	57.3	46.4	54.2	60.5	66.6	63.2	82.7	48.0	60.3	51.4
1244688	Not Used	Excluded by Method	3.8	7.6	7.3	7.2	12.7	5.9	6.5	6.4	3.2	6.7
1244684	Used	Unique	27.6	24.0	17.0	20.6	18.4	10.8	16.3	12.0	11.1	11.3

Average = 10.1 (> 10)

Average = 8.75 (< 10)

Normalization in PD 2.1



New normalization options:

1) Total Peptide Amount
2) Specific Protein Amount
3) None

If specific protein amount is chosen, the user can choose an indexed FASTA files with the list of proteins to use for normalization.

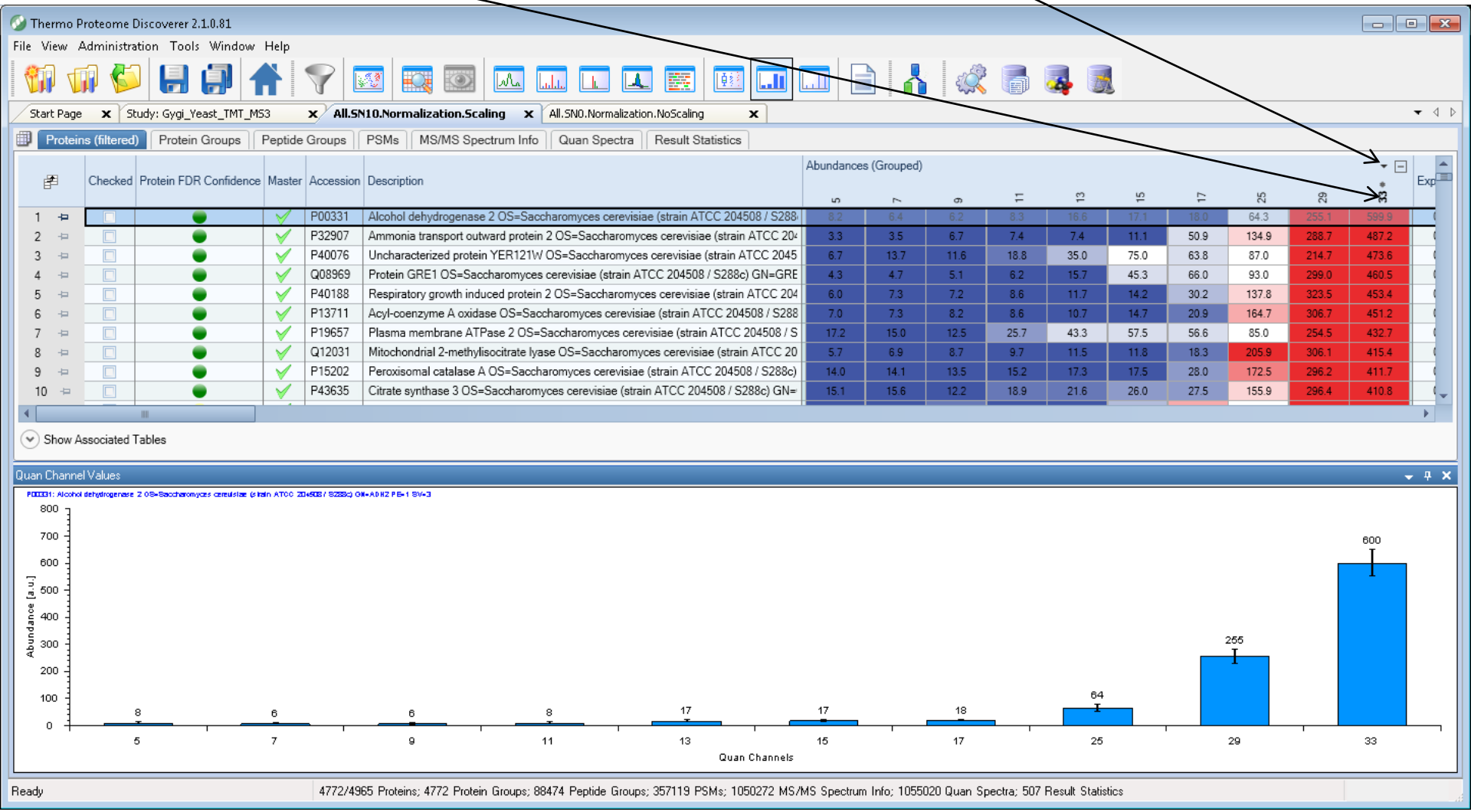
Scaling in PD 2.1

- Averaged scaled abundance equals 100 regardless of number of channels
- Scaled abundance values are displayed for peptide groups and proteins
- Samples or channels with higher abundance will be colored red while samples or channels with lower abundance are colored blue:

P19414	Aconitate hydratase, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 2045(	37.6	37.4	43.8	56.2	60.1	69.7	78.4	183.9	208.0	225.0
P38972	Phosphoribosylformylglycinamidine synthase OS=Saccharomyces cerevisiae (strain A	94.6	113.1	116.8	119.0	117.0	108.5	103.3	81.8	76.1	69.8
P00924	Enolase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ENO1 P	28.6	30.5	51.2	74.8	86.6	96.2	97.1	151.3	176.5	207.3
Q00402	Nuclear migration protein NUM1 OS=Saccharomyces cerevisiae (strain ATCC 204508	94.3	100.1	106.1	97.5	93.3	94.0	93.4	106.4	108.8	106.2
P38088	Glycine--tRNA ligase 1, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 20	111.9	106.8	108.8	108.8	107.5	105.6	103.4	85.9	82.7	78.7
P14540	Fructose-bisphosphate aldolase OS=Saccharomyces cerevisiae (strain ATCC 204508	101.6	99.5	99.8	109.5	106.5	104.6	100.3	93.7	90.2	94.1
P09436	Isoleucine--tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae (strain ATCC 204	104.0	100.2	98.8	116.0	115.2	112.7	110.9	86.2	79.8	76.1
P22202	Heat shock protein SSA4 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288(	27.1	14.4	18.7	97.0	132.4	149.4	164.6	140.6	130.7	124.9

Sorting scaled abundance columns in PD 2.1

- Select column name and click up or down triangle to sort:



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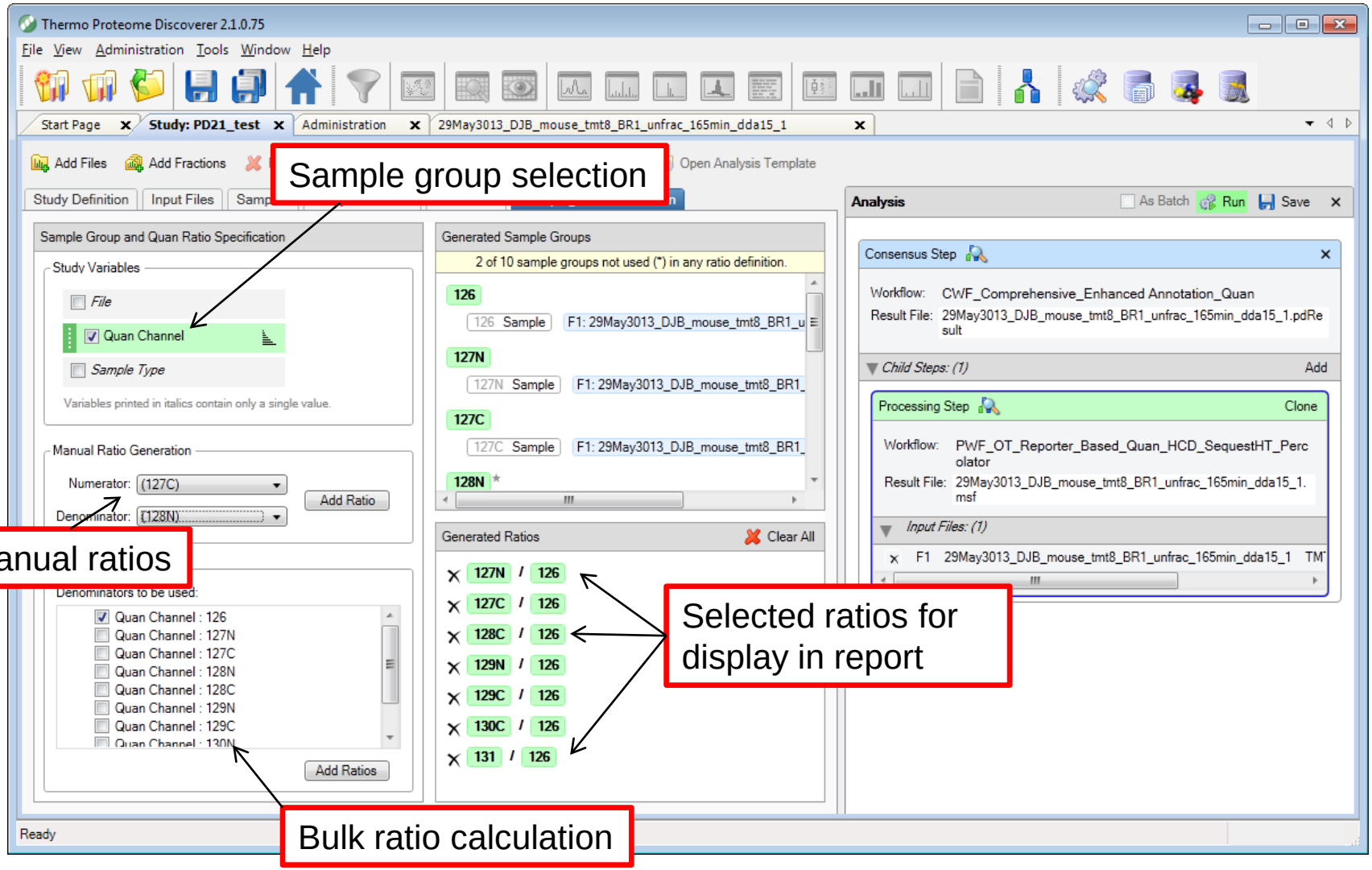
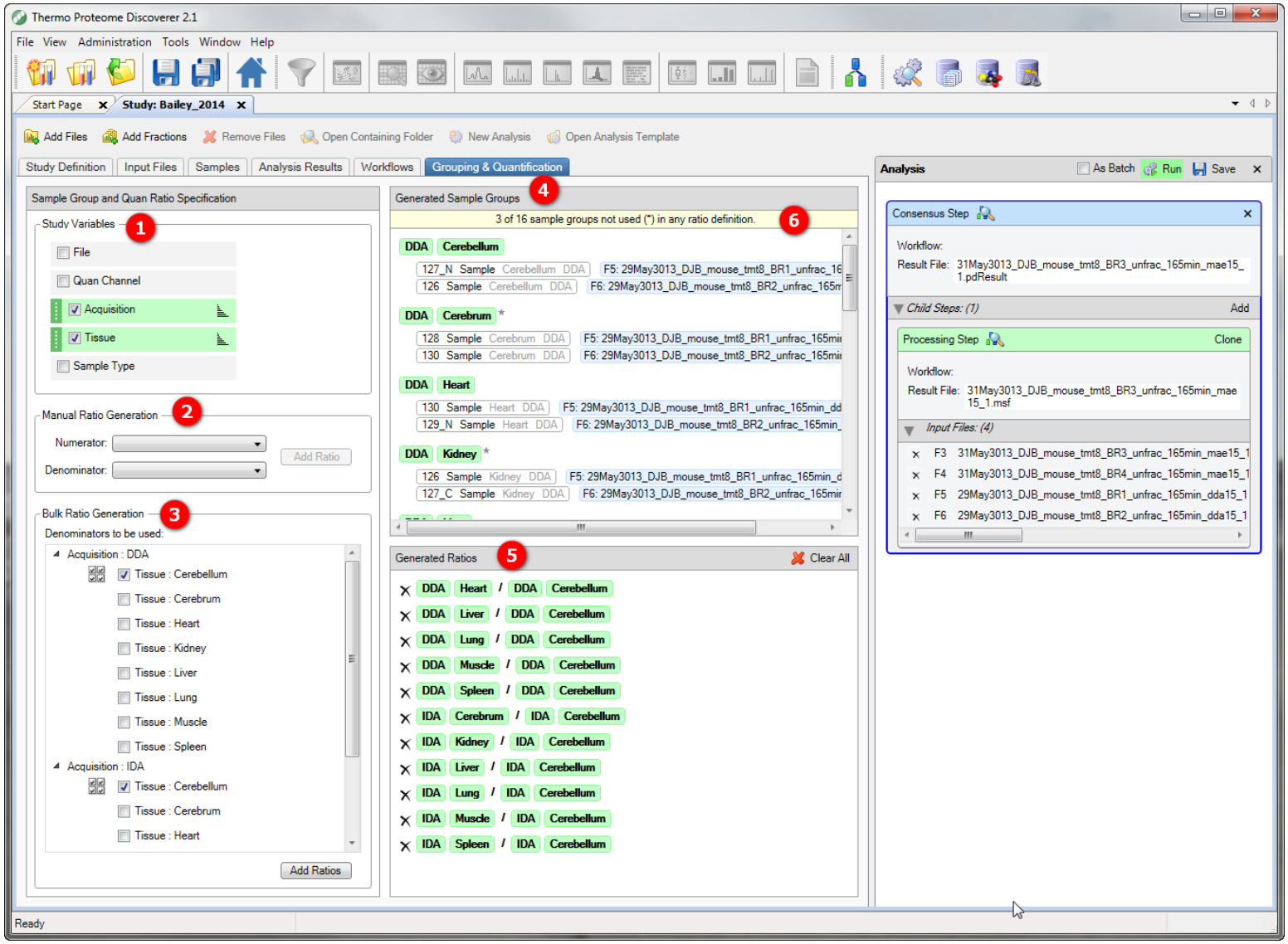
New custom ratio calculation in PD 2.1



Custom ratios in PD 2.1 – example 2



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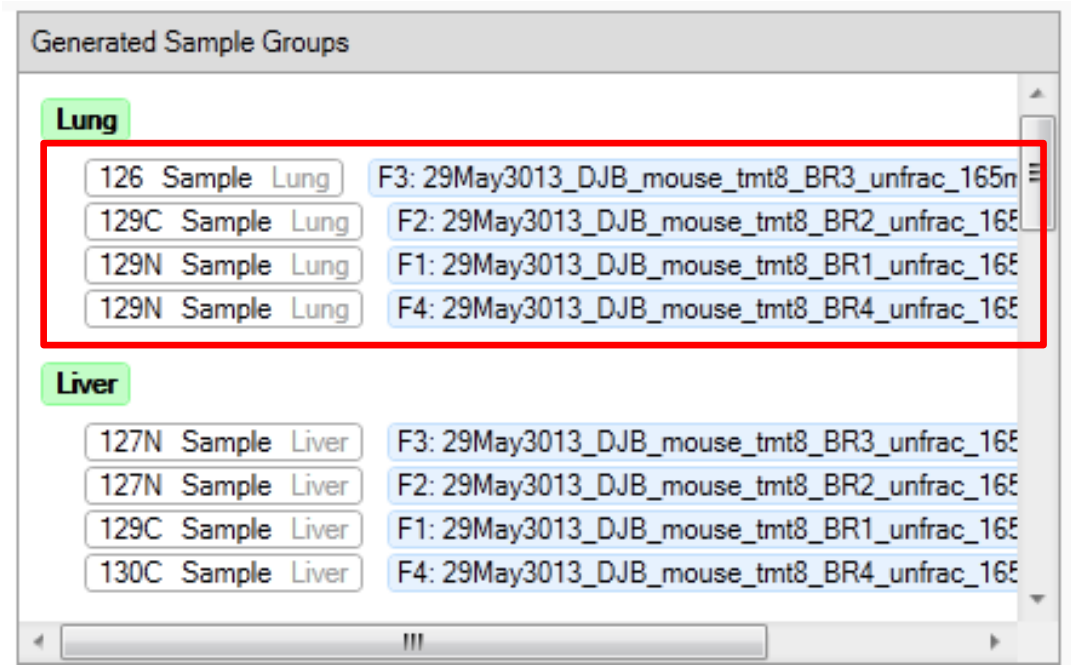
What about statistics?

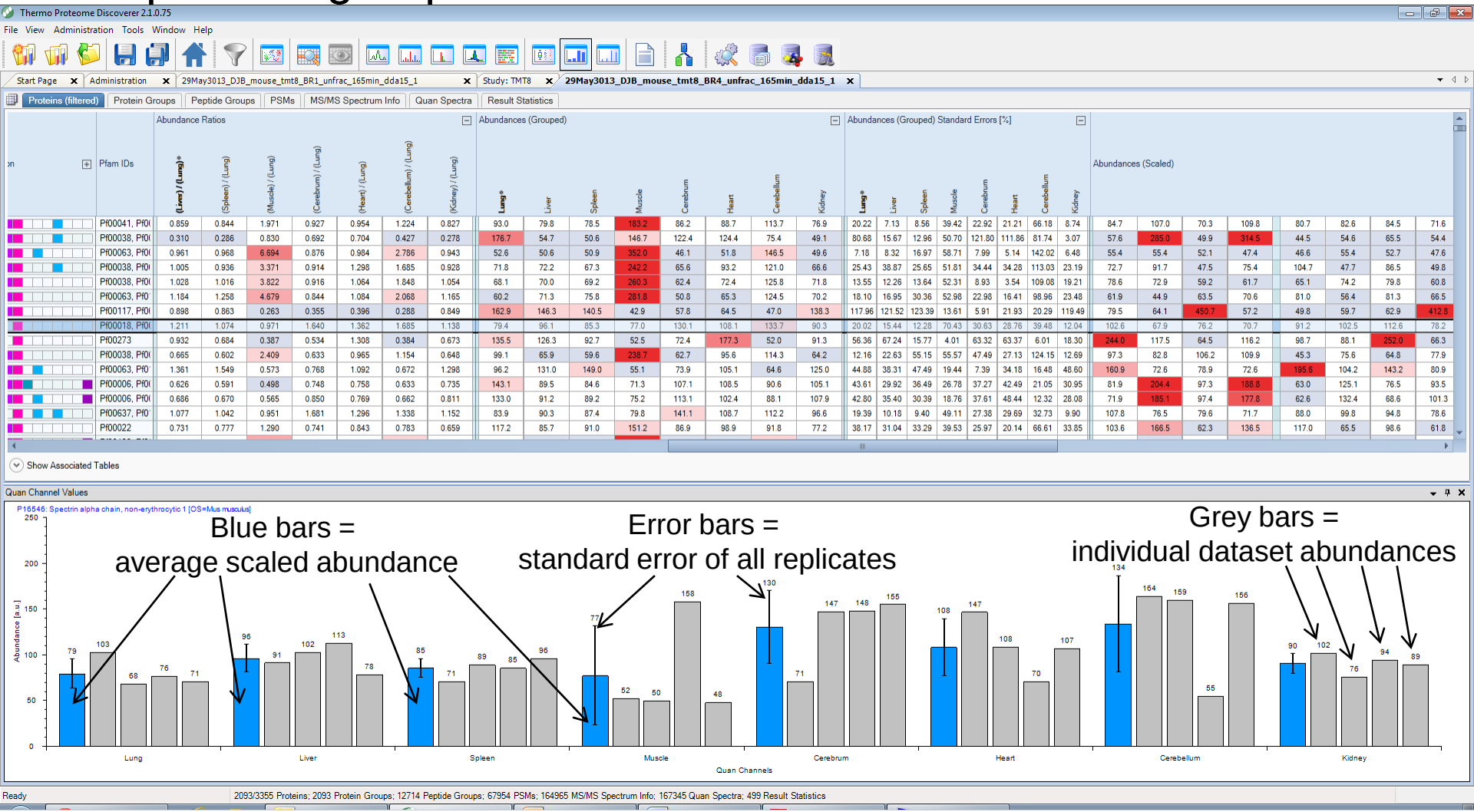
- Ratio variabilities no longer available for a single experiment
- However, when analyzing replicates, standard errors are calculated for ratios
- How do I know when I have replicate data?



- Value in final result will be the **average** of the four datasets
- Standard errors will be calculated for each peptide and protein across replicates

Results from biological replicate search

- Replicates grouped into ratios + standard errors



TMT 10-plex correction factor certificate

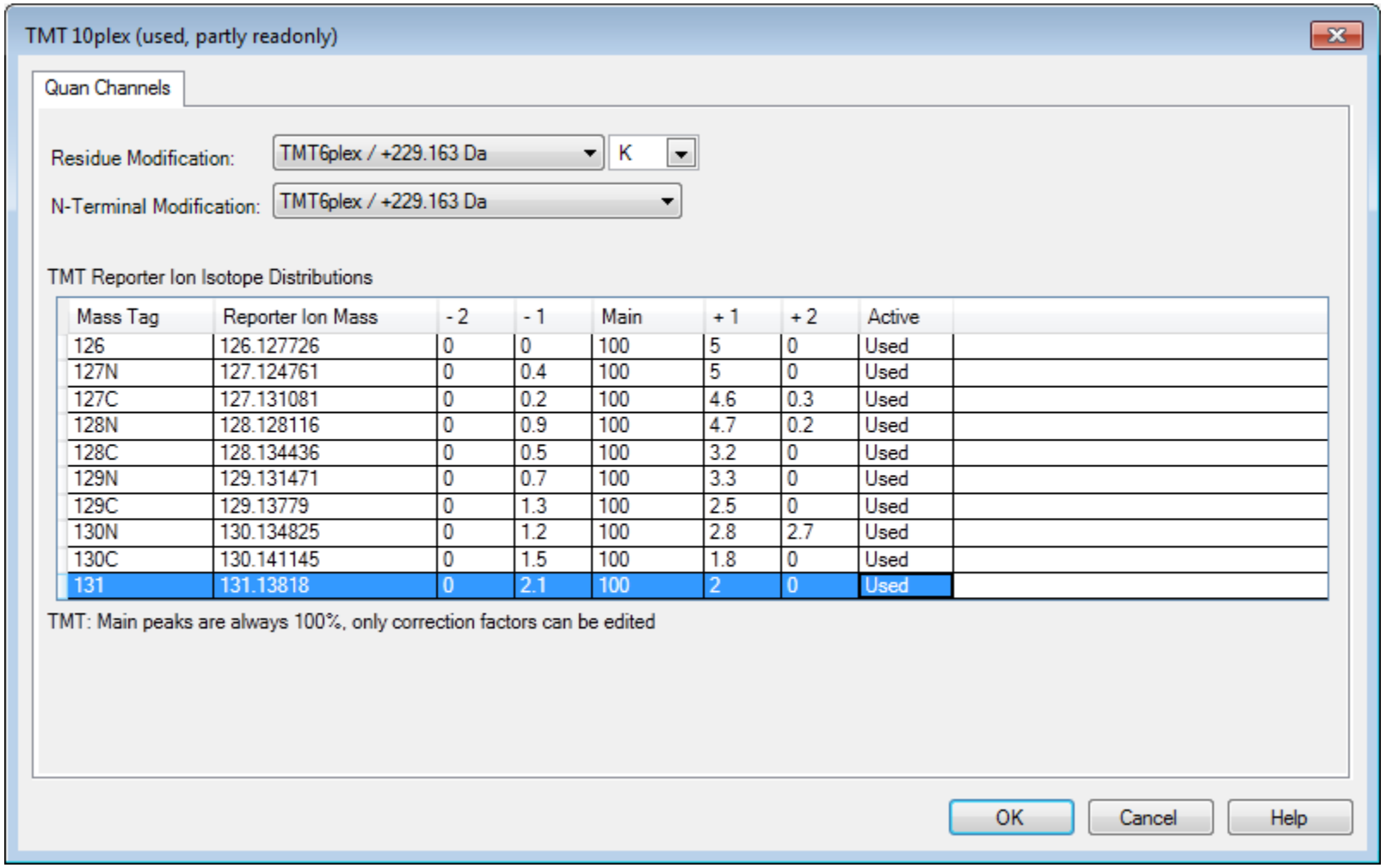
**Reporter Ion Isotopic Distributions:

Mass Tag	Reporter Ion	-2	-1	Monoisotopic	+1	+2
TMT^{10}-126	126.127726	0.0%	0.0%	**100%**	5.0% (127C)	0.0% (128N)
TMT^{10}-127N	127.124761	0.0%	0.4%	**100%**	5.0% (128N)	0.0% (128C)
TMT^{10}-127C	127.131081	0.0%	0.2% (126)	**100%**	4.6% (128C)	0.3% (129N)
TMT^{10}-128N	128.128116	0.0%	0.9% (127N)	**100%**	4.7% (129N)	0.2% (129C)
TMT^{10}-128C	128.134436	0.0% (126)	0.5% (127C)	**100%**	3.2% (129C)	0.0% (130N)
TMT^{10}-129N	129.131471	0.0% (127N)	0.7% (128N)	**100%**	3.3% (130N)	0.0% (130C)
TMT^{10}-129C	129.137790	0.0% (127C)	1.3% (128C)	**100%**	2.5% (130C)	0.0% (131)
TMT^{10}-130N	130.134825	0.0% (128N)	1.2% (129N)	**100%**	2.8% (131)	2.7%
TMT^{10}-130C	130.141145	0.0% (128C)	1.5% (129C)	**100%**	1.8%	0.0%
TMT^{10}-131	131.138180	0.0% (129N)	2.1% (130N)	**100%**	2.0%	0.0%

Stability: One year from date of product receipt.

Storage: Store at -20°C.

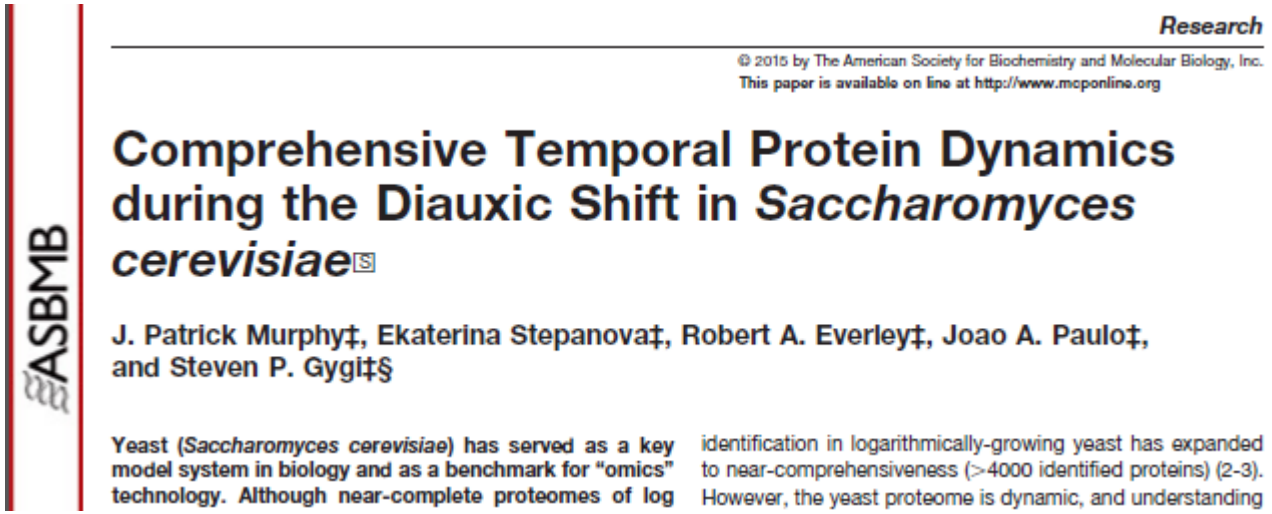
New user interface for adding correction factors



Correction factors are not required for high resolution TMT 6-plex or iodo-TMT experiments

Example Dataset – Gygi group MCP paper on diauxic shift in yeast

- TMT 10-plex experiment on an Orbitrap Fusion using the TMT MS3 method
- Monitored yeast protein abundances while monitoring glucose depletion
- 10 time points (5, 7, 9, 11, 13, 15, 17, 25, 29, 33 hr), 3 replicates, each with 12 fractions



ASBMB

Research

Comprehensive Temporal Protein Dynamics during the Diauxic Shift in *Saccharomyces cerevisiae*

J. Patrick Murphy‡, Ekaterina Stepanova‡, Robert A. Everley‡, Joao A. Paulo‡, and Steven P. Gygi‡§

Yeast (*Saccharomyces cerevisiae*) has served as a key model system in biology and as a benchmark for "omics" technology. Although near-complete proteomes of log identification in logarithmically-growing yeast has expanded to near-comprehensiveness (>4000 identified proteins) (2-3). However, the yeast proteome is dynamic, and understanding

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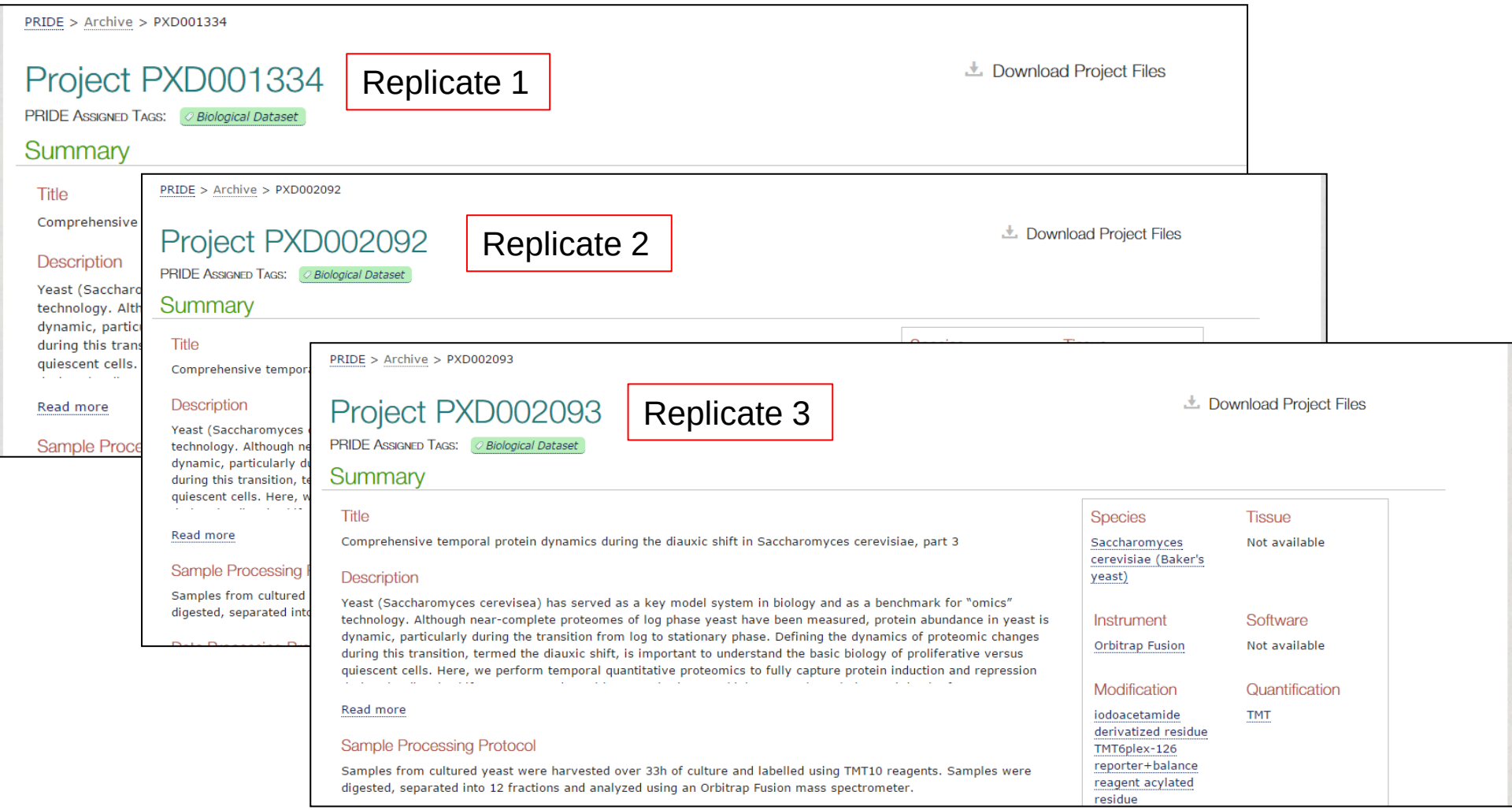
Yeast datasets are available for download from PRIDE



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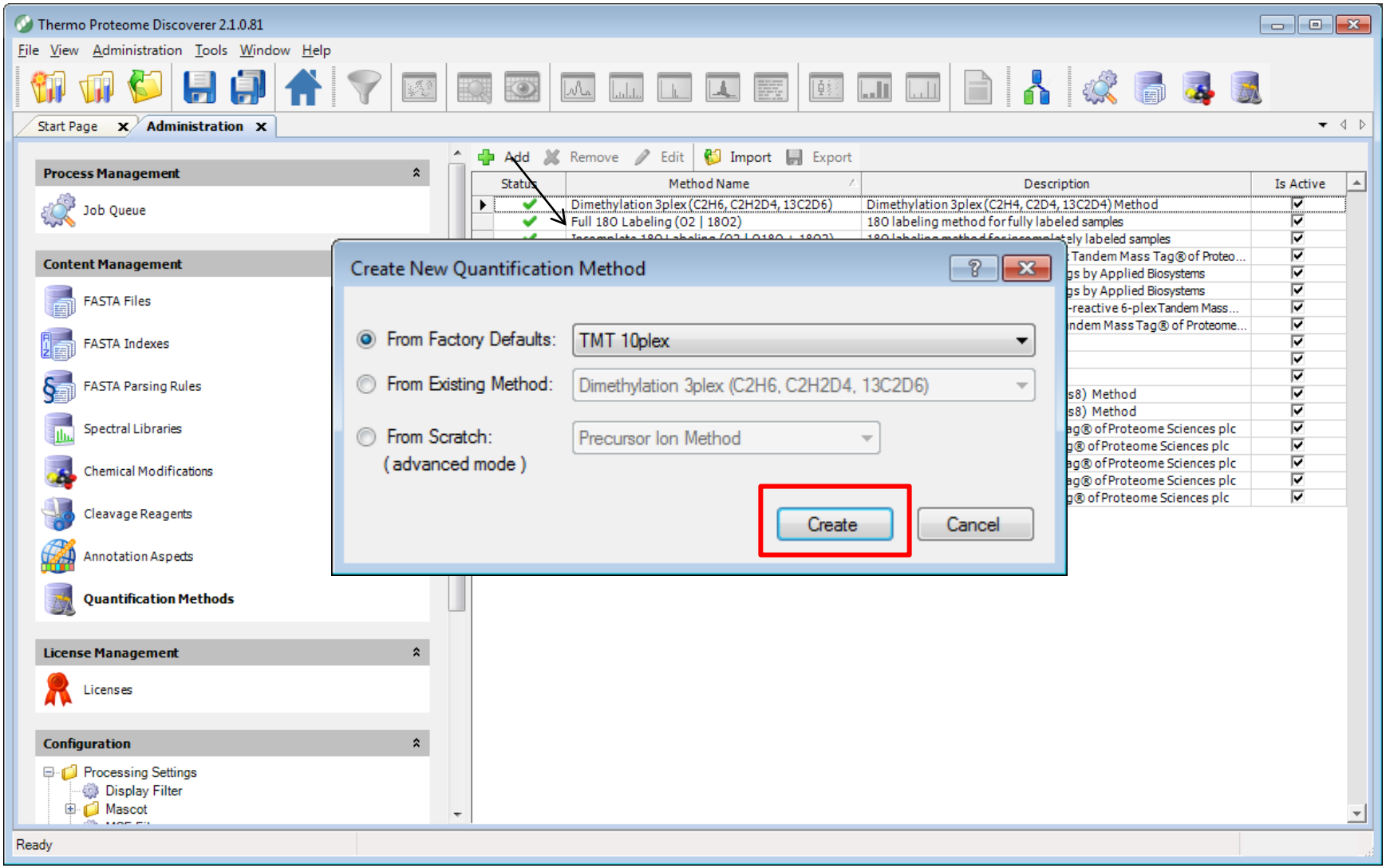
Step 1 – Create new quantitative method using correction factors from TMT CofA



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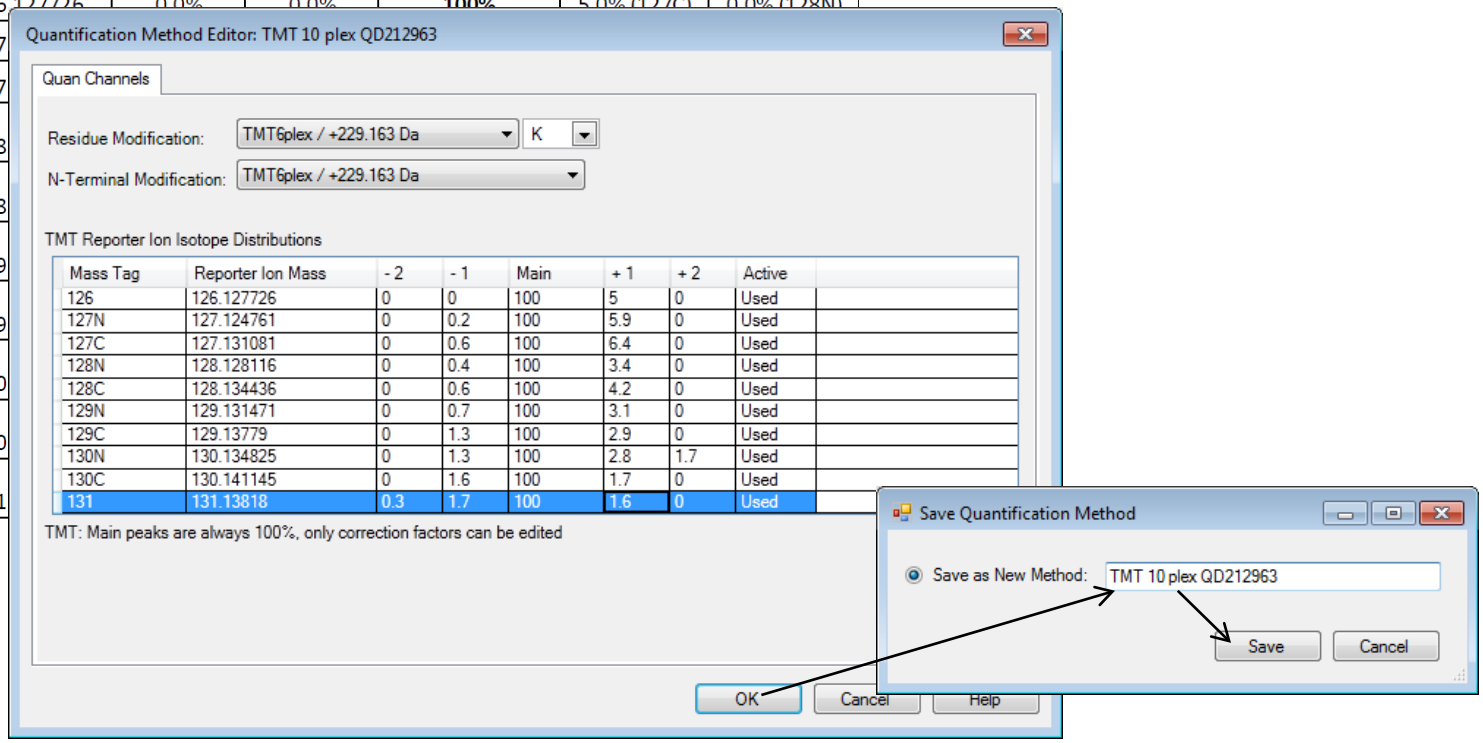
Step 1 (cont.) -

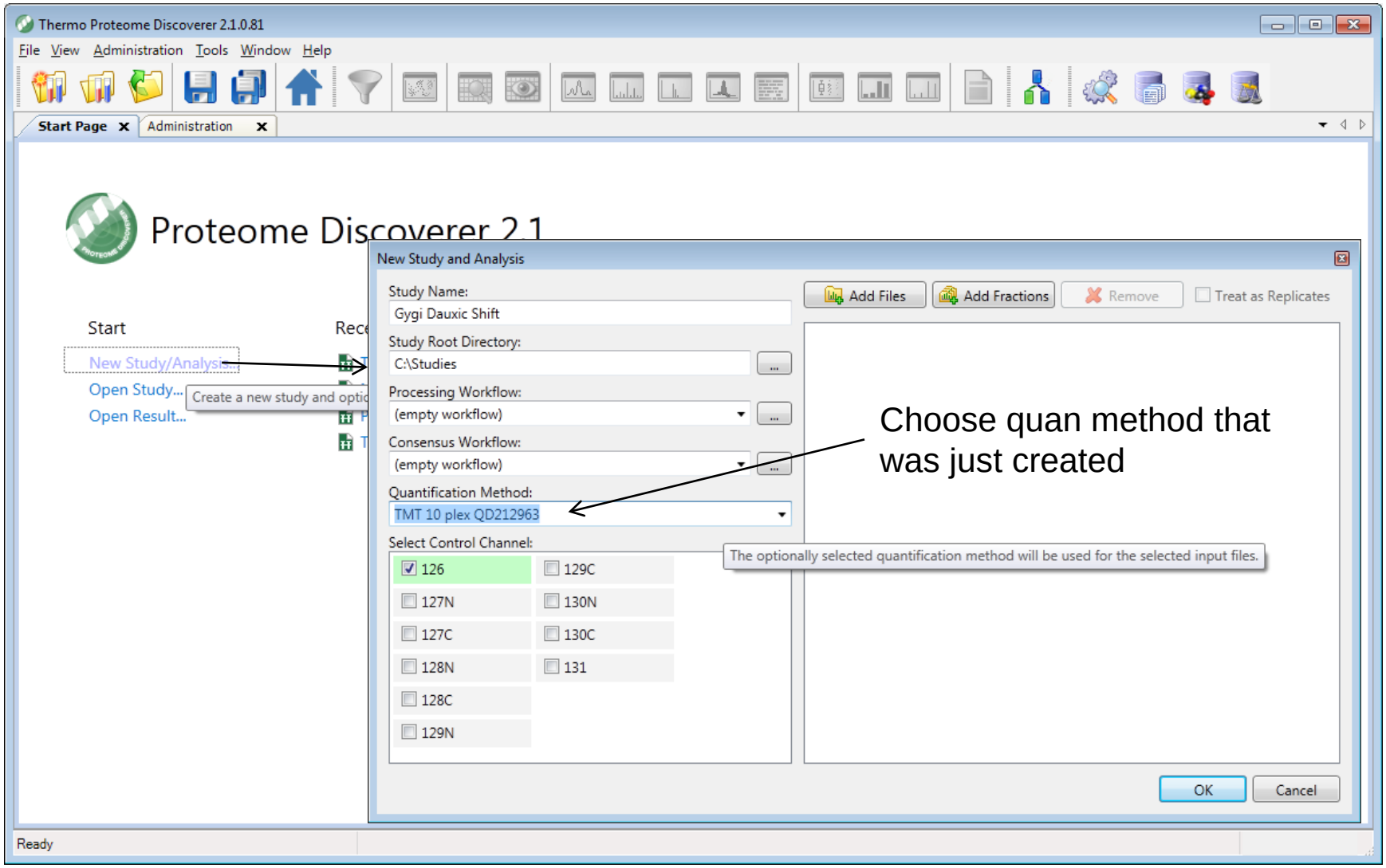
- Type in percentages exactly as shown in certificate (numbers between 0 and 100):

Mass Tag	Reporter Ion	-2	-1	Monoisotopic	+1	+2
TMT^{10}-126	126.127726	0.0%	0.0%	100%	5.0% (127C)	0.0% (128N)
TMT^{10}-127N	127					
TMT^{10}-127C	127					
TMT^{10}-128N	128					
TMT^{10}-128C	128					
TMT^{10}-129N	129					
TMT^{10}-129C	129					
TMT^{10}-130N	130					
TMT^{10}-130C	130					
TMT^{10}-131	131					



- Save as new method.

Step 2 - Create New Study



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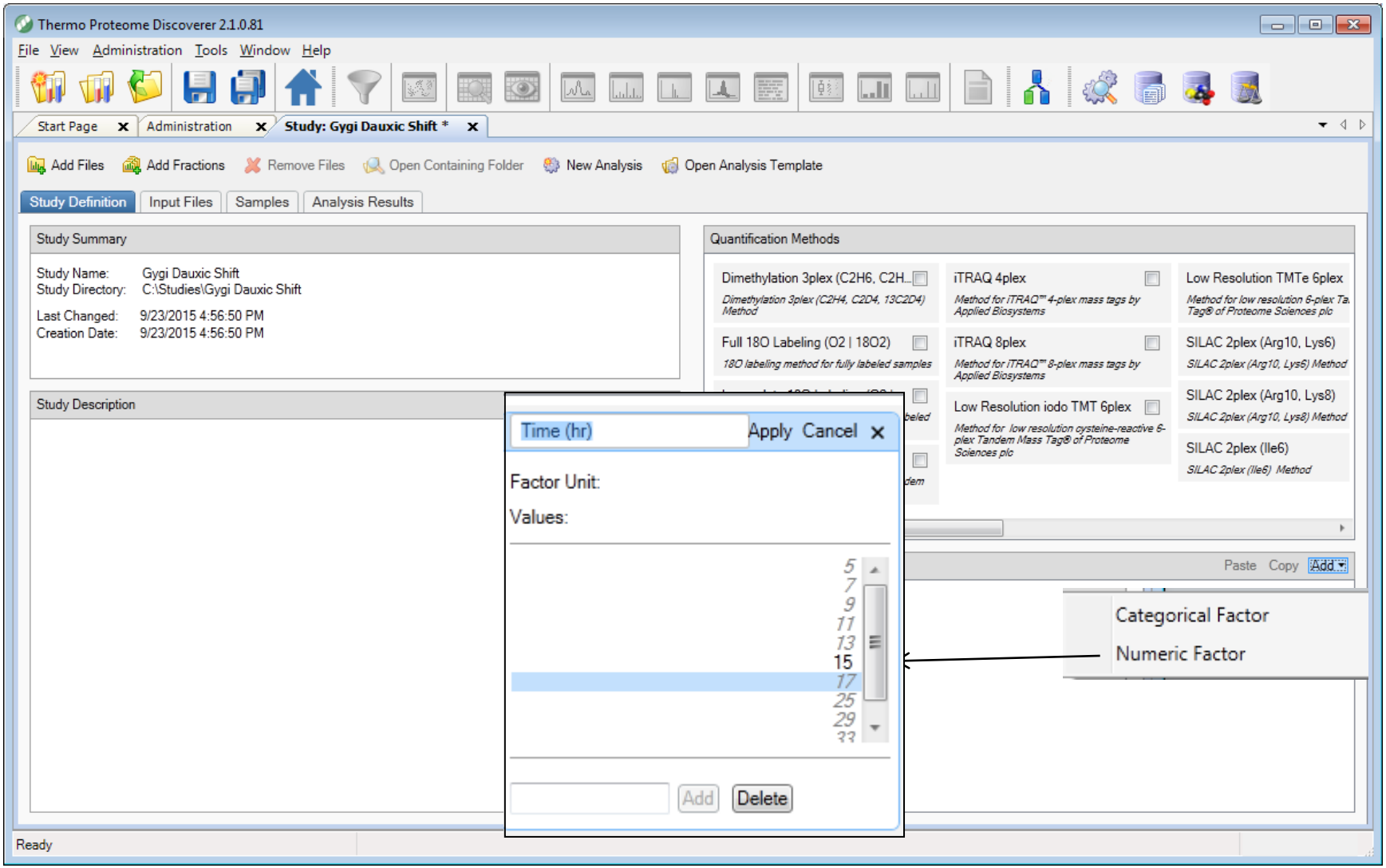
Step 3 – Set up study factors



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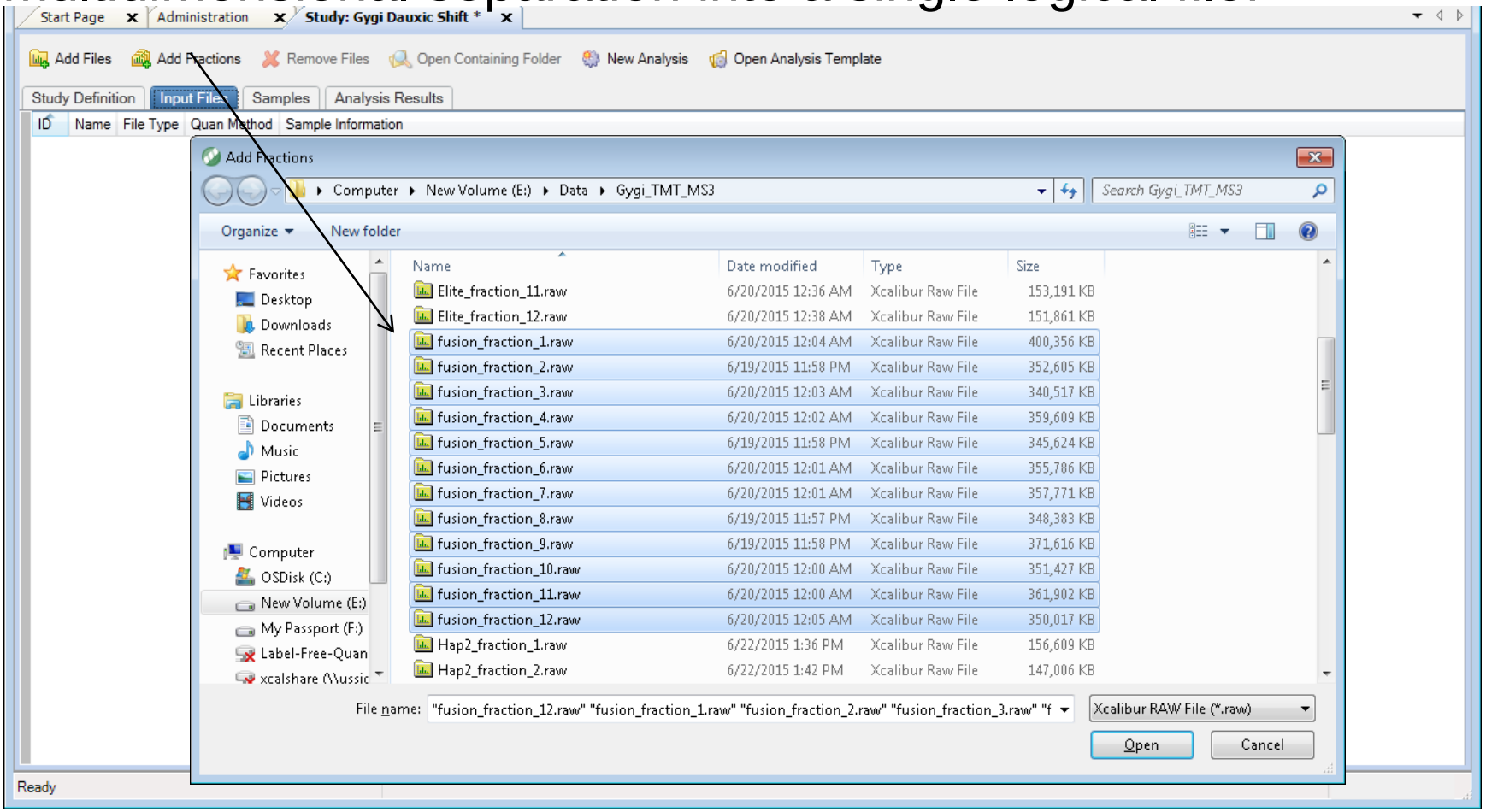
Step 4 – Import data files

- Choose “Add Fractions” to combine all data files from a multidimensional separation into a single logical file:



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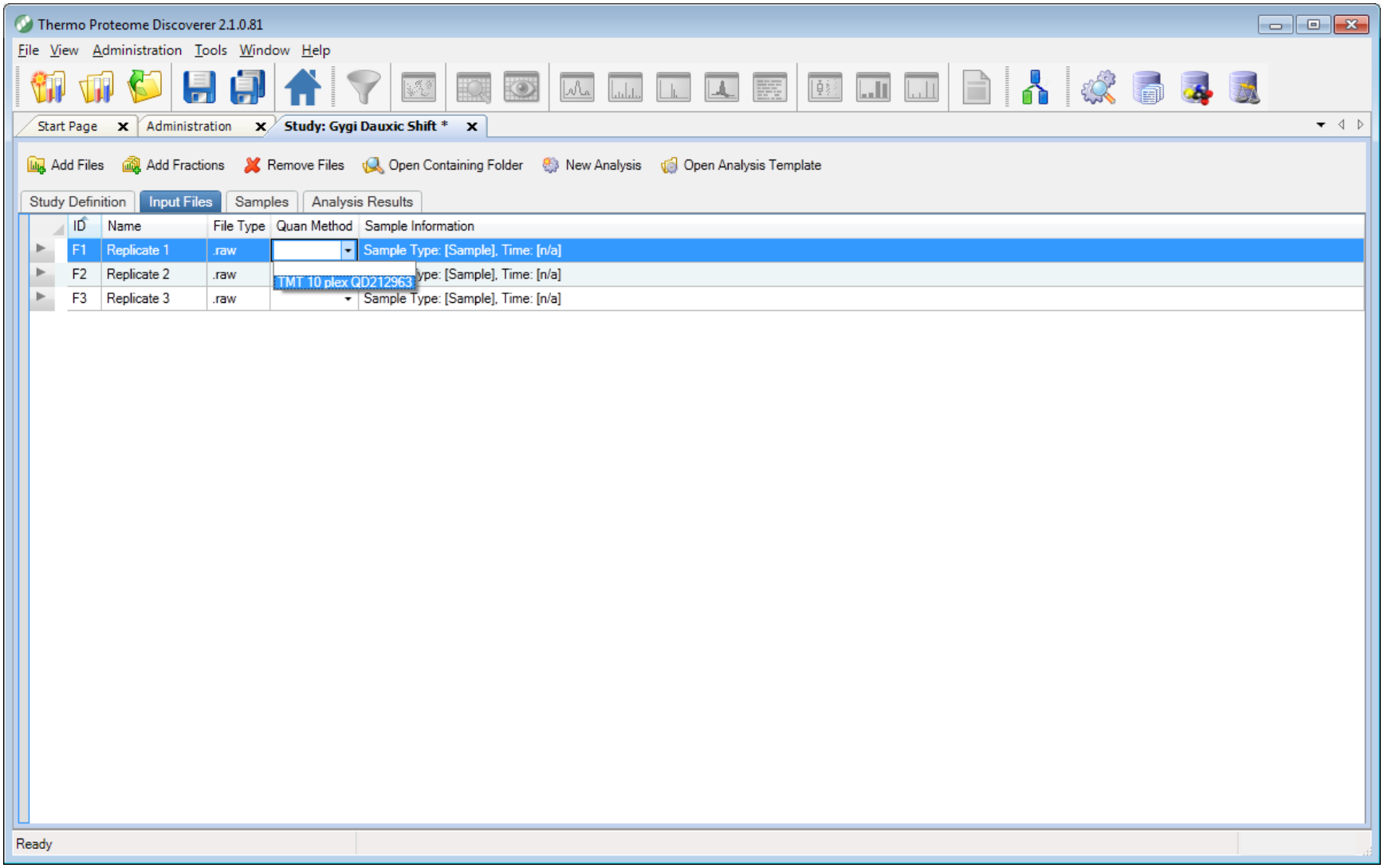
Step 5 – Assign Quan Method to each sample



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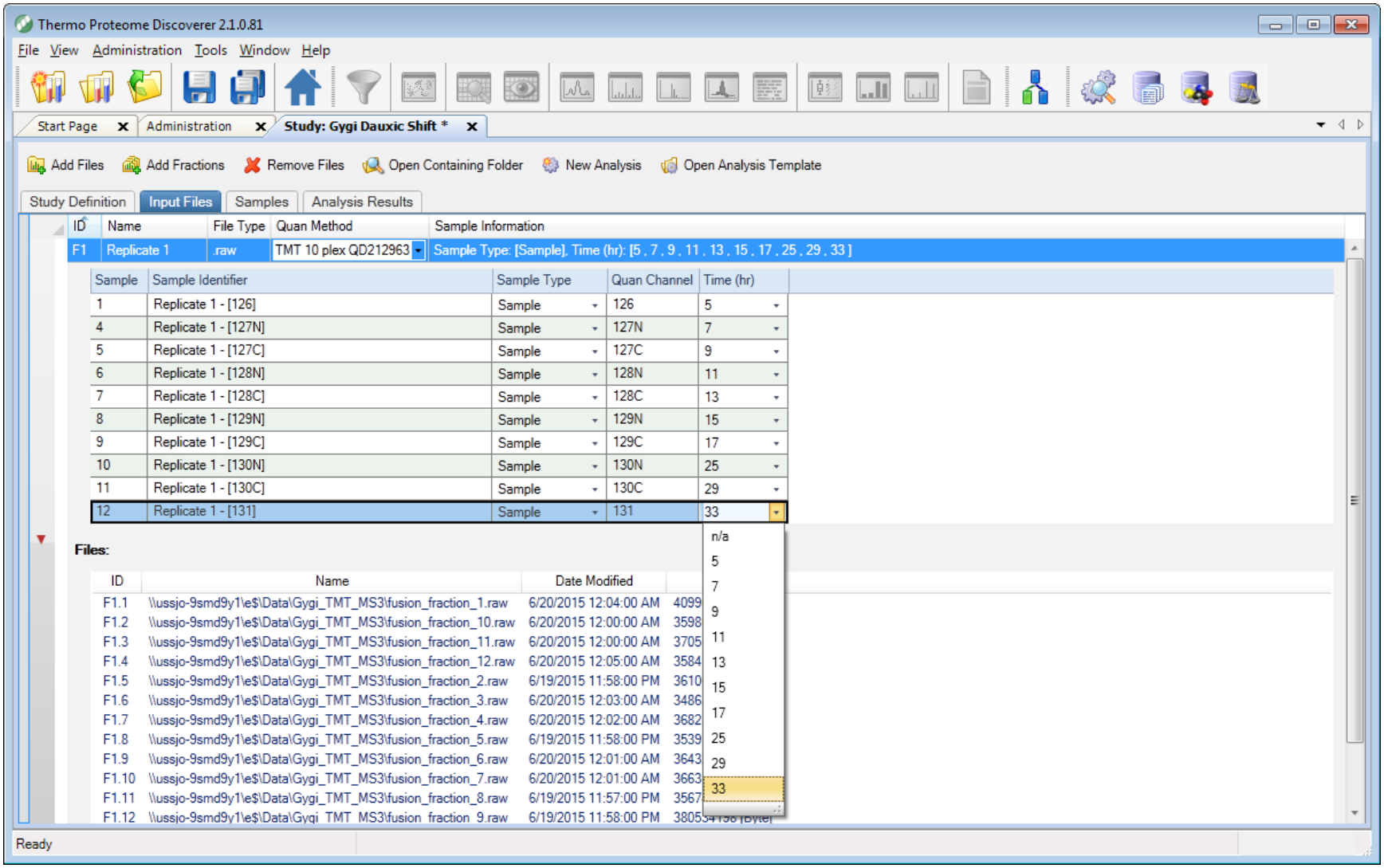
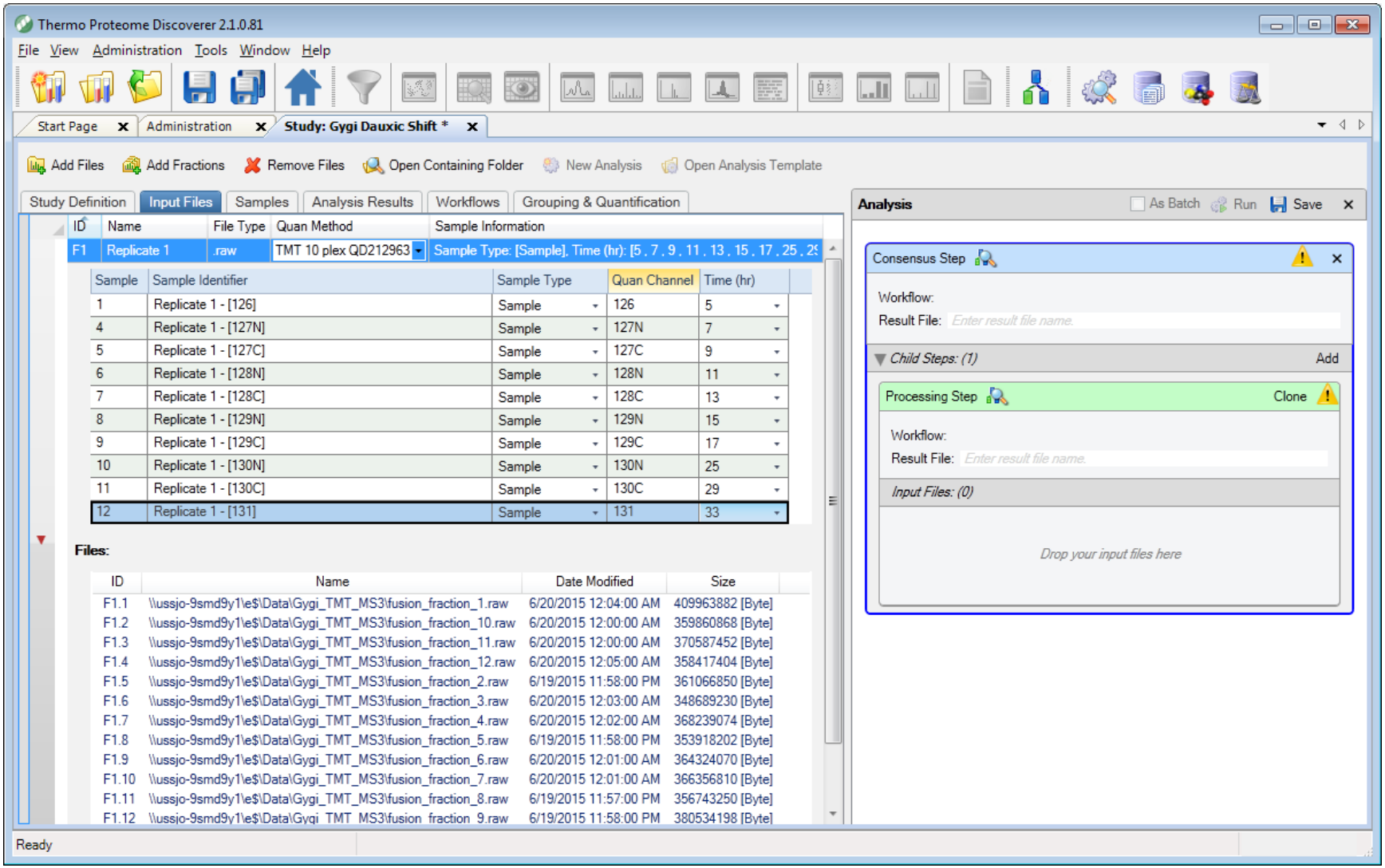
Step 6 - Assign study factors to reporter ions



Step 7 – Create New Analysis



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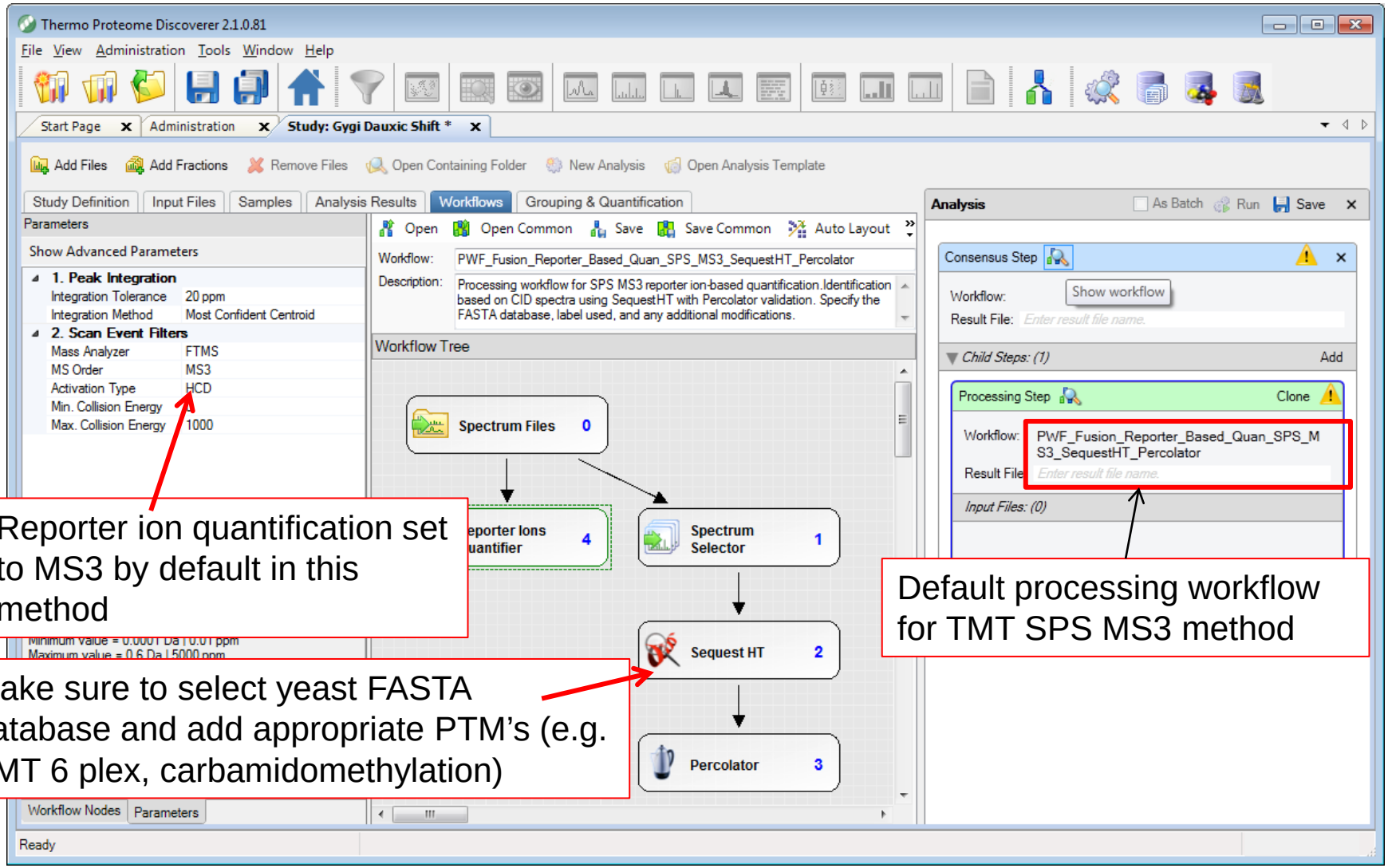
Step 8 – Choose processing workflow

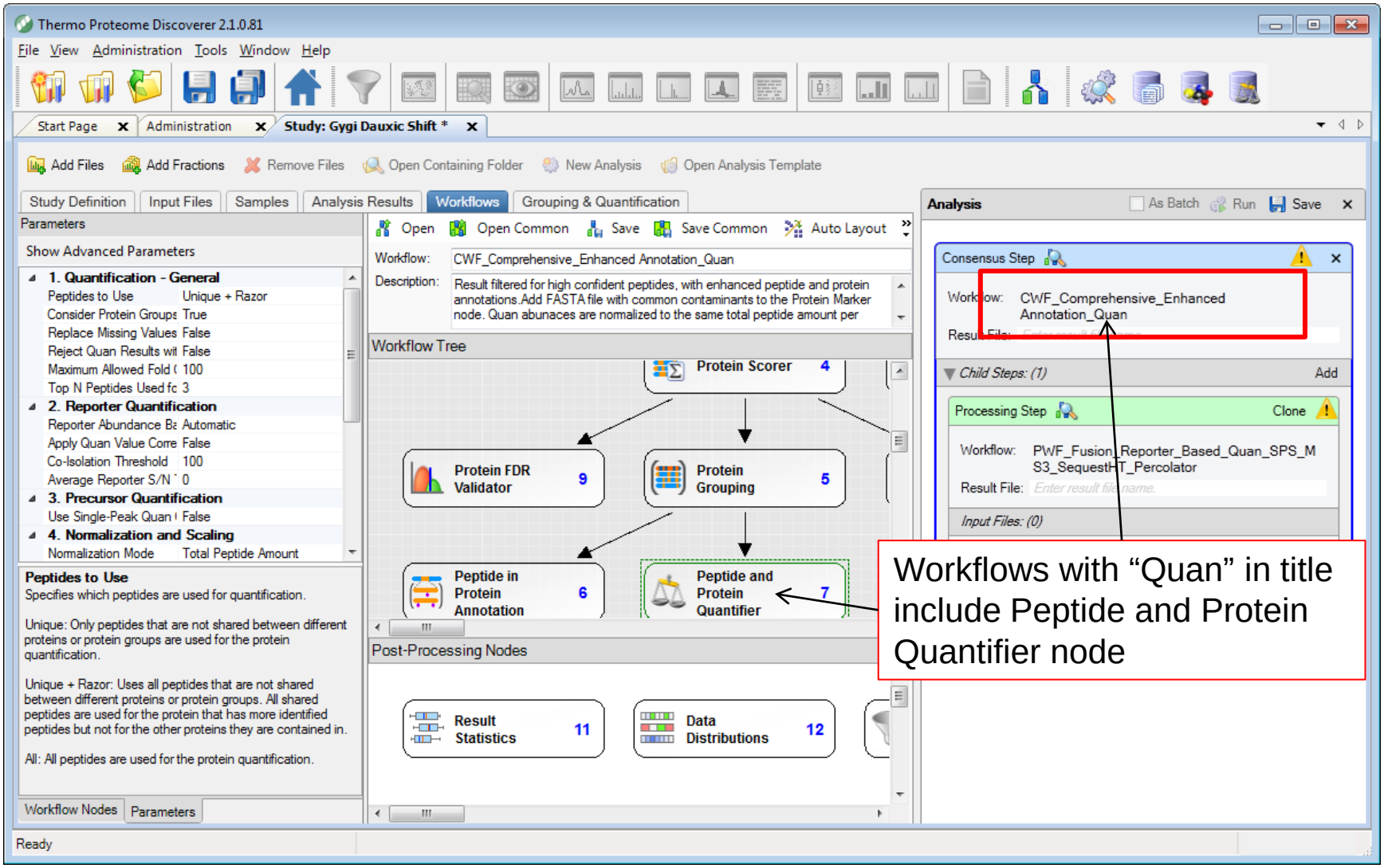


Reporter ion quantification set to MS3 by default in this method

Make sure to select yeast FASTA database and add appropriate PTM's (e.g. TMT 6 plex, carbamidomethylation)

Default processing workflow for TMT SPS MS3 method

Step 9 – Choose consensus workflow



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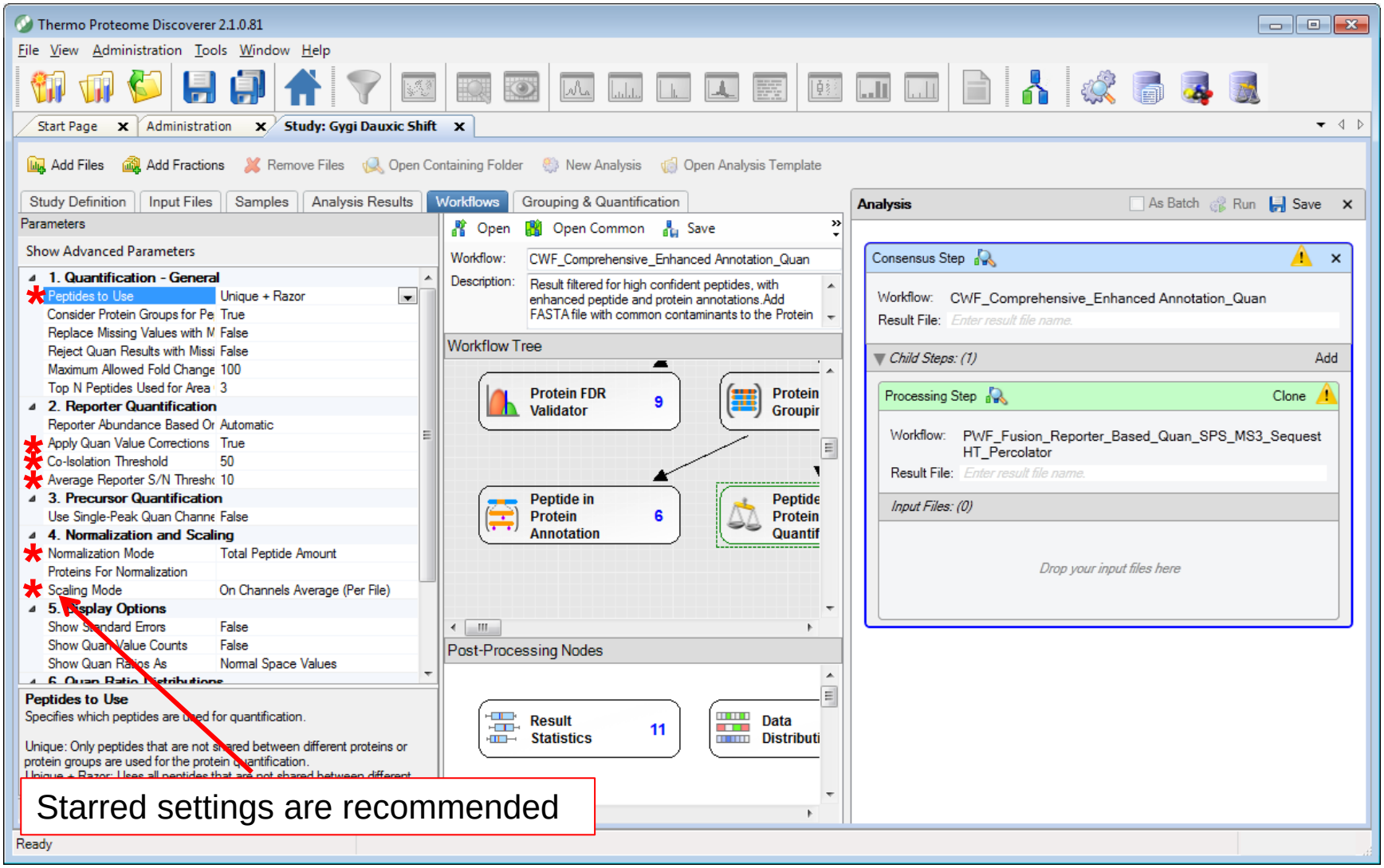
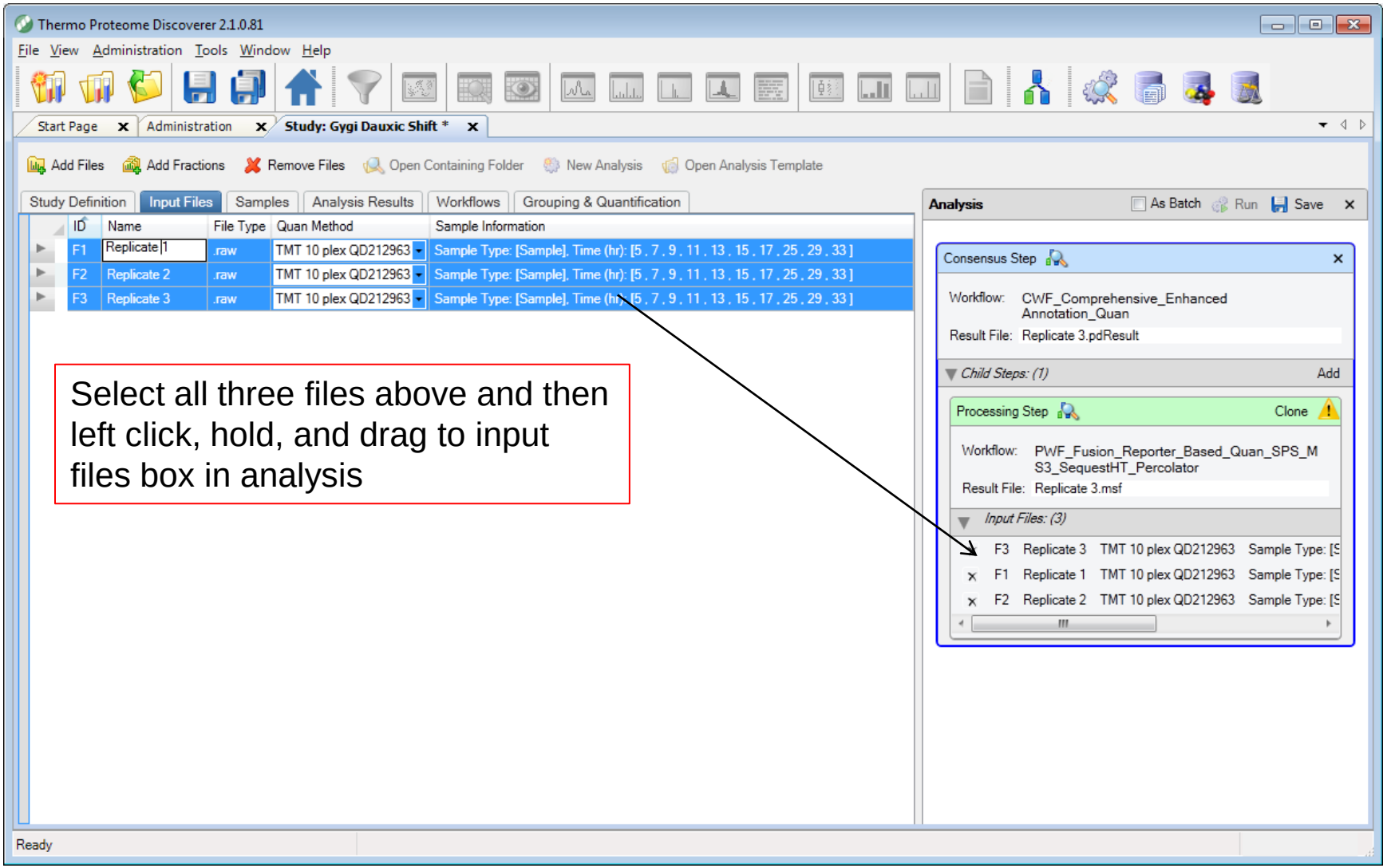
Step 10 – Modify Peptide and Protein Quantifier



Starred settings are recommended

Step 11- Drag files into analysis



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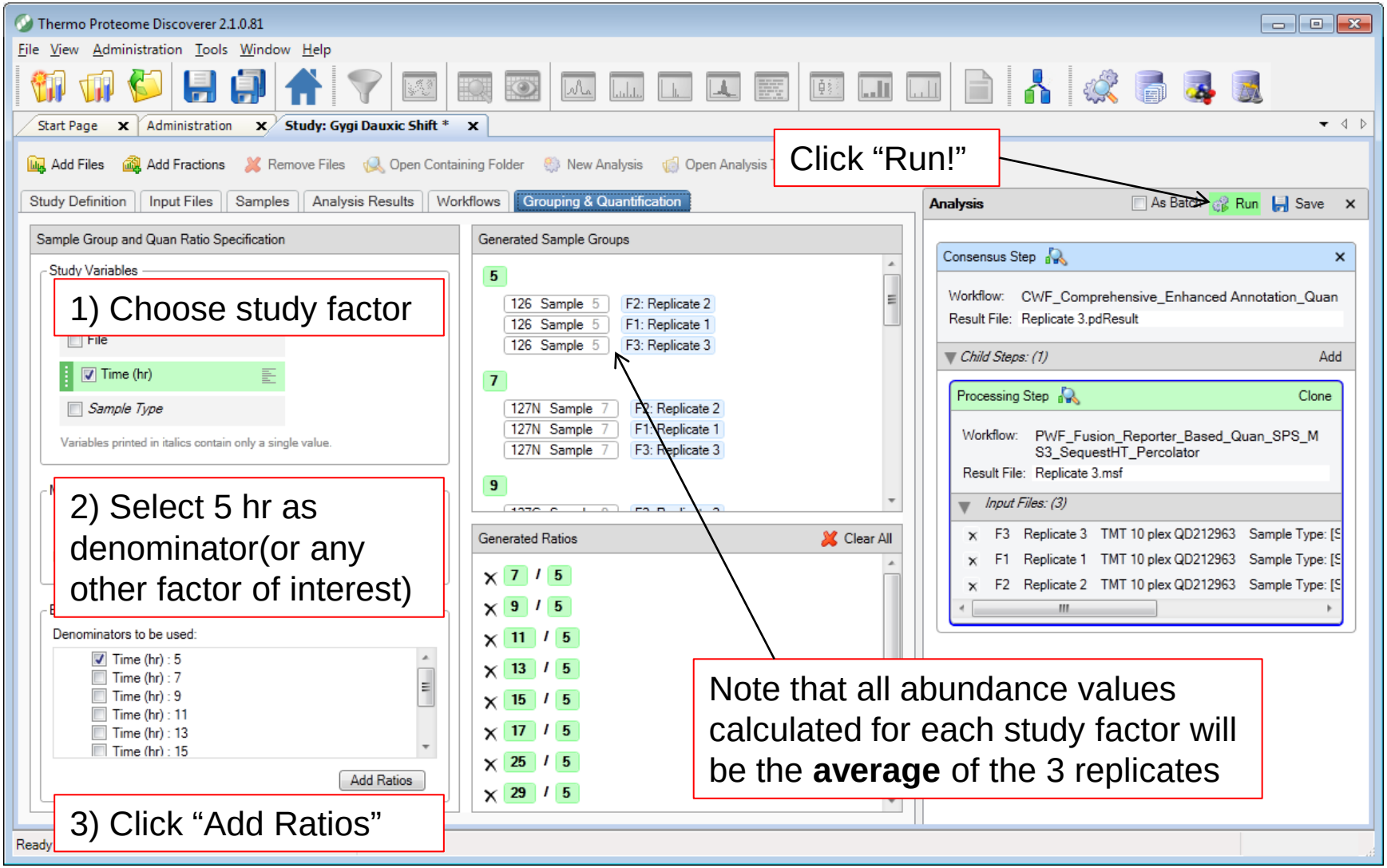
Step 12 – Grouping and Quantification tab



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Search results - >4700 proteins, >88000 unique peptides

Thermo Proteome Discoverer 2.1.0.81

File View Administration Tools Window Help

Start Page | Study: Gygi_Yeast_TMT_MS3 | All.SN10.Normalization.Scaling | All.SN0.Normalization.NoScaling | Study: Gygi Diauxic Shift

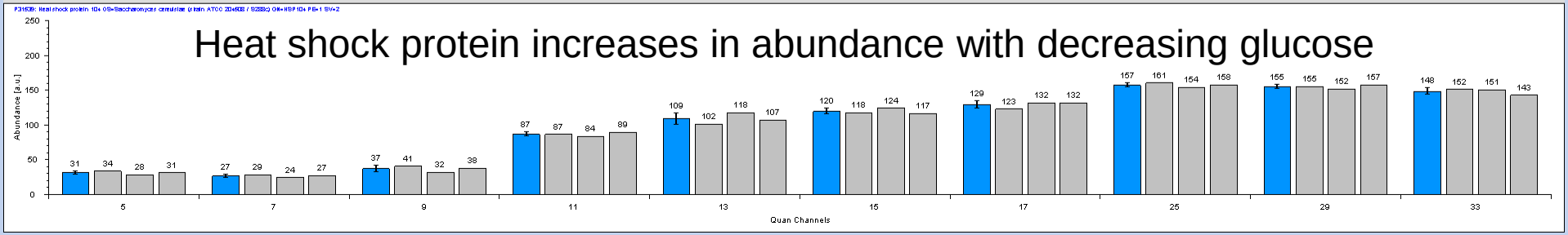
Proteins (filtered) | Protein Groups | Peptide Groups | PSMs | MS/MS Spectrum Info | Quan Spectra | Result Statistics

	Checked	Protein FDR Confidence	Master	Accession	Description	Abundances (Grouped) 5	7	9	11	13	15	17	25	29	33 *	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# AA
1				P07259	Protein URA2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=URA	58.9	44.5	59.1	111.5	139.4	131.3	126.3	113.0	108.0	107.8	0.000	1485.629	68%	159	1517	153	221
2				P19097	Fatty acid synthase subunit alpha OS=Saccharomyces cerevisiae (strain ATCC 20450	102.0	107.9	109.4	112.9	110.1	102.4	99.7	87.8	84.7	83.1	0.000	1477.540	62%	141	1339	140	188
3				P10591	Heat shock protein SSA1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	58.6	68.6	74.9	108.4	105.9	110.2	105.2	124.5	122.1	121.6	0.000	1328.855	90%	83	3055	21	642
4				P10592	Heat shock protein SSA2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	79.5	85.2	80.2	120.2	115.0	112.5	107.7	102.9	95.5	101.3	0.000	1286.627	90%	83	3061	19	639
5				Q00955	Acetyl-CoA carboxylase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	117.1	116.0	110.0	96.1	98.0	92.6	92.5	91.3	93.0	93.4	0.000	1252.103	66%	171	813	164	223
6				P00549	Pyruvate kinase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=(	91.1	90.5	90.2	113.8	126.7	120.0	108.9	90.7	82.5	85.6	0.000	1201.588	96%	68	3748	66	506
7				P15108	ATP-dependent molecular chaperone HSC82 OS=Saccharomyces cerevisiae (strain A	65.9	64.3	63.2	115.9	121.0	118.5	126.2	112.6	106.2	106.0	0.000	1196.036	82%	99	2393	19	709
8				P32324	Elongation factor 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=	113.7	105.1	101.2	115.1	118.3	113.2	109.7	81.1	71.6	71.1	0.000	1181.444	84%	89	2210	89	842
9				P02829	ATP-dependent molecular chaperone HSP82 OS=Saccharomyces cerevisiae (strain A	45.4	38.4	43.7	111.0	123.2	120.4	130.1	137.6	123.8	126.3	0.000	1144.380	79%	97	2265	17	709
10				P16521	Elongation factor 3A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN	131.0	115.4	107.4	119.2	113.1	104.8	102.8	75.1	67.1	64.1	0.000	1126.348	71%	86	1714	58	1044
11				P06105	Protein SCP160 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SC	102.6	115.3	121.1	114.7	114.1	106.2	101.2	80.3	73.9	70.7	0.000	1103.160	69%	118	860	118	1222
12				P00925	Enolase 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ENO2 P	91.1	92.5	91.8	112.0	114.8	108.8	108.8	92.5	91.8	95.9	0.000	1075.483	98%	63	4715	26	43
13				P07149	Fatty acid synthase subunit beta OS=Saccharomyces cerevisiae (strain ATCC 204508	101.4	107.4	111.4	112.3	108.1	103.1	102.7	86.5	84.3	82.9	0.000	1042.680	54%	117	832	117	205
14				P32589	Heat shock protein homolog SSE1 OS=Saccharomyces cerevisiae (strain ATCC 2045(	91.1	79.5	72.7	117.2	119.7	116.9	119.4	100.8	92.3	90.3	0.000	1005.152	81%	77	1295	66	693
15				P00560	Phosphoglycerate kinase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	82.6	91.5	96.8	111.8	104.0	105.5	104.3	98.1	98.6	106.8	0.000	948.671	96%	56	2924	56	416
16				P31539	Heat shock protein 104 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	31.2	26.7	37.1	86.5	109.0	119.7	129.0	157.5	154.9	148.4	0.000	939.395	77%	110	1005	109	908
17				P0CS90	Heat shock protein SSC1, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC	53.8	61.0	72.4	101.5	101.8	105.9	109.4	134.2	130.0	129.9	0.000	907.517	81%	78	1209	64	654
18				P02994	Elongation factor 1-alpha OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	133.6	109.7	97.8	113.6	102.7	100.7	99.7	82.3	77.6	82.2	0.000	898.150	88%	57	2719	57	458
19				P06169	Pyruvate decarboxylase isozyme 1 OS=Saccharomyces cerevisiae (strain ATCC 2045(	103.5	102.3	96.5	119.0	114.2	111.0	113.5	83.3	76.1	80.7	0.000	883.474	71%	53	2170	44	562
20				P40150	Heat shock protein SSB2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	99.5	102.7	98.3	106.5	111.9	104.9	101.0	94.5	90.8	89.8	0.000	879.364	76%	63	1927	6	613
21				P16861	ATP-dependent 6-phosphofructokinase subunit alpha OS=Saccharomyces cerevisiae (	87.5	89.7	93.8	104.2	113.7	112.0	112.0	100.0	92.5	94.6	0.000	858.615	70%	78	816	77	987
22				P11484	Heat shock protein SSB1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288	117.4	111.1	104.9	123.2	118.1	107.1	99.0	77.2	68.8	73.1	0.000	850.638	76%	63	1982	6	613
23				P17255	V-type proton ATPase catalytic subunit A OS=Saccharomyces cerevisiae (strain ATCC	90.0	88.8	94.8	108.0	118.9	114.4	110.9	97.4	89.4	87.5	0.000	845.933	67%	88	1018	88	107
24				Q02455	Protein MLP1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=MLP	97.8	104.1	110.3	118.6	113.8	110.8	103.4	83.0	80.1	78.1	0.000	844.791	67%	165	454	162	187

Show Associated Tables

Quan Channel Values

P31539: Heat shock protein 104 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP104 PE=1 SV=2



Ready

4772/4965 Proteins; 4772 Protein Groups; 88474 Peptide Groups; 357119 PSMs; 1050272 MS/MS Spectrum Info; 1055020 Quan Spectra; 507 Result Statistics

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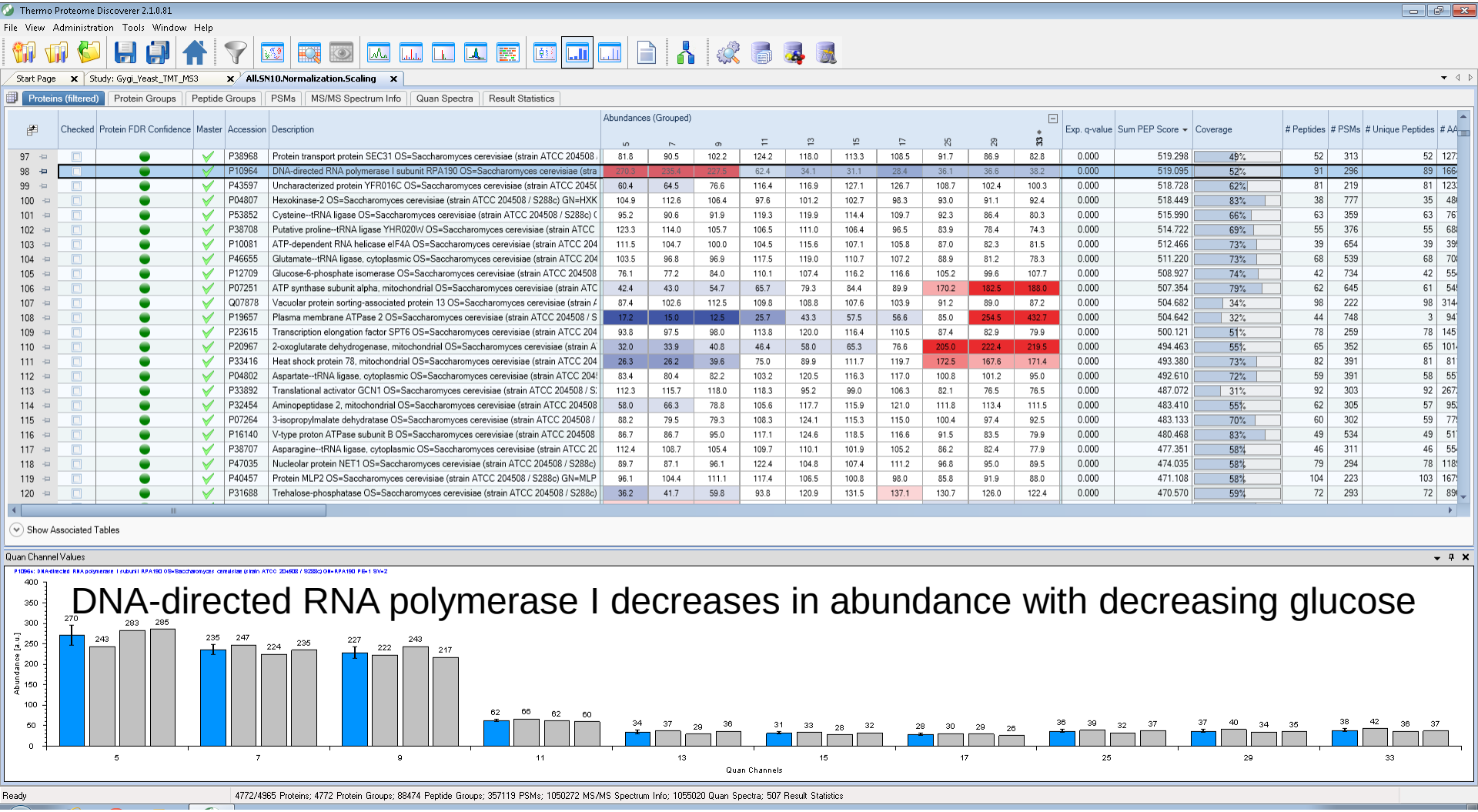
Search results - >4700 proteins, >88000 unique peptides



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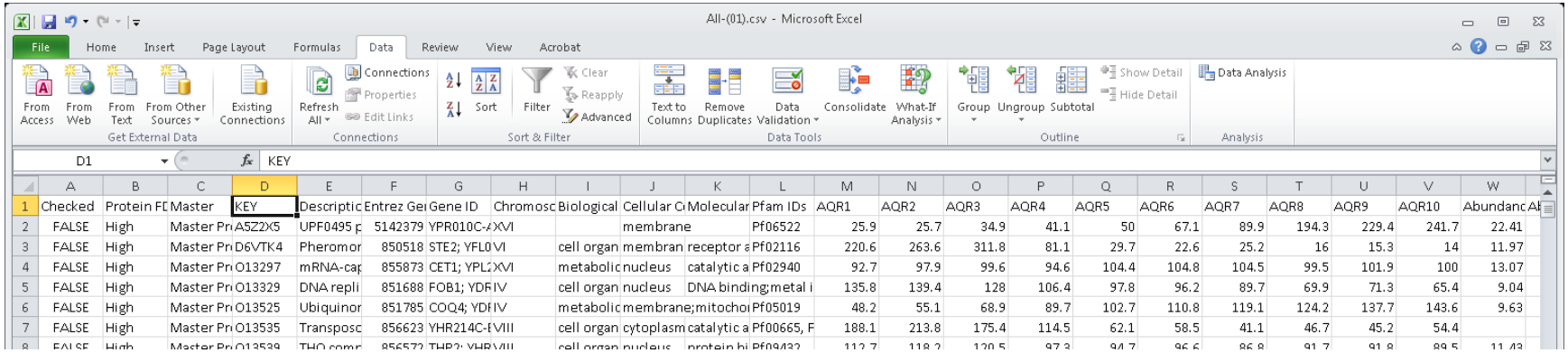
Extra credit – Profiling of scaled intensities in ProteinCenter

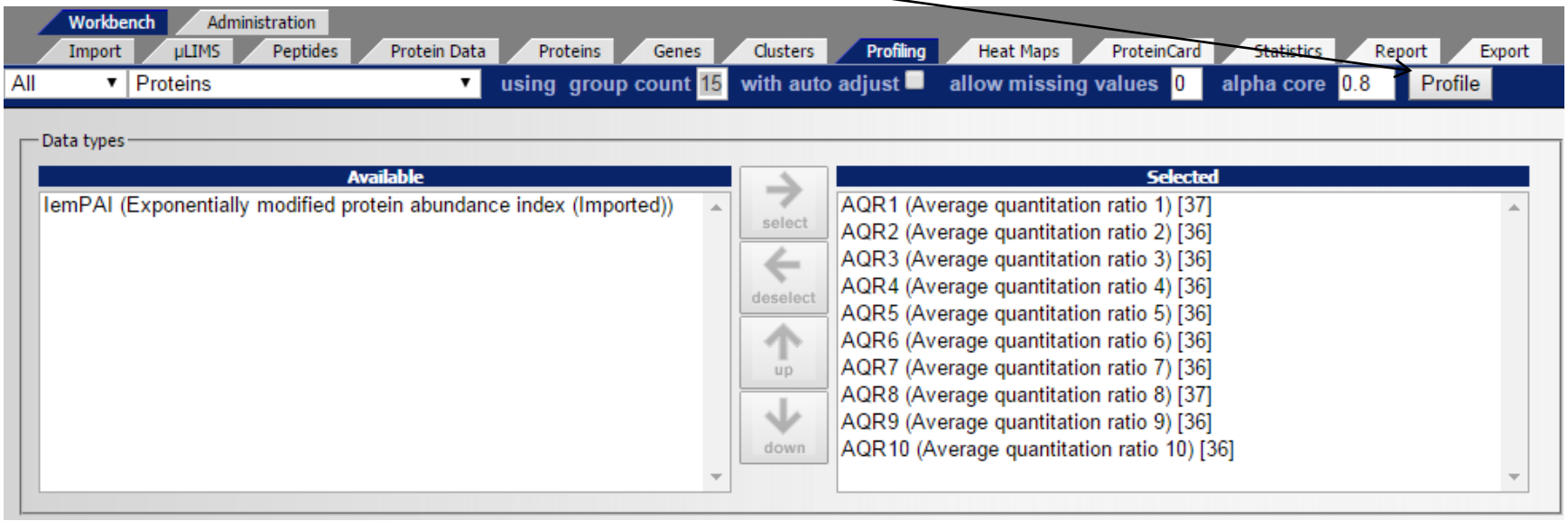
1) Export the proteins page to Excel
2) Rename the column with accession numbers as “KEY”
3) Rename the grouped abundance columns to AQR1, AQR2, AQR3, etc.



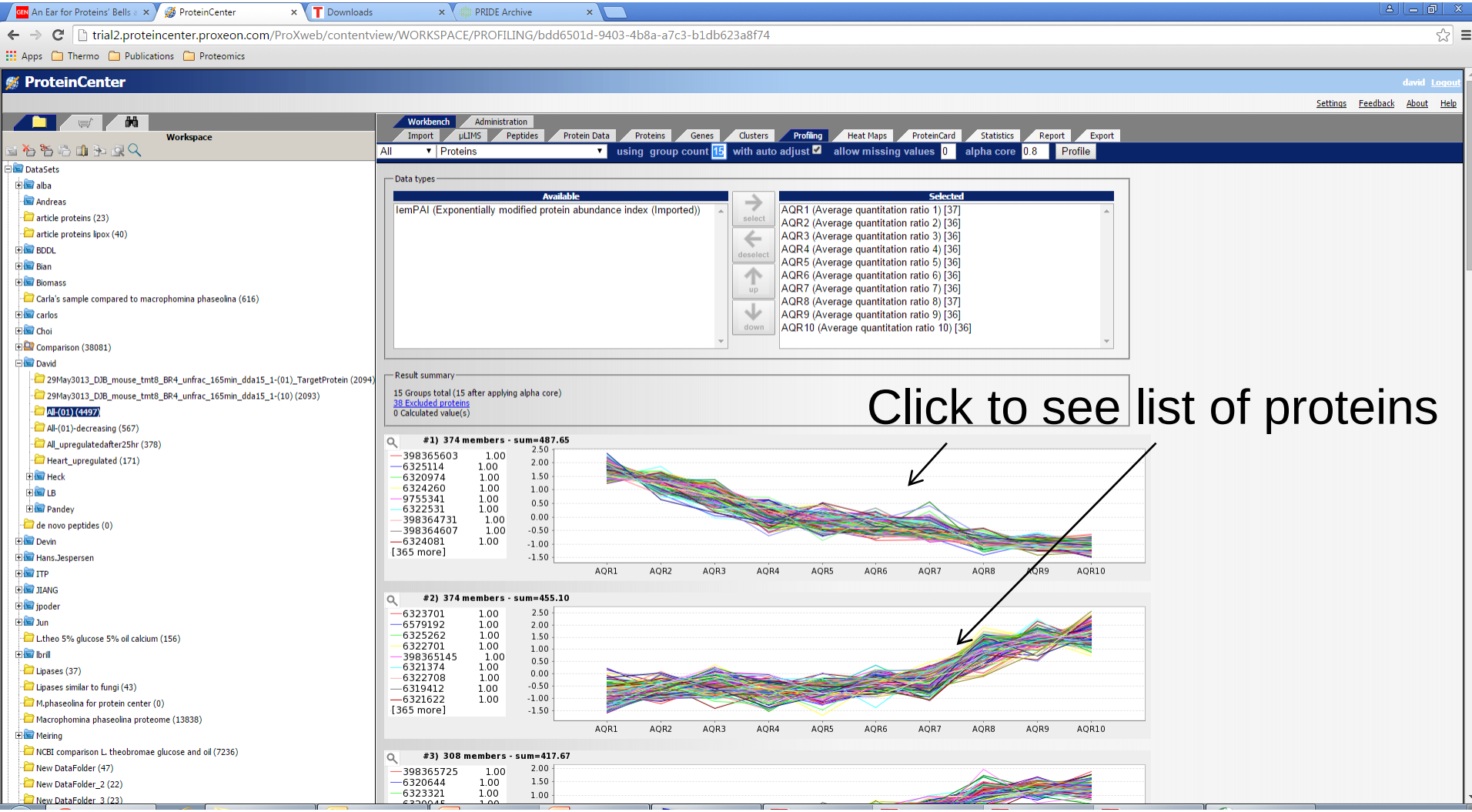
4) Save as .csv

Profiling in ProteinCenter

- Import .csv into ProteinCenter
- Click on the dataset and navigate to Profiling tab
- Move all “AQR#” values to “Selected”
- Choose an appropriate group count (10-15 works)
- Click “Profile”



Profiling results



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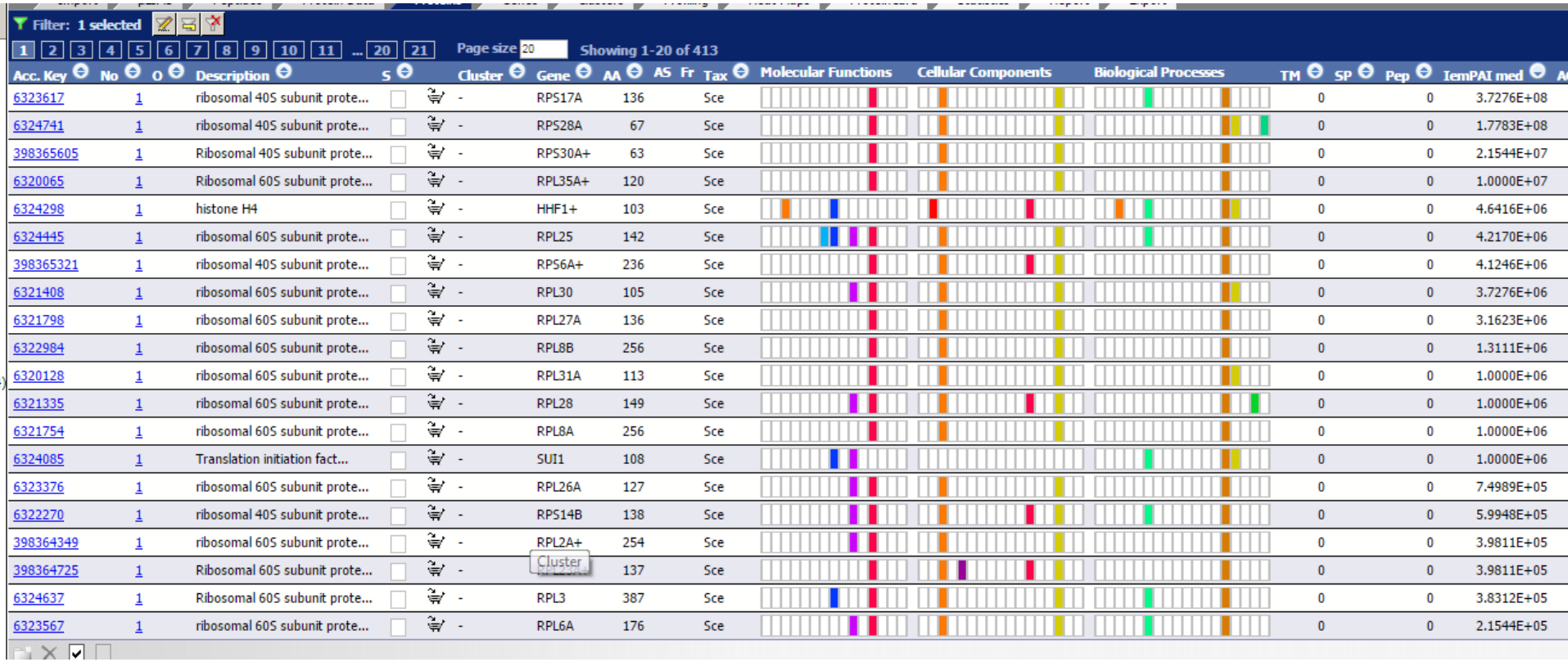
Cluster 1 – Proteins listed in order of decreasing emPAI



Filter: 1 selected

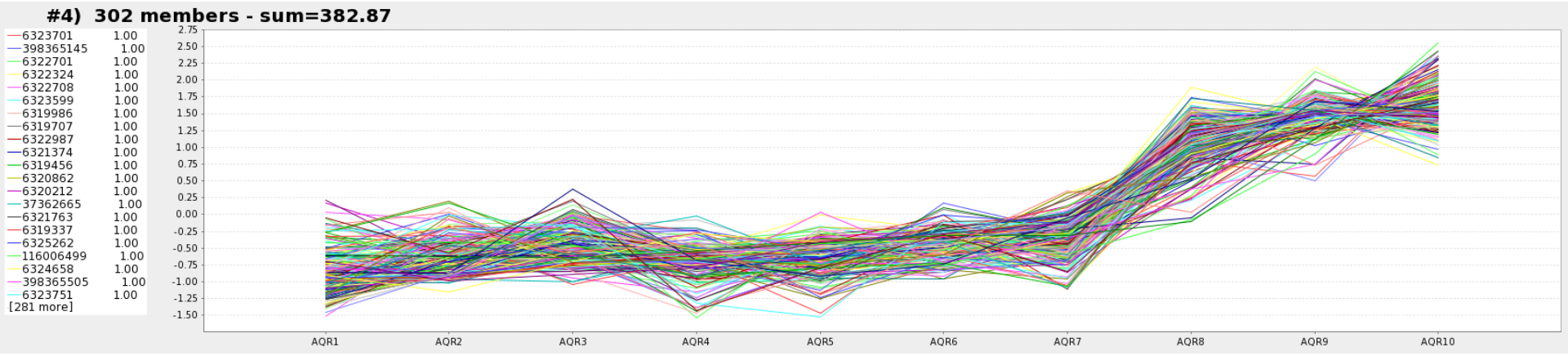
1 2 3 4 5 6 7 8 9 10 11 ... 20 21 Page size 20 Showing 1-20 of 413

Acc. Key	No	O	Description	S		Cluster	Gene	AA	AS	Fr	Tax	Molecular Functions	Cellular Components	Biological Processes	TM	SP	Pep	IemPAI med
6323617	1		ribosomal 40S subunit prote...			-	RPS17A	136			Sce				0		0	3.7276E+08
6324741	1		ribosomal 40S subunit prote...			-	RPS28A	67			Sce				0		0	1.7783E+08
398365605	1		Ribosomal 40S subunit prote...			-	RPS30A+	63			Sce				0		0	2.1544E+07
6320065	1		Ribosomal 60S subunit prote...			-	RPL35A+	120			Sce				0		0	1.0000E+07
6324298	1		histone H4			-	HHF1+	103			Sce				0		0	4.6416E+06
6324445	1		ribosomal 60S subunit prote...			-	RPL25	142			Sce				0		0	4.2170E+06
398365321	1		ribosomal 40S subunit prote...			-	RPS6A+	236			Sce				0		0	4.1246E+06
6321408	1		ribosomal 60S subunit prote...			-	RPL30	105			Sce				0		0	3.7276E+06
6321798	1		ribosomal 60S subunit prote...			-	RPL27A	136			Sce				0		0	3.1623E+06
6322984	1		ribosomal 60S subunit prote...			-	RPL8B	256			Sce				0		0	1.3111E+06
6320128	1		ribosomal 60S subunit prote...			-	RPL31A	113			Sce				0		0	1.0000E+06
6321335	1		ribosomal 60S subunit prote...			-	RPL28	149			Sce				0		0	1.0000E+06
6321754	1		ribosomal 60S subunit prote...			-	RPL8A	256			Sce				0		0	1.0000E+06
6324085	1		Translation initiation fact...			-	SUI1	108			Sce				0		0	1.0000E+06
6323376	1		ribosomal 60S subunit prote...			-	RPL26A	127			Sce				0		0	7.4989E+05
6322270	1		ribosomal 40S subunit prote...			-	RPS14B	138			Sce				0		0	5.9948E+05
398364349	1		ribosomal 60S subunit prote...			-	RPL2A+	254			Sce				0		0	3.9811E+05
398364725	1		Ribosomal 60S subunit prote...			-	[illegible]	137			Sce				0		0	3.9811E+05
6324637	1		Ribosomal 60S subunit prote...			-	RPL3	387			Sce				0		0	3.8312E+05
6323567	1		ribosomal 60S subunit prote...			-	RPL6A	176			Sce				0		0	2.1544E+05

Cluster

Cytoplasmic ribosomal proteins are highly overrepresented in this cluster. On average, ribosomal proteins are decreasing in abundance as the number of cells increase in time.

Cluster 4 – proteins that increase in abundance after glucose depletion



Over-represented Wiki pathways

Analysis data set: All-(01) Reference data set: Saccharomyces cerevisiae (SP)

1 2

Description	Occurrence	Count	Ref.
Principle Pathways of Carbon Metabolism (WP112)		22	
TCA Cycle (WP490)		18	
TCA Cycle - Detailed (WP296)		15	
Serine-isocitrate lyase pathway (WP390)		7	
Glycolysis and Gluconeogenesis (WP515)		6	
Fatty acid oxidation (WP91)		5	
Glutamate degradation VII (WP559)		5	
Gluconeogenesis (WP156)		4	
Glutamate degradation III (WP503)		3	
Superpathway of Glutamate Biosynthesis (WP191)		2	
Panothenate and Coenzyme A Biosynthesis (WP462)		2	
Glutamate biosynthesis (WP77)		2	
Cell Cycle and Cell Division (WP414)		2	
Mitochondrial tRNA Synthetases (WP62)		2	
Allantoin Degradation (WP328)		2	
Proteasome Degradation (WP158)		1	
Glucose Repression (WP2836)		1	
Anaerobic respiration (WP575)		1	
Aerobic Glycerol Catabolism (WP224)		1	
Sulfur degradation (WP440)		1	

Proteins from TCA Cycle are overrepresented

Filter: 1 selected

1 2 3 4 Page size: 20 Showing 1-20 of 79

Acc. Key	No	O	Description	S	Cluster	Gene	AA
6323335	1		aconitate hydratase ACO1		-	ACO1	778
6322765	1		Malate dehydrogenase MDH1		-	MDH1	334
6324328	1		citrate (Si)-synthase CIT1		-	CIT1	479
398365505	1		Phosphoenolpyruvate carboxy...		-	PCK1	549
398364491	1		Isocitrate lyase 1		-	ICL1	557
6324291	1		isocitrate dehydrogenase (N...		-	IDH1	360
6324212	1		malate synthase MLS1		-	MLS1	554
6322987	1		succinate dehydrogenase iro...		-	SDH2	266
6322066	1		alpha-ketoglutarate dehydro...		-	KGD1	1014
6323203	1		isocitrate dehydrogenase (N...		-	P2	412
6321376	1		pyruvate carboxylase 1		-	PYC1	1178
398365347	1		succinate--CoA ligase (GDP-...		-	LSC1	329
6319264	1		acetate--CoA ligase 1		-	ACS1	713
116006499	1		malate dehydrogenase MDH2		-	MDH2	377
6322701	1		Succinate dehydrogenase fla...		-	SDH1	640
6320125	1		malate dehydrogenase Mdh3		-	MDH3	343
6319850	1		citrate (Si)-synthase CIT2		-	CIT2	460
398366041	1		Fructose 1,6-bisphosphate 1...		-	FBP1	348
6320383	1		succinate dehydrogenase mem...		-	SDH4	181
6321755	1		glycerol kinase		-	GUT1	709

Add to basket

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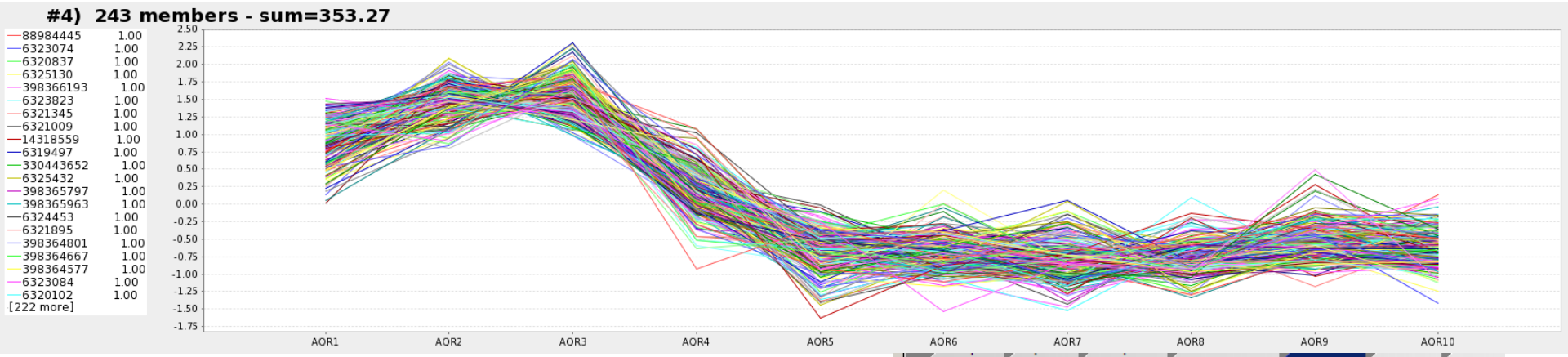
Cluster 5 – proteins that match glucose concentration



Over-represented Wiki pathways

Analysis data set: All-(01) **Reference data set:** Saccharomyces cerevisiae (SP)

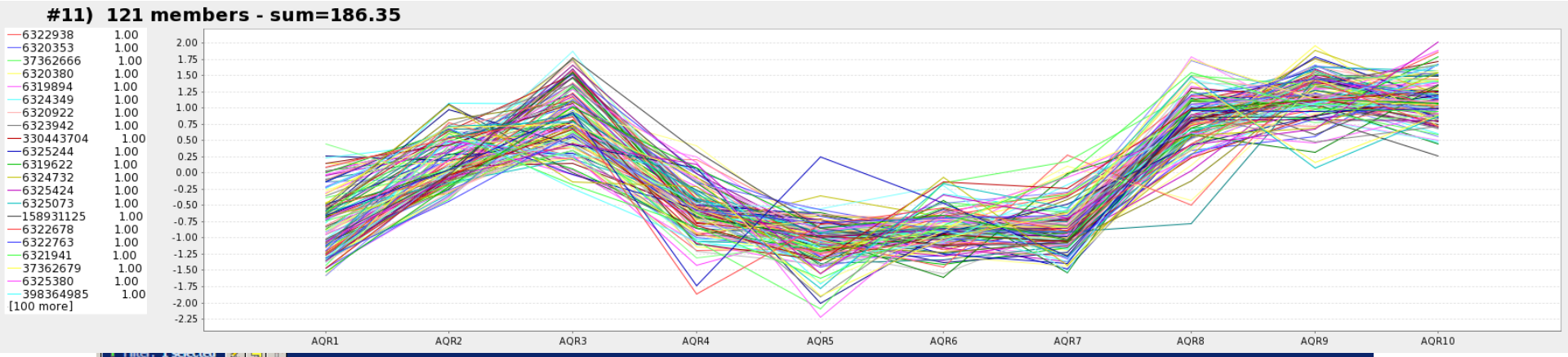
Description	Occurrence	Count	R
Cell Cycle and Cell Division (WP414)		13	
MAPK Signaling Pathway (WP510)		3	
Lipases biosynthesis (WP71)		1	
Nucleotide Metabolism (WP321)		1	
Triglyceride Biosynthesis (WP266)		1	
Genes of Meiotic Recombination (WP377)		1	
Riboflavin, FMN, and FAD Biosynthesis (WP381)		1	
Glucose Repression (WP2836)		1	
Protein Modifications (WP346)		1	

Filter: 1 selected | Work with proteins

1 2 3 4 5 Page size 20 Showing 1-20 of 84

Acc. Key	No	O	Description	S		Cluster	Gene
398366247	1		Pho81p	✔		-	PHO81
6323211	1		transcriptional regulator SWI6	✔		-	SWI6
6324944	1		Rad17p	✔		-	RAD17
6325062	1		Ddc1p	✔		-	DDC1
330443399	1		Brn1p	✔		-	BRN1
6319295	1		mitotic regulator LTE1	✔		-	LTE1
330443652	1		protein kinase HSL1	✔		-	HSL1
6322830	1		anaphase promoting complex ...	✔		-	CDC16
6323302	1		Ycs4p	✔		-	YCS4
6322304	1		cyclin-dependent protein se...	✔		-	FAR1
398365629	1		serine/threonine-protein ki...	✔		-	DBF2
6319751	1		Serine/threonine protein ki...	✔		-	CHK1
14318559	1		phosphate-sensing transcrip...	✔		-	PHO4

Cluster 11 – pattern not identified in Gygi paper



1 2 3 4 5 6 7 Page size 20 Showing 1-20 of 121

Acc. Key	No	O	Description	S		Cluster	Gene	AA	AS	Fr	Tax	Molecular Functions	Cellular Components	Biological Processes	TM	SP	Pep	IemPAI
6325073	1		putative mitochondrial 54S ...	✓		-	RTC6	93			Sce				0		0	
6323634	1		mitochondrial 54S ribosomal...	✓		-	MRPL39	70			Sce				0		0	
398364237	1		Mitochondrial 54S ribosomal...	✓		-	RML2	393			Sce				1	≀	0	
398366569	1		mitochondrial 54S ribosomal...	✓		-	MRP20	263			Sce				0		0	
6323644	1		Mix17p	✓		-	MIC17+	156			Sce				0		0	
6325380	1		Ctr1p	✓		-	CTR1								2		0	
398364681	1		Tec1p	✓		-	TEC1								0		0	
6324045	1		mitochondrial 54S ribosomal...	✓		-	MRPL1								1		0	
398366545	1		phenylpyruvate decarboxylas...	✓		-	ARO10								1		0	
6323942	1		mitochondrial 54S ribosomal...	✓		-	MRPL33	86			Sce				0		0	
37362666	1		mitochondrial 54S ribosomal...	✓		-	MRPL49	161			Sce				0		0	
6324732	1		mitochondrial 37S ribosomal...	✓		-	PET123	318			Sce				0		0	
6322755	1		Yju2p	✓		-	YJU2	278			Sce				0		0	
6322678	1		mitochondrial 54S ribosomal...	✓		-	MRPL38	138			Sce				0		0	
6324608	1		Akr2p	✓		-	AKR2	749			Sce				6		0	
398366647	1		mitochondrial 37S ribosomal...	✓		-	RSM28	361			Sce				0		0	
6321902	1		Erp5p	✓		-	ERP5	212			Sce				1	≀	0	
330443704	1		mitochondrial 54S ribosomal...	✓		-	MRPL22	309			Sce				0		0	
398365695	1		peptidylprolyl isomerase fa...	✓		-	CPR8	308			Sce				1	≀	0	
6319894	1		mitochondrial 54S ribosomal...	✓		-	IMG1	169			Sce				0		0	

Many mitochondrial ribosomal proteins

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Conclusions

- With scaled abundances, ratio calculations become less important
  - Immune to “0” values in the denominator
  - Can export for profiling
- New user interfaces are much easier to use than in previous releases
  - Ratio calculation page much improved over 2.0
  - Correction factors easy to add
- Best quantitative results from any PD release so far!