

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

Rosa Viner  
HUPO 2015

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# TMT quantification in PD 2.0 and previous

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- Using reporter ion intensity values from MS/MS of MS<sup>3</sup> 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

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- 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)

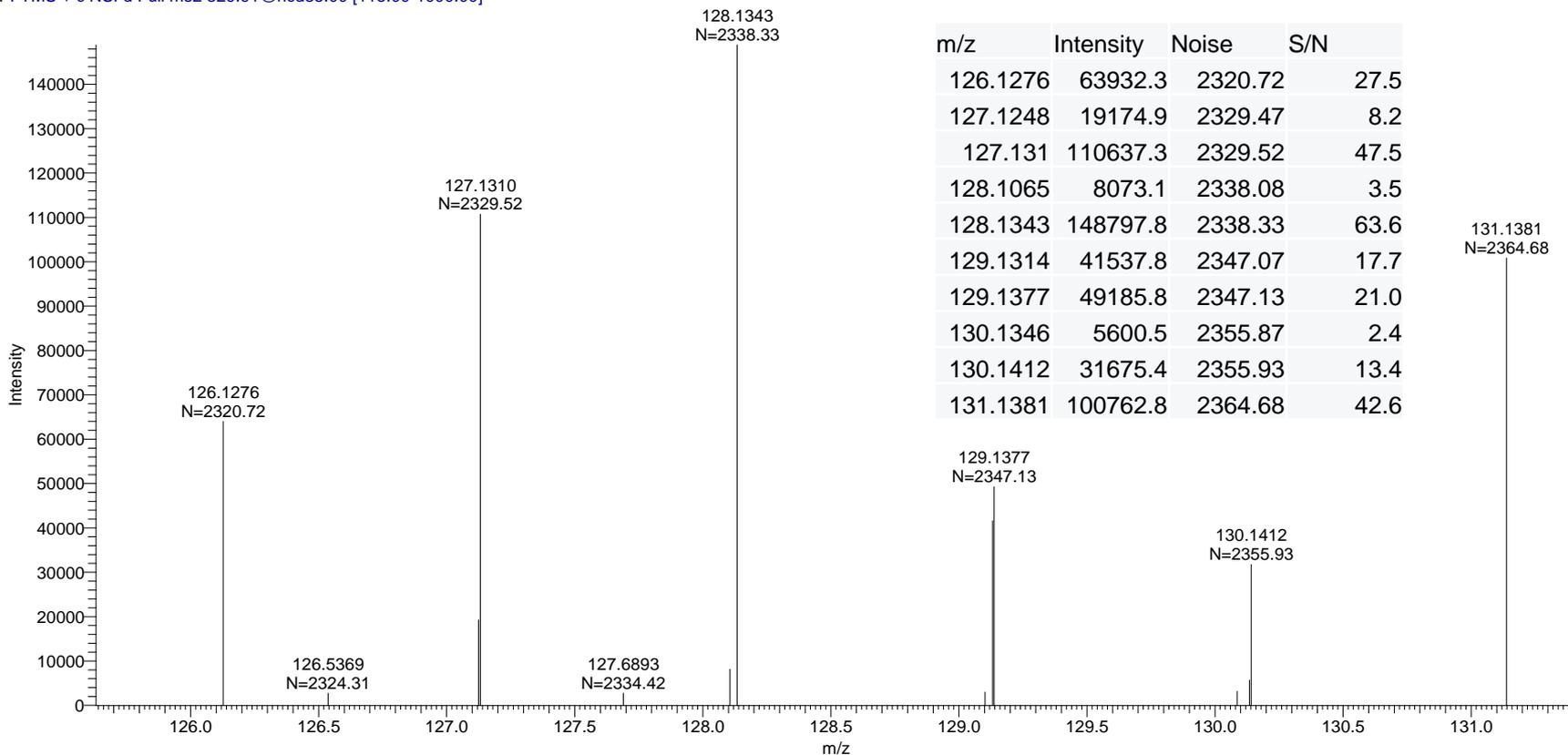
# Collaboration with Harvard Medical School for TMT quantification



# Gygi Method for TMT quantification – Step 1

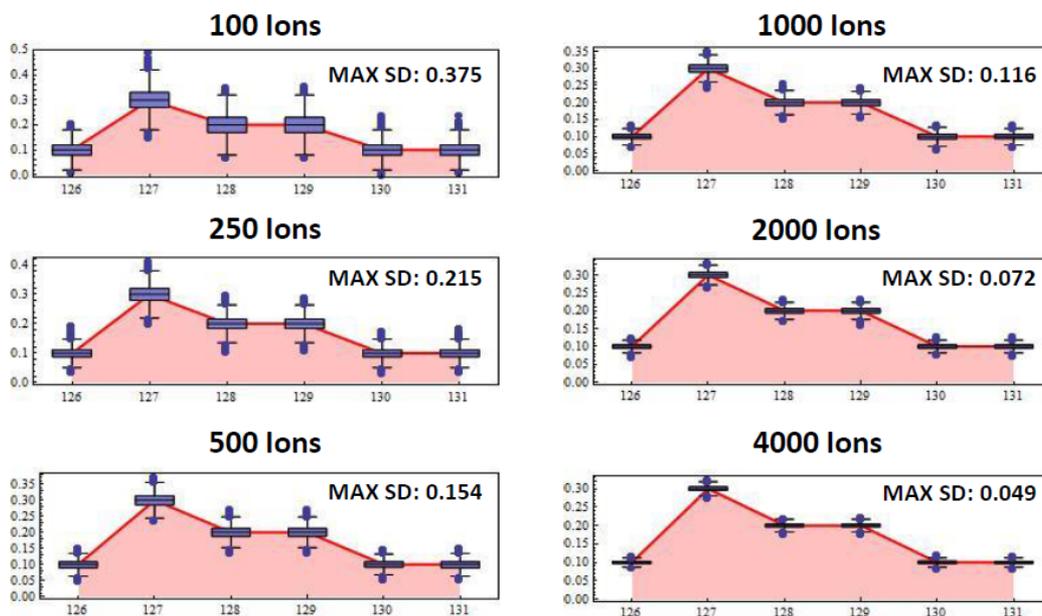
## 1. Extract S/N values for each reporter ion in the MS/MS data

29May3013\_DJB\_mouse\_tmt8\_BR1\_unfrac\_165min\_ddd15\_1 #8401 RT: 34.83 AV: 1 NL: 1.49E5  
T: FTMS + c NSI d Full ms2 329.91@hcd35.00 [115.00-1000.00]



# 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

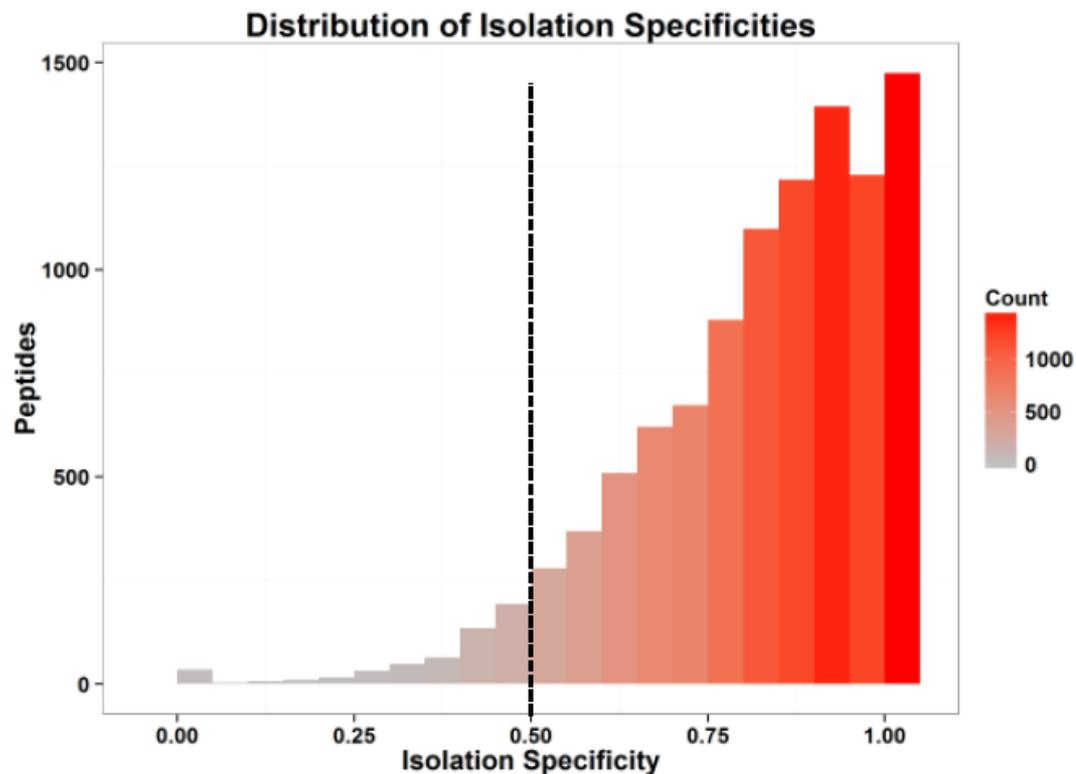
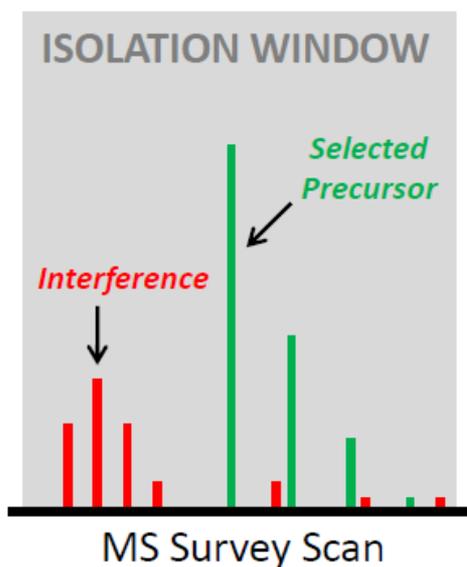
For a high-precision 10-plex TMT experiment, we recommend a minimum summed S/N of ~400 within each MS3 spectrum. This corresponds to less than 10% coefficient of variation between two equally abundant channels.

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



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

# 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
REVDDLGPVGDIIK	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
YGVIIIDEAHER	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

Thermo Proteome Discoverer 2.10.81

File View Administration Tools Window Help

Start Page x Study: Gvgl\_Yeast\_TMT\_MS3 x All.S/N1.Normalization,Scaling x All.S/N0.Normalization.NoScaling x

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

## Summed protein S/N

Checked	Protein FDR Confidence	Master	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	Abundances (Grouped)										# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Entr.
<input checked="" type="checkbox"/>	0.000	✓	P25694	Cell division control protein 48 OS=Saccharomyces cerevisiae	0.000	818.419	73%	28130.3	30091.9	32180.7	38165.6	42388.7	41167.8	40375.5	38894.7	38614.8	38099.7	79	718	79	1	835	91.9	4.94	851
<input checked="" type="checkbox"/>	0.000	✓	Q05022	rRNA biogenesis protein RRP5 OS=Saccharomyces cerevisiae	0.000	817.873	58%	42908.6	38246.6	36714.4	40878.2	40257.2	36858.1	35446.2	26201.8	24292.6	22995.1	125	523	124	1	1729	193.0	6.19	856
<input checked="" type="checkbox"/>	0.000	✓	P19414	Aconitate hydratase, mitochondrial OS=Saccharomyces cerevisiae	0.000	799.984	74%	18535.9	18474.8	21682.4	27740.0	29650.1	34353.7	38570.9	91021.0	102502.7	110973.6	78	969	75	1	778	85.3	8.07	851
<input checked="" type="checkbox"/>	0.000	✓	P38972	Phosphorylformylglycinamide synthase OS=Saccharomyces cerevisiae	0.000	783.400	60%	22502.5	26870.2	27499.1	28258.3	28013.2	25810.0	24575.1	19357.8	18004.9	16434.6	78	555	77	1	1358	148.8	5.27	852
<input checked="" type="checkbox"/>	0.000	✓	P00924	Enolase 1 OS=Saccharomyces cerevisiae (strain ATCC 20454)	0.000	770.445	97%	11822.9	12648.9	21357.4	31135.6	36019.6	39955.9	40285.5	63011.4	73283.0	86054.6	57	2470	20	1	437	46.8	6.62	853
<input checked="" type="checkbox"/>	0.000	✓	Q00402	Nuclear migration protein NUM1 OS=Saccharomyces cerevisiae	0.000	759.075	64%	14796.4	15665.8	16519.0	15197.5	14573.3	14656.1	14561.3	16626.6	17000.7	16564.1	125	389	122	1	2748	312.8	5.40	851
<input checked="" type="checkbox"/>	0.000	✓	P38088	Glycine-tRNA ligase 1, mitochondrial OS=Saccharomyces cerevisiae	0.000	728.809	80%	35215.9	33634.7	34081.4	34273.7	33639.7	32904.5	32294.8	26779.7	25887.6	24547.4	71	608	70	1	690	78.1	6.52	851
<input checked="" type="checkbox"/>	0.000	✓	P14540	Fructose-bisphosphate aldolase OS=Saccharomyces cerevisiae	0.000	728.331	86%	57950.8	56607.4	56806.4	62191.2	60472.2	59347.8	56981.8	53236.0	51271.7	53489.6	30	1708	30	1	359	39.6	5.78	853
<input checked="" type="checkbox"/>	0.000	✓	P09436	Isoleucine-tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae	0.000	716.056	65%	30403.4	29415.6	28969.9	33876.4	33861.1	33230.4	32680.9	25359.9	23540.4	22494.4	92	591	91	1	1072	122.9	6.06	852
<input checked="" type="checkbox"/>	0.000	✓	P22202	Heat shock protein SSA4 OS=Saccharomyces cerevisiae (strain ATCC 20454)	0.000	711.419	69%	5182.7	2766.3	3616.0	18581.6	25287.0	28607.8	31460.2	26986.7	25061.8	24025.8	66	1311	33	1	642	69.6	5.14	856
<input checked="" type="checkbox"/>	0.000	✓	P22515	Ubiquitin-activating enzyme E1 1 OS=Saccharomyces cerevisiae	0.000	703.813	58%	18447.7	19468.4	21025.9	29366.5	33146.9	32400.7	31477.7	28032.1	25491.8	25370.6	68	593	68	1	1024	114.2	5.11	853
<input checked="" type="checkbox"/>	0.000	✓	P00358	Glyceraldehyde 3-phosphate dehydrogenase 2 OS=Saccharomyces cerevisiae	0.000	703.786	66%	3413.6	2880.7	3746.6	3660.7	3037.6	2385.1	3183.6	3473.3	3038.4	3306.6	38	3731	4	1	233	26.8	6.66	853

Hide Associated Tables

Protein Groups Peptide Groups PSMs MS/MS Spectrum Info Annotated Modifications

## Peptide group S/N

Checked	Sequence in Protein	Positions in Protein	Protein Quan Usage	Confidence	Annotated Sequence	Modifications	Abundances (Grouped)										Modifications in Master Proteins	Quality PEP	Quality q-value	# Protein
<input checked="" type="checkbox"/>	KELEELTGVNRITDLHRDVI	[487-507]	Used	●	[K] ELEEELTGVNRITDLHRDVI	2xTMT6plex [N-Term; K21]	299.9	308.7	289.0	151.4	407.8	299.7	260.3	322.7	185.8	142.1		8.04e-06	0	
<input checked="" type="checkbox"/>	R.VISDDYYTSDSGTGVHNV	[308-339]	Used	●	[R] VISDDYYTSDSGTGVHNV	1xCarbamidomethyl [C30]; 2xTMT6plex	45.3	50.5	51.6	69.3	52.6	56.2	58.2	47.3	37.9	30.8		6.38e-10	0	
<input checked="" type="checkbox"/>	R.LYLINSPVLK.A	[627-636]	Used	●	[R] LYLINSPVLK.A	2xTMT6plex [N-Term; K10]	525.3	544.7	641.5	867.0	814.4	905.6	915.6	558.7	591.0	621.1		5.63e-05	0	
<input checked="" type="checkbox"/>	K.DVNDPAVTIGFNVIGQEK	[199-216]	Used	●	[K] DVNDPAVTIGFNVIGQEK	2xTMT6plex [N-Term; K18]	100.6	98.0	82.9	88.0	120.6	117.9	122.1	95.8	71.6	84.7		2.52e-05	0	
<input checked="" type="checkbox"/>	R.LYLINSPVLKAESLKF	[627-641]	Used	●	[R] LYLINSPVLKAESLKF	3xTMT6plex [N-Term; K10; K15]	27.9	25.8	21.2	35.5	52.7	42.6	33.2	21.1	27.2	20.0		3e-05	0	
<input checked="" type="checkbox"/>	K.NYPDPISVLNLY	[609-619]	Used	●	[K] NYPDPISVLNLY	2xTMT6plex [N-Term; K11]	711.4	675.2	766.8	851.4	866.6	857.3	1068.9	671.9	642.9	585.9		3.49e-06	0	
<input checked="" type="checkbox"/>	R.RFGWDTHGVPVIEHIDKK	[81-98]	Used	●	[R] RFGWDTHGVPVIEHIDKK	3xTMT6plex [N-Term; K17; K18]	1016.7	555.5	193.6	391.4	336.9	332.1	309.1	374.8	344.4	304.3		1.95e-05	0	
<input checked="" type="checkbox"/>	K.MSNIDFYDSDSVK.S	[675-687]	Used	●	[K] MSNIDFYDSDSVK.S	2xTMT6plex [N-Term; K13]	16.2	21.0	19.4	31.8	21.3	31.6	23.9	18.3	15.8	19.8		1.74e-07	0	
<input checked="" type="checkbox"/>	K.DALPSVTSEQVREY.FSG	[916-934]	Used	●	[K] DALPSVTSEQVREY.FSG	2xTMT6plex [N-Term; K19]	25.7	28.4	36.8	28.4	42.0	40.2	25.6	28.9	20.7	11.6		4.35e-05	0	

Hide Associated Tables

Proteins (filtered) Protein Groups PSMs MS/MS Spectrum Info

## PSM S/N values

Interference [%]	Average Reporter S/N	Ion Inject Time [ms]	RT [min]	First Scan	Spectrum File	Ions Matched	XCorr	Percolator q-Value	Percolator PEP	Reporter Quan Result ID	Peptide Quan Usage	Quan Info	126	127N	127C	128N	128C	129N	129C	130N	130C	131
0	24.9	43.147	135.5782	45999	fusion_fraction_3.raw	0/0	3.20	0	1.11e-05	1218812	Used	Unique	24.6	26.0	25.0	25.5	42.0	28.3	18.3	21.9	18.4	9.1
51	10.0	150.000	157.7240	59570	m04557.raw	0/0	3.32	8.33e-06	8.1e-05	1724394	Not Used	Excluded by Method	6.9	8.3	9.9	10.9	12.5	8.9	9.3	10.5	10.8	7.8

Ready 4772/4965 Proteins; 4772 Protein Groups; 88474 Peptide Groups; 357119 PSMs; 1050272 MS/MS Spectrum Info; 1055020 Quan Spectra; 447 Result Statistics

# Pros/Cons of the summed S/N approach

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## 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)

Show Advanced Parameters

<b>1. Quantification - General</b>	
Peptides to Use	Unique + Razor
Consider Protein Groups for Peptide	True
Replace Missing Values with Minimu	False
Reject Quan Results with Missing Ct	False
Maximum Allowed Fold Change	100
Top N Peptides Used for Area Calcu	3
<b>2. Reporter Quantification</b>	
Reporter Abundance Based On	Automatic
Apply Quan Value Corrections	False
Co-Isolation Threshold	100
Average Reporter S/N Threshold	0
<b>3. Precursor Quantification</b>	
Use Single-Peak Quan Channels	False
<b>4. Normalization and Scaling</b>	
Normalization Mode	Total Peptide Amount
Proteins For Normalization	
Scaling Mode	On Channels Average (Per File)
<b>5. Display Options</b>	
Show Standard Errors	False
Show Quan Value Counts	False
Show Quan Ratios As	Normal Space Values
<b>6. Quan Ratio Distributions</b>	
1st Fold Change Threshold	2
2nd Fold Change Threshold	4
3rd Fold Change Threshold	6
4th Fold Change Threshold	8
5th Fold Change Threshold	10

Razor peptides

Correction factors

Average S/N per channel

New normalization options

Scaling

Option to display log<sub>2</sub> 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

Quantification Method Editor: SILAC 2plex (Arg10, Lys6)

Quan Channels | Ratio Reporting | Ratio Calculation | Protein Quantification | Experimental Bias

Show the Raw Quan Values

**Minimum Quan Value Threshold:**

Replace Missing Quan Values With Minimum Intensity

Use Single-Peak Quan Channels

Apply Quan Value Corrections

Reject All Quan Values If Not All Quan Channels Are Present

Fold Change Threshold for Up-/Down-Regulation:

Maximum Allowed Fold Change:

Use Ratios Above Maximum Allowed Fold Change for Quantification

Percent Co-Isolation Excluding Peptides from Quantification:

## PD 2.0

Show Advanced Parameters

**1. Ratio Calculation**

**Minimum Quan Value Threshold** 0.0001

Replace Missing Quan Values With Minimum Intensity False

Reject All Quan Values If Not All Quan Channels Are I False

Maximum Allowed Fold Change 100

Use Ratios Above Maximum Allowed Fold Change for False

**1.1 Ratio Calculation for Precursor Quan**

Use Single-Peak Quan Channels False

**1.2 Ratio Calculation for Reporter Quan**

Apply Quan Value Corrections True

Co-Isolation Threshold 100

**2. Protein Quantification**

Use Only Unique Peptides True

Consider Proteins Groups for Peptide Uniqueness True

Top N Peptides Used for Area Calculation 3

**3. Normalization**

Experimental Bias Correction None

Minimum Ratio Count for Median Normalization 20

# 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:

Peptide	Quan Result ID	Peptide Quan Usage	Quan Info	126	127N	127C	128N	128C	129N	129C	130N	130C	131
			Average = 10.1 (> 10)										
	1486778	Used	Unique	19.0	23.0	19.6	19.0	17.3	16.7	12.4	17.0	14.0	9.3
	1244696	Used	Unique	12.5	19.9	21.7	12.0	17.4	11.0	15.6	17.2	20.5	18.3
	1244694	Not Used	Excluded by Method	3.6	10.8	8.8	15.8	11.9	12.5	9.4	7.0	11.1	10.3
	1244693	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
	1384282	Used	Unique	2.2	1.6	1.5	1.5	3.4	3.4	3.0	5.8		2.3
			Average = 8.75 (< 10)										
			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
	1244688	Not Used	Excluded by Method	57.3	46.4	54.2	60.5	66.6	63.2	82.7	48.0	60.3	51.4
	1244684	Used	Unique	3.8	7.6	7.3	7.2	12.7	5.9	6.5	6.4	3.2	6.7
			Unique	27.6	24.0	17.0	20.6	18.4	10.8	16.3	12.0	11.1	11.3

# Normalization in PD 2.1

Show Advanced Parameters	
<b>1. Quantification - General</b>	
Peptides to Use	Unique + Razor
Consider Protein Groups for Peptide	True
Replace Missing Values with Minimu	False
Reject Quan Results with Missing Ct	False
Maximum Allowed Fold Change	100
Top N Peptides Used for Area Calcu	3
<b>2. Reporter Quantification</b>	
Reporter Abundance Based On	Automatic
Apply Quan Value Corrections	False
Co-Isolation Threshold	100
Average Reporter S/N Threshold	0
<b>3. Precursor Quantification</b>	
Use Single-Peak Quan Channels	False
<b>4. Normalization and Scaling</b>	
Normalization Mode	Total Peptide Amount
Proteins For Normalization	
Scaling Mode	On Channels Average (Per File)
<b>5. Display Options</b>	
Show Standard Errors	False
Show Quan Value Counts	False
Show Quan Ratios As	Normal Space Values
<b>6. Quan Ratio Distributions</b>	
1st Fold Change Threshold	2
2nd Fold Change Threshold	4
3rd Fold Change Threshold	6
4th Fold Change Threshold	8
5th Fold Change Threshold	10

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.

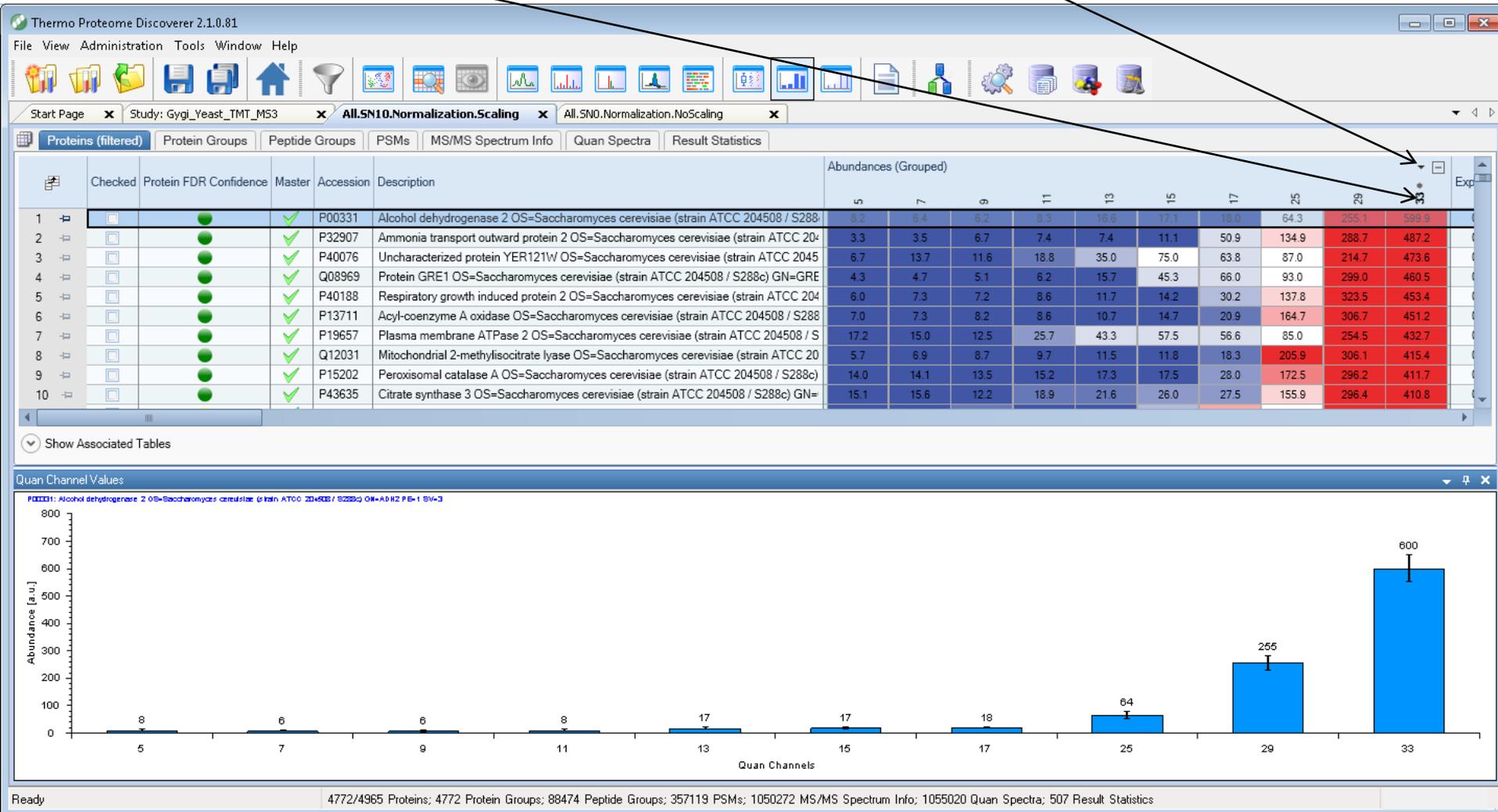
# Scaling in PD 2.1

- 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 204508)	37.6	37.4	43.8	56.2	60.1	69.7	78.4	183.9	208.0	225.0
P38972	Phosphoribosylformylglycinamide synthase OS=Saccharomyces cerevisiae (strain ATCC 204508)	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 204508)	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 204508)	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 / S288c)	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:



# New custom ratio calculation in PD 2.1

The screenshot displays the Thermo Proteome Discoverer 2.1.0.75 software interface. The main window is titled "Study: PD21\_test" and shows the "Sample Group and Quan Ratio Specification" panel. This panel includes "Study Variables" (File, **Quan Channel**, Sample Type), "Manual Ratio Generation" (Numerator: (127C), Denominator: (128N)), and "Denominators to be used:" (a list of channels from 126 to 130N). The "Generated Sample Groups" panel shows a list of sample groups (126, 127N, 127C, 128N\*) and "Generated Ratios" (127N / 126, 127C / 126, 128C / 126, 129N / 126, 129C / 126, 130C / 126, 131 / 126). The "Analysis" panel on the right shows a "Consensus Step" and a "Processing Step".

**Sample group selection**

**Manual ratios**

**Selected ratios for display in report**

**Bulk ratio calculation**

# Custom ratios in PD 2.1 – example 2

The screenshot displays the Thermo Proteome Discoverer 2.1 software interface. The main window is titled "Study: Bailey\_2014" and shows the "Grouping & Quantification" tab. The interface is divided into several panels:

- Sample Group and Quan Ratio Specification:** This panel contains "Study Variables" (1) with checkboxes for File, Quan Channel, Acquisition, Tissue, and Sample Type. Below it is "Manual Ratio Generation" (2) with dropdowns for Numerator and Denominator, and an "Add Ratio" button. The "Bulk Ratio Generation" (3) section includes "Denominators to be used" with a list of tissues (Cerebellum, Heart, Kidney, Liver, Lung, Muscle, Spleen) and checkboxes for Acquisition: DDA and IDA.
- Generated Sample Groups (4):** This panel shows a list of sample groups. A yellow banner at the top indicates "3 of 16 sample groups not used (\*) in any ratio definition." (6). The list includes groups for Cerebellum, Cerebrum, Heart, and Kidney, with sample IDs and acquisition parameters.
- Generated Ratios (5):** This panel shows a list of custom ratios, such as "DDA Heart / DDA Cerebellum", "DDA Liver / DDA Cerebellum", "DDA Lung / DDA Cerebellum", "DDA Muscle / DDA Cerebellum", "DDA Spleen / DDA Cerebellum", "IDA Cerebrum / IDA Cerebellum", "IDA Kidney / IDA Cerebellum", "IDA Liver / IDA Cerebellum", "IDA Lung / IDA Cerebellum", "IDA Muscle / IDA Cerebellum", and "IDA Spleen / IDA Cerebellum". A "Clear All" button is visible.
- Analysis:** This panel on the right shows a "Consensus Step" dialog box with a "Processing Step" (Clone) and a list of "Input Files" (4) including sample IDs and acquisition parameters.

# 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?

The screenshot shows a window titled "Generated Sample Groups" with two sections: "Lung" and "Liver". The "Lung" section is highlighted with a green background and contains four rows of sample information, each with a red border around it. The "Liver" section also contains four rows of sample information. Each row consists of a sample ID, the word "Sample", the organ name, and a file path.

Sample ID	Sample	Organ	File Path
126	Sample	Lung	F3: 29May3013_DJB_mouse_tmt8_BR3_unfrac_165n
129C	Sample	Lung	F2: 29May3013_DJB_mouse_tmt8_BR2_unfrac_165
129N	Sample	Lung	F1: 29May3013_DJB_mouse_tmt8_BR1_unfrac_165
129N	Sample	Lung	F4: 29May3013_DJB_mouse_tmt8_BR4_unfrac_165
<b>Liver</b>			
127N	Sample	Liver	F3: 29May3013_DJB_mouse_tmt8_BR3_unfrac_165
127N	Sample	Liver	F2: 29May3013_DJB_mouse_tmt8_BR2_unfrac_165
129C	Sample	Liver	F1: 29May3013_DJB_mouse_tmt8_BR1_unfrac_165
130C	Sample	Liver	F4: 29May3013_DJB_mouse_tmt8_BR4_unfrac_165

- 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

Thermo Proteome Discoverer 2.1.0.75

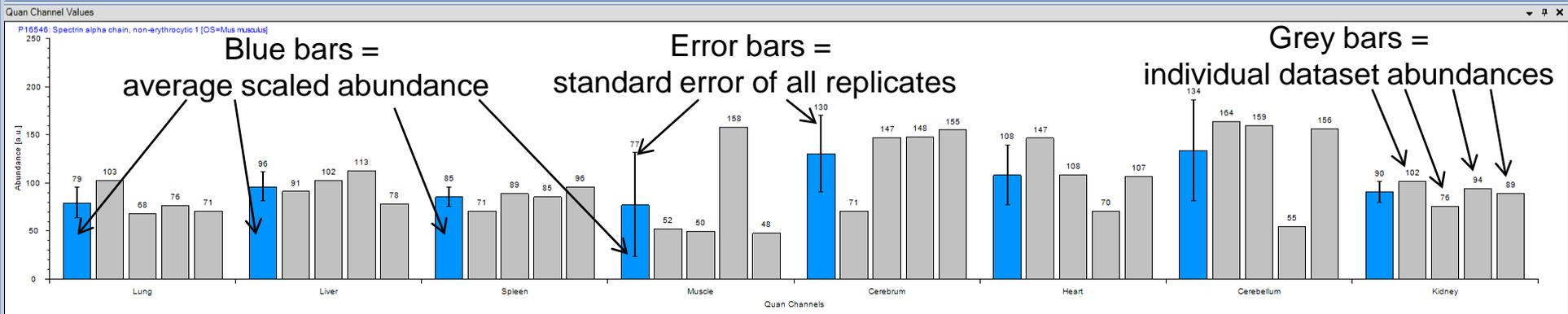
File View Administration Tools Window Help

Start Page Administration 29May3013\_DJB\_mouse\_tmt8\_BR1\_unfrac\_165min\_dda15\_1 Study: TMT8 29May3013\_DJB\_mouse\_tmt8\_BR4\_unfrac\_165min\_dda15\_1

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

Pfam IDs	Abundance Ratios							Abundances (Grouped)							Abundances (Grouped) Standard Errors [%]							Abundances (Scaled)									
	(Lung) / (Lung)*	(Spleen) / (Lung)	(Muscle) / (Lung)	(Cerebrum) / (Lung)	(Heart) / (Lung)	(Cerebellum) / (Lung)	(Kidney) / (Lung)	Lung*	Liver	Spleen	Muscle	Cerebrum	Heart	Cerebellum	Kidney	Lung*	Liver	Spleen	Muscle	Cerebrum	Heart	Cerebellum	Kidney	Lung*	Liver	Spleen	Muscle	Cerebrum	Heart	Cerebellum	Kidney
PF00041, PFI01	0.859	0.844	1.971	0.927	0.954	1.224	0.827	93.0	79.8	78.5	183.2	86.2	88.7	113.7	76.9	20.22	7.13	8.56	39.42	22.92	21.21	66.18	8.74	84.7	107.0	70.3	109.8	80.7	82.6	84.5	71.6
PF00038, PFI01	0.310	0.286	0.830	0.692	0.704	0.427	0.278	176.7	54.7	50.6	146.7	122.4	124.4	75.4	49.1	80.68	15.67	12.96	50.70	121.80	111.86	81.74	3.07	57.6	285.0	49.9	314.5	44.5	54.6	65.5	54.4
PF00063, PFI01	0.961	0.968	6.894	0.876	0.984	2.786	0.943	52.6	50.6	50.9	352.0	46.1	51.8	146.5	49.6	7.18	8.32	16.97	58.71	7.99	5.14	142.02	6.48	55.4	55.4	52.1	47.4	46.6	55.4	52.7	47.6
PF00038, PFI01	1.005	0.936	3.371	0.914	1.298	1.685	0.928	71.8	72.2	67.3	242.2	65.6	93.2	121.0	66.6	25.43	38.87	25.65	51.81	34.44	34.28	113.03	23.19	72.7	91.7	47.5	75.4	104.7	47.7	86.5	49.8
PF00038, PFI01	1.028	1.016	3.822	0.916	1.064	1.848	1.054	68.1	70.0	69.2	260.3	62.4	72.4	125.8	71.8	13.55	12.26	13.64	52.31	8.93	3.54	109.08	19.21	78.6	72.9	59.2	61.7	65.1	74.2	79.8	60.8
PF00063, PFI01	1.184	1.258	4.679	0.844	1.084	2.068	1.165	60.2	71.3	75.8	281.8	50.8	65.3	124.5	70.2	18.10	16.95	30.36	52.98	22.98	16.41	98.96	23.48	61.9	44.9	63.5	70.6	81.0	56.4	81.3	66.5
PF00117, PFI01	0.898	0.863	0.263	0.355	0.396	0.288	0.849	162.9	146.3	140.5	42.9	57.8	64.5	47.0	138.3	117.96	121.52	123.39	13.61	5.91	21.93	20.29	119.49	79.5	64.1	450.7	57.2	49.8	59.7	62.9	412.8
PF00018, PFI01	1.211	1.074	0.971	1.640	1.362	1.685	1.138	79.4	96.1	85.3	77.0	130.1	108.1	133.7	90.3	20.02	15.44	12.28	70.43	30.63	28.76	39.48	12.04	102.6	67.9	76.2	70.7	91.2	102.5	112.6	78.2
PF00273	0.932	0.684	0.387	0.534	1.308	0.384	0.673	135.5	126.3	92.7	52.5	72.4	177.3	52.0	91.3	56.36	67.24	15.77	4.01	63.32	63.37	6.01	18.30	244.0	117.5	64.5	116.2	98.7	88.1	252.0	66.3
PF00038, PFI01	0.665	0.602	2.409	0.633	0.965	1.154	0.648	99.1	65.9	59.6	238.7	62.7	95.6	114.3	64.2	12.16	22.63	55.15	55.57	47.49	27.13	124.15	12.69	97.3	82.8	106.2	109.9	45.3	75.6	64.8	77.9
PF00063, PFI01	1.361	1.549	0.573	0.768	1.092	0.672	1.298	96.2	131.0	149.0	55.1	73.9	105.1	64.6	125.0	44.88	38.31	47.49	19.44	7.39	34.18	16.48	48.60	160.9	72.6	78.9	72.6	195.6	104.2	143.2	80.9
PF00006, PFI01	0.626	0.591	0.498	0.748	0.758	0.633	0.735	143.1	89.5	84.6	71.3	107.1	108.5	90.6	105.1	43.61	29.92	36.49	26.78	37.27	42.49	21.05	30.95	81.9	204.4	97.3	188.8	63.0	125.1	76.5	93.5
PF00006, PFI01	0.686	0.670	0.565	0.850	0.769	0.682	0.811	133.0	91.2	89.2	75.2	113.1	102.4	88.1	107.9	42.80	35.40	30.39	18.76	37.61	48.44	12.32	28.08	71.9	185.1	97.4	177.8	62.6	132.4	68.6	101.3
PF00637, PFI01	1.077	1.042	0.951	1.681	1.296	1.338	1.152	83.9	90.3	87.4	79.8	141.1	108.7	112.2	96.6	19.39	10.18	9.40	49.11	27.38	29.69	32.73	9.90	107.8	76.5	79.6	71.7	88.0	99.8	94.8	78.6
PF00022	0.731	0.777	1.290	0.741	0.843	0.783	0.659	117.2	85.7	91.0	151.2	86.9	98.9	91.8	77.2	38.17	31.04	33.29	39.53	25.97	20.14	66.61	33.85	103.6	166.5	62.3	136.5	117.0	65.5	98.6	61.8

Show Associated Tables



Ready 2093/3355 Proteins; 2093 Protein Groups; 12714 Peptide Groups; 67954 PSMs; 164965 MS/MS Spectrum Info; 167345 Quan Spectra; 499 Result Statistics

# TMT 10-plex correction factor certificate

**\*\*Reporter Ion Isotopic Distributions:**

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

TMT 10plex (used, partly readonly)

Quan Channels

Residue Modification: TMT6plex / +229.163 Da K

N-Terminal Modification: TMT6plex / +229.163 Da

TMT Reporter Ion Isotope Distributions

Mass Tag	Reporter Ion Mass	- 2	- 1	Main	+ 1	+ 2	Active
126	126.127726	0	0	100	5	0	Used
127N	127.124761	0	0.4	100	5	0	Used
127C	127.131081	0	0.2	100	4.6	0.3	Used
128N	128.128116	0	0.9	100	4.7	0.2	Used
128C	128.134436	0	0.5	100	3.2	0	Used
129N	129.131471	0	0.7	100	3.3	0	Used
129C	129.13779	0	1.3	100	2.5	0	Used
130N	130.134825	0	1.2	100	2.8	2.7	Used
130C	130.141145	0	1.5	100	1.8	0	Used
131	131.13818	0	2.1	100	2	0	Used

TMT: Main peaks are always 100%, only correction factors can be edited

OK Cancel Help

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



*Research*

© 2015 by The American Society for Biochemistry and Molecular Biology, Inc.  
This paper is available on line at <http://www.mcponline.org>

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

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

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

# Yeast datasets are available for download from PRIDE

PRIDE > Archive > PXD001334

Project PXD001334 **Replicate 1** [Download Project Files](#)

PRIDE ASSIGNED TAGS: [Biological Dataset](#)

### Summary

PRIDE > Archive > PXD002092

Project PXD002092 **Replicate 2** [Download Project Files](#)

PRIDE ASSIGNED TAGS: [Biological Dataset](#)

### Summary

PRIDE > Archive > PXD002093

Project PXD002093 **Replicate 3** [Download Project Files](#)

PRIDE ASSIGNED TAGS: [Biological Dataset](#)

### Summary

**Title**  
Comprehensive temporal protein dynamics during the diauxic shift in *Saccharomyces cerevisiae*, part 3

**Description**  
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 phase yeast have been measured, protein abundance in yeast is dynamic, particularly during the transition from log to stationary phase. Defining the dynamics of proteomic changes during this transition, termed the diauxic shift, is important to understand the basic biology of proliferative versus quiescent cells. Here, we perform temporal quantitative proteomics to fully capture protein induction and repression during this transition.

**Read more**

**Sample Processing Protocol**  
Samples from cultured yeast were harvested over 33h of culture and labelled using TMT10 reagents. Samples were digested, separated into 12 fractions and analyzed using an Orbitrap Fusion mass spectrometer.

<b>Species</b> <a href="#">Saccharomyces cerevisiae (Baker's yeast)</a>	<b>Tissue</b> Not available
<b>Instrument</b> <a href="#">Orbitrap Fusion</a>	<b>Software</b> Not available
<b>Modification</b> <a href="#">iodoacetamide derivatized residue</a> <a href="#">TMT6plex-126 reporter+balance reagent acylated residue</a>	<b>Quantification</b> <a href="#">TMT</a>

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

The screenshot shows the Thermo Proteome Discoverer 2.1.0.81 interface. The 'Administration' tab is active, displaying a table of existing methods. A dialog box titled 'Create New Quantification Method' is overlaid on the table. The dialog has three radio buttons: 'From Factory Defaults' (selected), 'From Existing Method', and 'From Scratch (advanced mode)'. The 'From Factory Defaults' dropdown is set to 'TMT 10plex'. The 'Create' button is highlighted with a red box.

Status	Method Name	Description	Is Active
✓	Dimethylation 3plex (C2H6, C2H2D4, 13C2D6)	Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method	✓
✓	Full 180 Labeling (O2   18O2)	180 labeling method for fully labeled samples	✓
✓	Incomplete 180 Labeling (O2 L18O + 18O2)	180 labeling method for incompletely labeled samples	✓
		Tandem Mass Tag® of Proteo...	✓
		gs by Applied Biosystems	✓
		gs by Applied Biosystems	✓
		-reactive 6-plex Tandem Mass...	✓
		Tandem Mass Tag® of Proteome...	✓
			✓
			✓
		s8) Method	✓
		s8) Method	✓
		ag® of Proteome Sciences plc	✓
		ag® of Proteome Sciences plc	✓
		ag® of Proteome Sciences plc	✓
		ag® of Proteome Sciences plc	✓
		ag® of Proteome Sciences plc	✓

# 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 <sup>10</sup> -126	126.127726	0.0%	0.0%	100%	5.0% (127C)	0.0% (128N)
TMT <sup>10</sup> -127N	127					
TMT <sup>10</sup> -127C	127					
TMT <sup>10</sup> -128N	128					
TMT <sup>10</sup> -128C	128					
TMT <sup>10</sup> -129N	129					
TMT <sup>10</sup> -129C	129					
TMT <sup>10</sup> -130N	130					
TMT <sup>10</sup> -130C	130					
TMT <sup>10</sup> -131	131					

The screenshot shows the 'Quantification Method Editor: TMT 10 plex QD212963' window. The 'Quan Channels' tab is active, showing 'Residue Modification: TMT6plex / +229.163 Da' and 'N-Terminal Modification: TMT6plex / +229.163 Da'. Below is a table titled 'TMT Reporter Ion Isotope Distributions' with columns for Mass Tag, Reporter Ion Mass, -2, -1, Main, +1, +2, and Active. The row for mass tag 131 is highlighted in blue, showing values 0.3, 1.7, 100, 1.6, and 0. A 'Save Quantification Method' dialog box is open over the editor, with the text 'Save as New Method: TMT 10 plex QD212963' and 'Save' and 'Cancel' buttons. Arrows point from the dialog box to the 'OK' button of the editor window.

Mass Tag	Reporter Ion Mass	- 2	- 1	Main	+ 1	+ 2	Active
126	126.127726	0	0	100	5	0	Used
127N	127.124761	0	0.2	100	5.9	0	Used
127C	127.131081	0	0.6	100	6.4	0	Used
128N	128.128116	0	0.4	100	3.4	0	Used
128C	128.134436	0	0.6	100	4.2	0	Used
129N	129.131471	0	0.7	100	3.1	0	Used
129C	129.13779	0	1.3	100	2.9	0	Used
130N	130.134825	0	1.3	100	2.8	1.7	Used
130C	130.141145	0	1.6	100	1.7	0	Used
131	131.13818	0.3	1.7	100	1.6	0	Used

TMT: Main peaks are always 100%, only correction factors can be edited

- Save as new method.

# Step 2 - Create New Study

Thermo Proteome Discoverer 2.1.0.81

File View Administration Tools Window Help

Start Page Administration

Proteome Discoverer 2.1

Start

New Study/Analysis... Create a new study and options

Open Study... Open Result...

**New Study and Analysis**

Study Name: Gygi Dauxic Shift

Study Root Directory: C:\Studies

Processing Workflow: (empty workflow)

Consensus Workflow: (empty workflow)

Quantification Method: TMT 10 plex QD212963

Select Control Channel:

<input checked="" type="checkbox"/> 126	<input type="checkbox"/> 129C
<input type="checkbox"/> 127N	<input type="checkbox"/> 130N
<input type="checkbox"/> 127C	<input type="checkbox"/> 130C
<input type="checkbox"/> 128N	<input type="checkbox"/> 131
<input type="checkbox"/> 128C	
<input type="checkbox"/> 129N	

Buttons: Add Files, Add Fractions, Remove, Treat as Replicates

Buttons: OK, Cancel

Ready

Choose quan method that was just created

The optionally selected quantification method will be used for the selected input files.

# Step 3 – Set up study factors

The screenshot shows the Thermo Proteome Discoverer 2.1.0.81 interface. The main window displays the 'Study: Gygi Dauxic Shift' configuration. The 'Study Summary' section shows the study name, directory, and dates. The 'Quantification Methods' section lists various methods like Dimethylation 3plex, Full 18O Labeling, iTRAQ 4plex, iTRAQ 8plex, and Low Resolution TMT 6plex. A dialog box is open for adding a factor, with 'Time (hr)' selected as the factor unit and a list of values including 5, 7, 9, 11, 13, 15, 17, 25, 29, and 33. The value 17 is highlighted. A legend indicates 'Categorical Factor' and 'Numeric Factor'.

Study Summary

Study Name: Gygi Dauxic Shift  
Study Directory: C:\Studies\Gygi Dauxic Shift  
Last Changed: 9/23/2015 4:56:50 PM  
Creation Date: 9/23/2015 4:56:50 PM

Quantification Methods

Dimethylation 3plex (C2H6, C2H...  
*Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method*

Full 18O Labeling (O2 | 18O2)  
*18O labeling method for fully labeled samples*

iTRAQ 4plex  
*Method for iTRAQ™ 4-plex mass tags by Applied Biosystems*

iTRAQ 8plex  
*Method for iTRAQ™ 8-plex mass tags by Applied Biosystems*

Low Resolution TMT 6plex  
*Method for low resolution 6-plex Tandem Mass Tag® of Proteome Sciences plc*

SILAC 2plex (Arg10, Lys6)  
*SILAC 2plex (Arg10, Lys6) Method*

SILAC 2plex (Arg10, Lys8)  
*SILAC 2plex (Arg10, Lys8) Method*

SILAC 2plex (Ile6)  
*SILAC 2plex (Ile6) Method*

Low Resolution iodo TMT 6plex  
*Method for low resolution cysteine-reactive 6-plex Tandem Mass Tag® of Proteome Sciences plc*

Factor Unit: Time (hr)

Values:

5  
7  
9  
11  
13  
15  
17  
25  
29  
33

Apply Cancel x

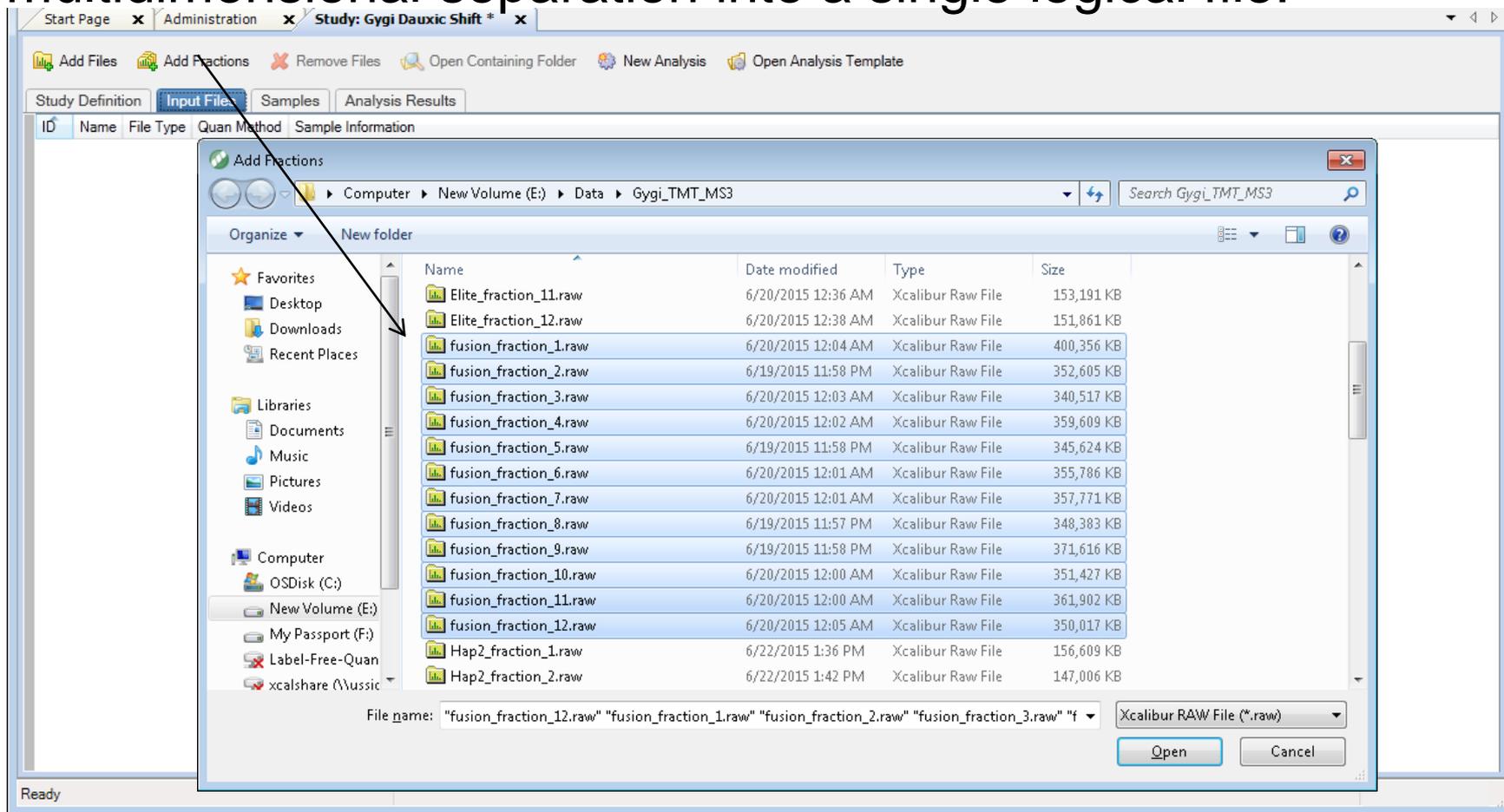
Paste Copy Add

Categorical Factor  
Numeric Factor

Ready

# Step 4 – Import data files

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



# Step 5 – Assign Quan Method to each sample

The screenshot shows the Thermo Proteome Discoverer 2.1.0.81 software interface. The main window displays the 'Input Files' tab, which contains a table of samples. The table has columns for ID, Name, File Type, Quan Method, and Sample Information. Three samples are listed: F1 (Replicate 1), F2 (Replicate 2), and F3 (Replicate 3), all with a File Type of .raw. The 'Quan Method' column for F1 is currently empty, and a dropdown menu is open, showing 'TMT\_10 plex QD212963' as the selected option. The 'Sample Information' column for all samples shows 'Sample Type: [Sample], Time: [n/a]'. The status bar at the bottom left indicates 'Ready'.

ID	Name	File Type	Quan Method	Sample Information
F1	Replicate 1	.raw		Sample Type: [Sample], Time: [n/a]
F2	Replicate 2	.raw	TMT_10 plex QD212963	Sample Type: [Sample], Time: [n/a]
F3	Replicate 3	.raw		Sample Type: [Sample], Time: [n/a]

# Step 6 - Assign study factors to reporter ions

Thermo Proteome Discoverer 2.1.0.81

File View Administration Tools Window Help

Start Page Administration **Study: Gygi Dauxic Shift \***

Add Files Add Fractions Remove Files Open Containing Folder New Analysis Open Analysis Template

Study Definition **Input Files** Samples Analysis Results

ID	Name	File Type	Quan Method	Sample Information
F1	Replicate 1	.raw	TMT 10 plex QD212963	Sample Type: [Sample], Time (hr): [5 . 7 . 9 . 11 . 13 . 15 . 17 . 25 . 29 . 33]

Sample	Sample Identifier	Sample Type	Quan Channel	Time (hr)
1	Replicate 1 - [126]	Sample	126	5
4	Replicate 1 - [127N]	Sample	127N	7
5	Replicate 1 - [127C]	Sample	127C	9
6	Replicate 1 - [128N]	Sample	128N	11
7	Replicate 1 - [128C]	Sample	128C	13
8	Replicate 1 - [129N]	Sample	129N	15
9	Replicate 1 - [129C]	Sample	129C	17
10	Replicate 1 - [130N]	Sample	130N	25
11	Replicate 1 - [130C]	Sample	130C	29
12	Replicate 1 - [131]	Sample	131	33

**Files:**

ID	Name	Date Modified	n/a
F1.1	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_1.raw	6/20/2015 12:04:00 AM	4099
F1.2	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_10.raw	6/20/2015 12:00:00 AM	3598
F1.3	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_11.raw	6/20/2015 12:00:00 AM	3705
F1.4	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_12.raw	6/20/2015 12:05:00 AM	3584
F1.5	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_2.raw	6/19/2015 11:58:00 PM	3610
F1.6	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_3.raw	6/20/2015 12:03:00 AM	3486
F1.7	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_4.raw	6/20/2015 12:02:00 AM	3682
F1.8	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_5.raw	6/19/2015 11:58:00 PM	3539
F1.9	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_6.raw	6/20/2015 12:01:00 AM	3643
F1.10	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_7.raw	6/20/2015 12:01:00 AM	3663
F1.11	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_8.raw	6/19/2015 11:57:00 PM	3567
F1.12	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_9.raw	6/19/2015 11:58:00 PM	3805

Ready

# Step 7 – Create New Analysis

Thermo Proteome Discoverer 2.1.0.81

File View Administration Tools Window Help

Start Page Administration Study: Gygi Dauxic Shift

Add Files Add Fractions Remove Files Open Containing Folder New Analysis Open Analysis Template

Study Definition Input Files Samples Analysis Results Workflows Grouping & Quantification

ID	Name	File Type	Quan Method	Sample Information
F1	Replicate 1	.raw	TMT 10 plex QD212963	Sample Type: [Sample], Time (hr): [5 . 7 . 9 . 11 . 13 . 15 . 17 . 25 . 29]

Sample	Sample Identifier	Sample Type	Quan Channel	Time (hr)
1	Replicate 1 - [126]	Sample	126	5
4	Replicate 1 - [127N]	Sample	127N	7
5	Replicate 1 - [127C]	Sample	127C	9
6	Replicate 1 - [128N]	Sample	128N	11
7	Replicate 1 - [128C]	Sample	128C	13
8	Replicate 1 - [129N]	Sample	129N	15
9	Replicate 1 - [129C]	Sample	129C	17
10	Replicate 1 - [130N]	Sample	130N	25
11	Replicate 1 - [130C]	Sample	130C	29
12	Replicate 1 - [131]	Sample	131	33

Files:

ID	Name	Date Modified	Size
F1.1	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_1.raw	6/20/2015 12:04:00 AM	409963882 [Byte]
F1.2	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_10.raw	6/20/2015 12:00:00 AM	359860868 [Byte]
F1.3	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_11.raw	6/20/2015 12:00:00 AM	370587452 [Byte]
F1.4	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_12.raw	6/20/2015 12:05:00 AM	358417404 [Byte]
F1.5	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_2.raw	6/19/2015 11:58:00 PM	361066850 [Byte]
F1.6	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_3.raw	6/20/2015 12:03:00 AM	348689230 [Byte]
F1.7	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_4.raw	6/20/2015 12:02:00 AM	368239074 [Byte]
F1.8	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_5.raw	6/19/2015 11:58:00 PM	353918202 [Byte]
F1.9	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_6.raw	6/20/2015 12:01:00 AM	364324070 [Byte]
F1.10	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_7.raw	6/20/2015 12:01:00 AM	366356810 [Byte]
F1.11	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_8.raw	6/19/2015 11:57:00 PM	356743250 [Byte]
F1.12	\\ussjo-9smd9y1e\Data\Gygi_TMT_MS3\fusion_fraction_9.raw	6/19/2015 11:58:00 PM	380534198 [Byte]

Ready

Analysis

Consensus Step

Workflow:

Result File: Enter result file name.

Child Steps: (1)

Processing Step Clone

Workflow:

Result File: Enter result file name.

Input Files: (0)

Drop your input files here

# Step 8 – Choose processing workflow

The screenshot displays the Thermo Proteome Discoverer 2.1.0.81 interface. The main window shows the 'Workflows' tab with a workflow tree containing the following steps: Spectrum Files (0), Reporter Ions Quantifier (4), Spectrum Selector (1), Sequest HT (2), and Percolator (3). The 'Sequest HT' step is highlighted with a red box and an arrow pointing to a callout box. The 'Parameters' panel on the left shows the 'Scan Event Filters' section with 'MS Order' set to 'MS3'. The 'Analysis' window on the right shows the 'Consensus Step' configuration with the workflow name 'PWF\_Fusion\_Reporter\_Based\_Quan\_SPS\_MS3\_SequestHT\_Percolator' highlighted in a red box. A red arrow points from this box to another callout box.

**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

The screenshot displays the Thermo Proteome Discoverer 2.1.0.81 software interface. The main window shows the 'Workflows' tab with a workflow tree for 'CWF\_Comprehensive\_Enhanced Annotation\_Quan'. The workflow tree includes nodes: Protein Scorer (4), Protein FDR Validator (9), Protein Grouping (5), Peptide in Protein Annotation (6), and Peptide and Protein Quantifier (7). The 'Peptide and Protein Quantifier' node is highlighted with a green dashed border. A red box highlights the 'Consensus Step' dialog box, which shows the selected workflow: 'CWF\_Comprehensive\_Enhanced Annotation\_Quan'. A red callout box with an arrow points to the 'Peptide and Protein Quantifier' node, containing the text: 'Workflows with “Quan” in title include Peptide and Protein Quantifier node'. The left sidebar shows parameters for '1. Quantification - General', '2. Reporter Quantification', '3. Precursor Quantification', and '4. Normalization and Scaling'. The bottom status bar shows 'Ready'.

# Step 10 – Modify Peptide and Protein Quantifier

The screenshot displays the Thermo Proteome Discoverer 2.1.0.81 software interface. The main window is titled "Study: Gygi Dauxic Shift". The "Workflows" tab is active, showing a workflow tree with nodes: Protein FDR Validator (9), Protein Grouping (10), Peptide in Protein Annotation (6), and Peptide Protein Quantifier (7). The "Peptide Protein Quantifier" node is highlighted with a dashed green border. The "Parameters" panel on the left shows various settings, with several items marked with a red asterisk to indicate recommended settings:

- 1. Quantification - General**
  - Peptides to Use: Unique + Razor (marked with a red asterisk)
  - Consider Protein Groups for Pe: True
  - Replace Missing Values with M: False
  - Reject Quan Results with Missi: False
  - Maximum Allowed Fold Change: 100
  - Top N Peptides Used for Area: 3
- 2. Reporter Quantification**
  - Reporter Abundance Based Or: Automatic
  - Apply Quan Value Corrections: True (marked with a red asterisk)
  - Co-Isolation Threshold: 50 (marked with a red asterisk)
  - Average Reporter S/N Thresh: 10 (marked with a red asterisk)
- 3. Precursor Quantification**
  - Use Single-Peak Quan Chann: False
- 4. Normalization and Scaling**
  - Normalization Mode: Total Peptide Amount (marked with a red asterisk)
  - Proteins For Normalization: (empty)
  - Scaling Mode: On Channels Average (Per File) (marked with a red asterisk)
- 5. Display Options**
  - Show Standard Errors: False
  - Show Quan Value Counts: False
  - Show Quan Results As: Normal Space Values
- 6. Quan Ratio Distributions**

The "Peptides to Use" section is expanded, showing the following text:

**Peptides to Use**  
Specifies which peptides are used for quantification.  
Unique: Only peptides that are not shared between different proteins or protein groups are used for the protein quantification.  
Unique + Razor: Uses all peptides that are not shared between different

A red arrow points from the "Peptides to Use" section to a red-bordered box at the bottom of the screen containing the text: "Starred settings are recommended".

The "Analysis" panel on the right shows a "Consensus Step" window with the following details:

- Workflow: CWF\_Comprehensive\_Enhanced Annotation\_Quan
- Result File: Enter result file name.
- Child Steps: (1) Add
  - Processing Step (Clone) (marked with a red asterisk)
    - Workflow: PWF\_Fusion\_Reporter\_Based\_Quan\_SPS\_MS3\_Sequest HT\_Percolator
    - Result File: Enter result file name.
    - Input Files: (0)
    - Drop your input files here

# Step 11- Drag files into analysis

The screenshot displays the Thermo Proteome Discoverer 2.1.0.81 interface. The 'Input Files' tab is active, showing a table with three rows representing replicates. A red box highlights the instruction: 'Select all three files above and then left click, hold, and drag to input files box in analysis'. An arrow points from this box to the 'Input Files' section of the 'Analysis' panel on the right, where the three replicates are listed under 'Input Files: (3)'. The 'Analysis' panel also shows a 'Consensus Step' and a 'Processing Step'.

ID	Name	File Type	Quan Method	Sample Information
F1	Replicate 1	.raw	TMT 10 plex QD212963	Sample Type: [Sample], Time (hr): [5, 7, 9, 11, 13, 15, 17, 25, 29, 33]
F2	Replicate 2	.raw	TMT 10 plex QD212963	Sample Type: [Sample], Time (hr): [5, 7, 9, 11, 13, 15, 17, 25, 29, 33]
F3	Replicate 3	.raw	TMT 10 plex QD212963	Sample Type: [Sample], Time (hr): [5, 7, 9, 11, 13, 15, 17, 25, 29, 33]

**Select all three files above and then left click, hold, and drag to input files box in analysis**

**Analysis** [As Batch] [Run] [Save] [X]

Consensus Step [X]

Workflow: CWF\_Comprehensive\_Enhanced  
Annotation\_Quan  
Result File: Replicate 3.pdResult

Child Steps: (1) Add

Processing Step [Clone] [Warning]

Workflow: PWF\_Fusion\_Reporter\_Based\_Quan\_SPS\_M  
S3\_SequestHT\_Percolator  
Result File: Replicate 3.msf

Input Files: (3)

- F3 Replicate 3 TMT 10 plex QD212963 Sample Type: [S
- x F1 Replicate 1 TMT 10 plex QD212963 Sample Type: [S
- x F2 Replicate 2 TMT 10 plex QD212963 Sample Type: [S

Ready

# Step 12 – Grouping and Quantification tab

Thermo Proteome Discoverer 2.1.0.81

File View Administration Tools Window Help

Start Page Administration Study: Gygi Dauxic Shift \* Analysis

Add Files Add Fractions Remove Files Open Containing Folder New Analysis Open Analysis

Study Definition Input Files Samples Analysis Results Workflows **Grouping & Quantification** Analysis

Sample Group and Quan Ratio Specification

Study Variables

1) Choose study factor

File

Time (hr)

Sample Type

Variables printed in italics contain only a single value.

2) Select 5 hr as denominator(or any other factor of interest)

Denominators to be used:

Time (hr) : 5

Time (hr) : 7

Time (hr) : 9

Time (hr) : 11

Time (hr) : 13

Time (hr) : 15

Add Ratios

3) Click "Add Ratios"

Generated Sample Groups

5

126 Sample 5 F2: Replicate 2

126 Sample 5 F1: Replicate 1

126 Sample 5 F3: Replicate 3

7

127N Sample 7 F2: Replicate 2

127N Sample 7 F1: Replicate 1

127N Sample 7 F3: Replicate 3

9

127C Sample 9 F2: Replicate 2

127C Sample 9 F1: Replicate 1

127C Sample 9 F3: Replicate 3

Generated Ratios

Clear All

× 7 / 5

× 9 / 5

× 11 / 5

× 13 / 5

× 15 / 5

× 17 / 5

× 25 / 5

× 29 / 5

Note that all abundance values calculated for each study factor will be the **average** of the 3 replicates

Click "Run!"

Analysis

As Batch Run Save

Consensus Step

Workflow: CWF\_Comprehensive\_Enhanced Annotation\_Quan

Result File: Replicate 3.pdResult

Child Steps: (1)

Processing Step Clone

Workflow: PWF\_Fusion\_Reporter\_Based\_Quan\_SPS\_M S3\_SequestHT\_Percolator

Result File: Replicate 3.msfr

Input Files: (3)

× F3 Replicate 3 TMT 10 plex QD212963 Sample Type: [S

× F1 Replicate 1 TMT 10 plex QD212963 Sample Type: [S

× F2 Replicate 2 TMT 10 plex QD212963 Sample Type: [S

# Search results - >4700 proteins, >88000 unique peptides

Thermo Proteome Discoverer 2.1.0.81

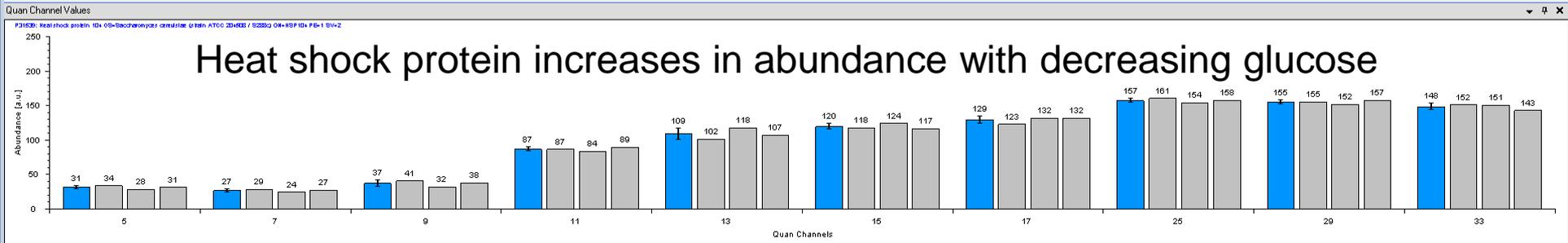
File View Administration Tools Window Help

Start Page Study: Gygi\_Yeast\_TMT\_MS3 All.SN10.Normalization.Scaling All.SN10.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)														Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# AA
					5	7	9	11	13	15	17	19	21	23	25	27	29	31							
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P19097	Fatty acid synthase subunit alpha OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=FAA1	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P10591	Heat shock protein SSA1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSA1	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	64				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P10592	Heat shock protein SSA2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSA2	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	63				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Q00955	Acetyl-CoA carboxylase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ACC1	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P00549	Pyruvate kinase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PK1	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	50				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P15108	ATP-dependent molecular chaperone HSC82 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSC82	65.9	64.3	63.2	115.9	121.0	118.5	126.2	112.6	106.2	106.0	0.000	1196.036	92%	99	2393	19	70				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P32324	Elongation factor 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=EF2	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	84				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P02829	ATP-dependent molecular chaperone HSP82 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP82	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	70				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P16521	Elongation factor 3A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=EF3A	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	104				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P06105	Protein SCP160 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SCP160	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	122				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P00925	Enolase 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=ENO2	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P07149	Fatty acid synthase subunit beta OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=FAA2	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P32589	Heat shock protein homolog SSE1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSE1	91.1	79.5	72.7	117.2	119.7	119.7	119.4	100.8	92.3	90.3	0.000	1005.152	81%	77	1295	66	69				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P00560	Phosphoglycerate kinase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PGK1	82.6	91.5	96.8	111.8	104.0	105.5	104.3	98.1	86.6	106.8	0.000	948.671	96%	56	2924	56	41				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P31539	Heat shock protein 104 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HSP104	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	90				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P0C590	Heat shock protein SSC1, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSC1	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	65				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P02994	Elongation factor 1-alpha OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=EF1A	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	45				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P06169	Pyruvate decarboxylase isozyme 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=DC1	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	56				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P40150	Heat shock protein SSB2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSB2	99.5	102.7	98.3	105.5	111.9	104.9	101.0	94.5	90.8	89.8	0.000	879.364	76%	63	1927	6	61				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P16961	ATP-dependent 6-phosphofructokinase subunit alpha OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=PFK1A	87.5	89.7	93.8	104.2	113.7	112.0	112.0	100.0	82.5	94.6	0.000	858.615	70%	78	816	77	98				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P11484	Heat shock protein SSB1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=SSB1	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	61				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	P17255	V-type proton ATPase catalytic subunit A OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=V-ATPase	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				
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Q02455	Protein MLP1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=MLP1	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



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

# Search results - >4700 proteins, >88000 unique peptides

Thermo Proteome Discoverer 2.1.0.81

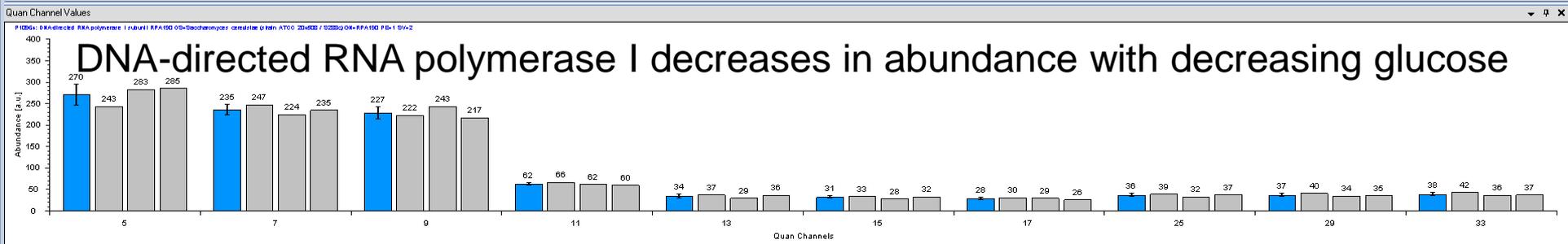
File View Administration Tools Window Help

Start Page Study: Gvgl\_Yeast\_TMT\_MS3 All.SN10.Normalization.Scaling

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

Checked	Protein FDR Confidence	Master	Accession	Description	Abundances (Grouped)										Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# AA
					5	6	7	8	9	10	11	12	13	14							
<input checked="" type="checkbox"/>	100%	✓	P38968	Protein transport protein SEC31 OS=Saccharomyces cerevisiae (strain ATCC 204508)	81.8	90.5	102.2	124.2	118.0	113.3	108.5	91.7	86.9	82.8	0.000	519.298	49%	52	313	52	127
<input checked="" type="checkbox"/>	100%	✓	P10964	DNA-directed RNA polymerase I subunit RPA190 OS=Saccharomyces cerevisiae (stra	270.3	235.4	227.5	62.4	34.1	31.1	28.4	36.1	36.6	38.2	0.000	519.095	52%	91	296	89	166
<input checked="" type="checkbox"/>	100%	✓	P43597	Uncharacterized protein YFR016C OS=Saccharomyces cerevisiae (strain ATCC 20450	80.4	64.5	76.6	116.4	116.9	127.1	126.7	108.7	102.4	100.3	0.000	518.728	62%	81	219	81	123
<input checked="" type="checkbox"/>	100%	✓	P04807	Hexokinase-2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=HXK	104.9	112.6	106.4	97.6	101.2	102.7	99.3	93.0	91.1	92.4	0.000	518.449	83%	38	777	35	49
<input checked="" type="checkbox"/>	100%	✓	P53852	Cysteine-tRNA ligase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	95.2	90.6	91.9	119.3	119.9	114.4	109.7	92.3	86.4	80.3	0.000	515.990	66%	63	359	63	76
<input checked="" type="checkbox"/>	100%	✓	P38708	Putative proline-tRNA ligase YHR020W OS=Saccharomyces cerevisiae (strain ATCC	123.3	114.0	105.7	106.5	111.0	106.4	96.5	83.9	78.4	74.3	0.000	514.722	69%	55	376	55	68
<input checked="" type="checkbox"/>	100%	✓	P10081	ATP-dependent RNA helicase eIF4A OS=Saccharomyces cerevisiae (strain ATCC 204	111.5	104.7	100.0	104.5	115.6	107.1	105.8	87.0	82.3	81.5	0.000	512.466	73%	39	654	39	39
<input checked="" type="checkbox"/>	100%	✓	P46655	Glutamate-tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae (strain ATCC 204	103.5	96.8	96.9	117.5	119.0	110.7	107.2	88.9	81.2	78.3	0.000	511.220	74%	68	539	68	70
<input checked="" type="checkbox"/>	100%	✓	P12709	Glucose-6-phosphate isomerase OS=Saccharomyces cerevisiae (strain ATCC 204508	76.1	77.2	84.0	110.1	107.4	116.2	116.6	105.2	99.6	107.7	0.000	508.927	74%	42	734	42	55
<input checked="" type="checkbox"/>	100%	✓	P07251	ATP synthase subunit alpha, mitochondrial OS=Saccharomyces cerevisiae (strain ATC	42.4	43.0	54.7	65.7	79.3	84.4	89.9	170.2	182.5	188.0	0.000	507.354	79%	62	645	61	54
<input checked="" type="checkbox"/>	100%	✓	Q07878	Vacuolar protein sorting-associated protein 13 OS=Saccharomyces cerevisiae (strain /	87.4	102.6	112.5	109.8	108.8	107.6	103.9	91.2	89.0	87.2	0.000	504.682	34%	98	222	98	314
<input checked="" type="checkbox"/>	100%	✓	P19657	Plasma membrane ATPase 2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S	17.2	15.0	12.5	25.7	43.3	57.5	56.6	85.0	254.5	432.7	0.000	504.642	32%	44	748	3	94
<input checked="" type="checkbox"/>	100%	✓	P23615	Transcription elongation factor SPT6 OS=Saccharomyces cerevisiae (strain ATCC 204	93.8	97.5	96.0	113.8	120.0	116.4	110.5	87.4	82.9	79.9	0.000	500.121	51%	78	259	78	145
<input checked="" type="checkbox"/>	100%	✓	P20967	2-oxoglutarate dehydrogenase, mitochondrial OS=Saccharomyces cerevisiae (strain A	32.0	33.9	40.8	46.4	58.0	65.3	76.6	205.0	222.4	219.5	0.000	494.463	55%	65	352	65	101
<input checked="" type="checkbox"/>	100%	✓	P33416	Heat shock protein 78, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204	26.3	26.2	39.6	75.0	89.9	111.7	119.7	172.5	167.6	171.4	0.000	493.380	73%	82	391	81	81
<input checked="" type="checkbox"/>	100%	✓	P04802	Aspartate-tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae (strain ATCC 204	83.4	80.4	82.2	103.2	120.5	116.3	117.0	100.8	101.2	95.0	0.000	492.610	72%	59	391	58	55
<input checked="" type="checkbox"/>	100%	✓	P33892	Translational activator GCN1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S	112.3	115.7	118.0	118.3	95.2	99.0	106.3	82.1	76.5	76.5	0.000	487.072	31%	92	303	92	267
<input checked="" type="checkbox"/>	100%	✓	P32454	Aminopeptidase 2, mitochondrial OS=Saccharomyces cerevisiae (strain ATCC 204508	58.0	66.3	78.8	105.6	117.7	115.9	121.0	111.8	113.4	111.5	0.000	483.410	55%	62	305	57	95
<input checked="" type="checkbox"/>	100%	✓	P07264	3-isopropylmalate dehydratase OS=Saccharomyces cerevisiae (strain ATCC 204508 /	88.2	79.5	79.3	108.3	124.1	115.3	115.0	100.4	97.4	92.5	0.000	483.133	70%	60	302	59	77
<input checked="" type="checkbox"/>	100%	✓	P16140	V-type proton ATPase subunit B OS=Saccharomyces cerevisiae (strain ATCC 204508	86.7	86.7	95.0	117.1	124.6	118.5	116.6	91.5	83.5	79.9	0.000	480.468	83%	49	534	49	51
<input checked="" type="checkbox"/>	100%	✓	P38707	Asparagine-tRNA ligase, cytoplasmic OS=Saccharomyces cerevisiae (strain ATCC 20	112.4	108.7	105.4	109.7	110.1	101.9	105.2	86.2	82.4	77.9	0.000	477.351	56%	46	311	46	55
<input checked="" type="checkbox"/>	100%	✓	P47035	Nucleolar protein NET1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	89.7	87.1	96.1	122.4	104.8	107.4	111.2	96.8	95.0	89.5	0.000	474.035	58%	79	294	78	118
<input checked="" type="checkbox"/>	100%	✓	P40457	Protein MLP2 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=MLP	96.1	104.4	111.1	117.4	106.5	100.8	99.0	85.8	91.9	88.0	0.000	471.108	58%	104	223	103	167
<input checked="" type="checkbox"/>	100%	✓	P31688	Trehalose-phosphatase OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c)	36.2	41.7	59.8	93.8	120.9	131.5	137.1	130.7	126.0	122.4	0.000	470.570	59%	72	293	72	89

Show Associated Tables



# 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.

The screenshot shows a Microsoft Excel spreadsheet titled "All-(01).csv - Microsoft Excel". The ribbon includes File, Home, Insert, Page Layout, Formulas, Data, Review, View, and Acrobat. The Data ribbon is active, showing options like Sort, Filter, Text to Columns, and Data Tools. The spreadsheet has columns labeled A through W. Column D is highlighted and labeled "KEY". The data rows contain protein information such as "Checked", "Protein FC", "Master", "Descriptio", "Entrez Ge", "Gene ID", "Chromosc", "Biological", "Cellular C", "Molecular", "Pfam IDs", and abundance values (AQR1-AQR10). The last column, W, is labeled "AbundancA".

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Checked	Protein FC	Master	KEY	Descriptio	Entrez Ge	Gene ID	Chromosc	Biological	Cellular C	Molecular	Pfam IDs	AQR1	AQR2	AQR3	AQR4	AQR5	AQR6	AQR7	AQR8	AQR9	AQR10	AbundancA
2	FALSE	High	Master Pri	A5Z2X5	UPF0495	5142379	YPR010C-A	XVI		membrane		Pf06522	25.9	25.7	34.9	41.1	50	67.1	89.9	194.3	229.4	241.7	22.41
3	FALSE	High	Master Pri	D6VTK4	Pheromor	850518	STE2; YFL0VI		cell organ	membran	receptor	Pf02116	220.6	263.6	311.8	81.1	29.7	22.6	25.2	16	15.3	14	11.97
4	FALSE	High	Master Pri	O13297	mRNA-cap	855873	CET1; YPL2XVI		metabolic	nucleus	catalytic	Pf02940	92.7	97.9	99.6	94.6	104.4	104.8	104.5	99.5	101.9	100	13.07
5	FALSE	High	Master Pri	O13329	DNA repli	851688	FOB1; YDF1V		cell organ	nucleus	DNA binding	metal i	135.8	139.4	128	106.4	97.8	96.2	89.7	69.9	71.3	65.4	9.04
6	FALSE	High	Master Pri	O13525	Ubiquinor	851785	COQ4; YDF1V		metabolic	membrane;	mitochoi	Pf05019	48.2	55.1	68.9	89.7	102.7	110.8	119.1	124.2	137.7	143.6	9.63
7	FALSE	High	Master Pri	O13535	Transposc	856623	YHR214C-F	VIII		cell organ	cytoplasm	catalytic	Pf00665	188.1	213.8	175.4	114.5	62.1	58.5	41.1	46.7	45.2	54.4
8	FALSE	High	Master Pri	O13539	THO comp	855577	THP2; YHR214I		cell organ	nucleus	protein hi	Pf09432	112.7	118.2	120.5	97.2	94.7	96.5	86.8	91.7	91.8	89.5	11.83

- 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”

The screenshot shows the ProteinCenter Profiling interface. The top navigation bar includes tabs for Workbench, Administration, Import,  $\mu$ LIMS, Peptides, Protein Data, Proteins, Genes, Clusters, Profiling (active), Heat Maps, ProteinCard, Statistics, Report, and Export. Below the navigation bar, there are several settings: 'All' (dropdown), 'Proteins' (dropdown), 'using group count 15', 'with auto adjust' (checkbox), 'allow missing values 0', 'alpha core 0.8', and a 'Profile' button. The main area is titled 'Data types' and is divided into two columns: 'Available' and 'Selected'. The 'Available' column contains 'lemPAI (Exponentially modified protein abundance index (Imported))'. The 'Selected' column contains ten entries: 'AQR1 (Average quantitation ratio 1) [37]', 'AQR2 (Average quantitation ratio 2) [36]', 'AQR3 (Average quantitation ratio 3) [36]', 'AQR4 (Average quantitation ratio 4) [36]', 'AQR5 (Average quantitation ratio 5) [36]', 'AQR6 (Average quantitation ratio 6) [36]', 'AQR7 (Average quantitation ratio 7) [36]', 'AQR8 (Average quantitation ratio 8) [37]', 'AQR9 (Average quantitation ratio 9) [36]', and 'AQR10 (Average quantitation ratio 10) [36]'. Between the columns are four buttons: 'select', 'deselect', 'up', and 'down'. An arrow from the 'Profile' button in the top bar points to the 'Profile' button in the interface.

# Profiling results

ProteinCenter

Workbench Administration

Import LIMS Peptides Protein Data Proteins Genes Clusters Profiling Heat Maps ProteinCard Statistics Report Export

All Proteins using group count 16 with auto adjust allow missing values 0 alpha core 0.8 Profile

Data types

Available	Selected
lemPAI (Exponentially modified protein abundance index (Imported))	AQR1 (Average quantitation ratio 1) [37]
	AQR2 (Average quantitation ratio 2) [36]
	AQR3 (Average quantitation ratio 3) [36]
	AQR4 (Average quantitation ratio 4) [36]
	AQR5 (Average quantitation ratio 5) [36]
	AQR6 (Average quantitation ratio 6) [36]
	AQR7 (Average quantitation ratio 7) [36]
	AQR8 (Average quantitation ratio 8) [37]
	AQR9 (Average quantitation ratio 9) [36]
	AQR10 (Average quantitation ratio 10) [36]

Result summary

15 Groups total (15 after applying alpha core)  
38 Excluded proteins  
0 Calculated value(s)

#1 374 members - sum=487.65

Protein ID	Value
398365603	1.00
6325114	1.00
6320974	1.00
6324260	1.00
9755341	1.00
6322531	1.00
398364731	1.00
398364607	1.00
6324081	1.00
[365 more]	

#2 374 members - sum=455.10

Protein ID	Value
6323701	1.00
6579192	1.00
6325262	1.00
6322701	1.00
398365145	1.00
6321374	1.00
6322708	1.00
6319412	1.00
6321622	1.00
[365 more]	

#3 308 members - sum=417.67

Protein ID	Value
398365725	1.00
6320644	1.00
6323321	1.00
6320644	1.00
[307 more]	

Click to see list of proteins

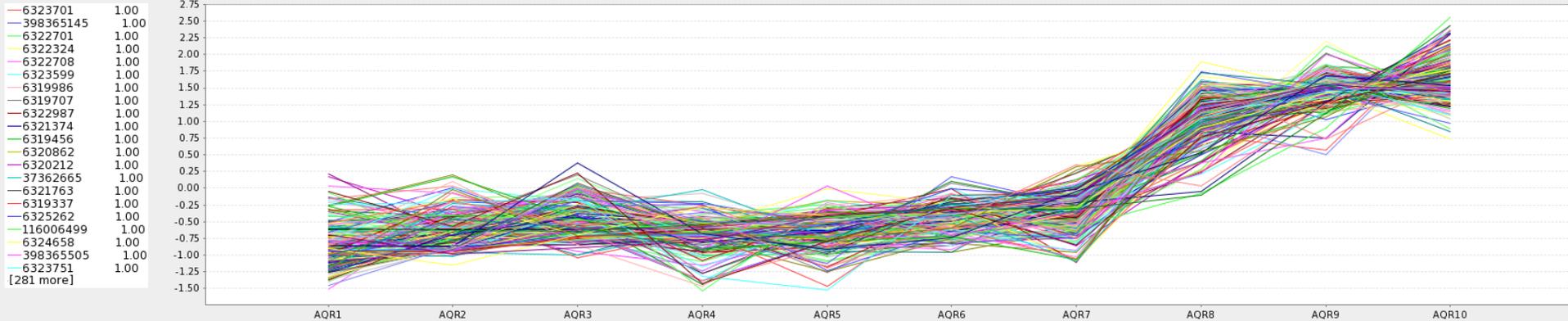
# Cluster 1 – Proteins listed in order of decreasing emPAI

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

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

#4) 302 members - sum=382.87



## Over-represented Wiki pathways

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

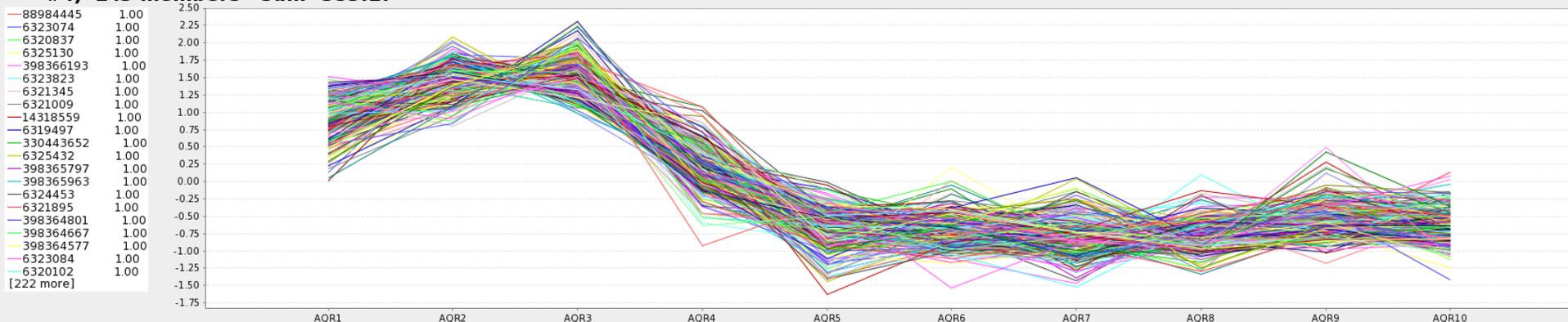
Description	Occurrence	Count	Ref.
Principle Pathways of Carbon Metabolism (WP112)		22	
TCA Cycle (WP490)		18	
TCA Cycle - Detailed (WP296)		15	
Serine-isocitrate lyase pathway (WP390)		7	
Glycolysis and Gluconeogenesis (WPS15)		6	
Fatty acid oxidation (WP91)		5	
Glutamate degradation VII (WP559)		5	
Gluconeogenesis (WP156)		4	
Glutamate degradation III (WP503)		3	
Superpathway of Glutamate Biosynthesis (WP191)		2	
Pantothenate 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 (WPS75)		1	
Aerobic Glycerol Catabolism (WP224)		1	
Sulfur degradation (WP440)		1	

Proteins from TCA Cycle are overrepresented

Acc. Key	No	Description	Cluster	Gene	AA
6323335	1	aconitate hydratase ACO1	-	ACO1	778
6322765	1	Malate dehydrogenase MDH1	-	MDH1	334
6324328	1	citrate (5i)-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...	-	ICDH2	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 (5i)-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

# Cluster 5 – proteins that match glucose concentration

#4) 243 members - sum=353.27



## 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)	<div style="width: 100%; height: 10px; background-color: red;"></div>	13	
MAPK Signaling Pathway (WP510)	<div style="width: 25%; height: 10px; background-color: red;"></div>	3	
Lipases biosynthesis (WP71)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Nucleotide Metabolism (WP321)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Triglyceride Biosynthesis (WP266)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Genes of Meiotic Recombination (WP377)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Riboflavin, FMN, and FAD Biosynthesis (WP381)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Glucose Repression (WP2836)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	
Protein Modifications (WP346)	<div style="width: 5%; height: 10px; background-color: red;"></div>	1	

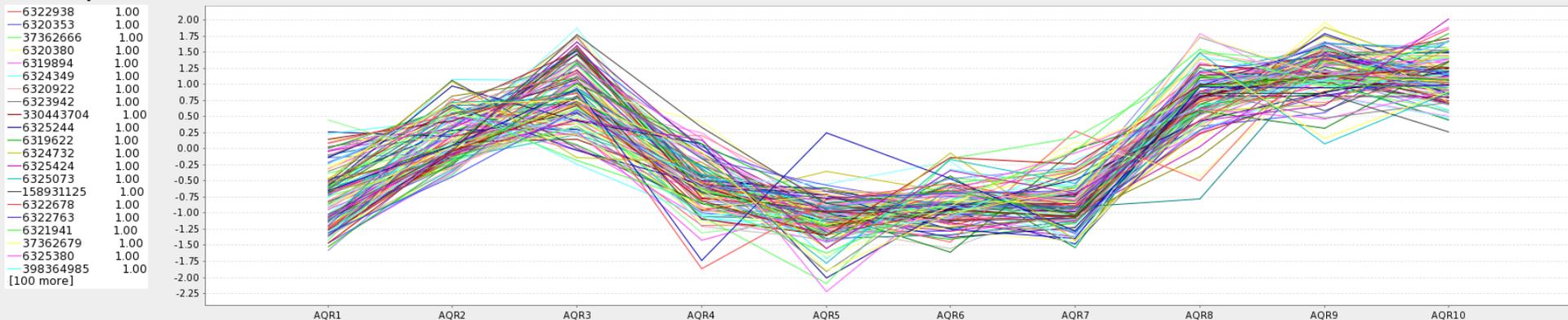
Filter: 1 selected

Page size: 20 Showing 1-20 of 84

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

# Cluster 11 – pattern not identified in Gygi paper

#11) 121 members - sum=186.35



- 6322938 1.00
- 6320353 1.00
- 37362666 1.00
- 6320380 1.00
- 6319894 1.00
- 6324349 1.00
- 6320922 1.00
- 6323942 1.00
- 330443704 1.00
- 6325244 1.00
- 6319622 1.00
- 6324732 1.00
- 6325424 1.00
- 6325073 1.00
- 158931125 1.00
- 6322678 1.00
- 6322763 1.00
- 6321941 1.00
- 37362679 1.00
- 6325380 1.00
- 398364985 1.00
- [100 more]

Acc. Key	No	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		Sc						0		0
6323634	1	mitochondrial 54S ribosomal...	✓	-	MRPL39	70		Sc						0		0
398364237	1	Mitochondrial 54S ribosomal...	✓	-	RML2	393		Sc					1			0
398366569	1	mitochondrial 54S ribosomal...	✓	-	MRP20	263		Sc						0		0
6323644	1	Mix17p	✓	-	MIC17	156		Sc						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...	✓	-	PRO10								1			0
6323942	1	mitochondrial 54S ribosomal...	✓	-	MRPL33	86		Sc						0		0
37362666	1	mitochondrial 54S ribosomal...	✓	-	MRPL49	161		Sc						0		0
6324732	1	mitochondrial 37S ribosomal...	✓	-	PET123	318		Sc						0		0
6322755	1	Yju2p	✓	-	YJU2	278		Sc						0		0
6322678	1	mitochondrial 54S ribosomal...	✓	-	MRPL38	138		Sc						0		0
6324608	1	Akr2p	✓	-	AKR2	749		Sc					6			0
398366647	1	mitochondrial 37S ribosomal...	✓	-	R5M28	361		Sc						0		0
6321902	1	Erp5p	✓	-	ERP5	212		Sc					1			0
330443704	1	mitochondrial 54S ribosomal...	✓	-	MRPL22	309		Sc						0		0
398365695	1	peptidylprolyl isomerase fa...	✓	-	CPR8	308		Sc					1			0
6319894	1	mitochondrial 54S ribosomal...	✓	-	IMG1	169		Sc						0		0

Many mitochondrial ribosomal proteins

# Conclusions

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- 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!