Supplementary Information
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A. SOP for the preparation of the yeast protein lysate

Reagents

YPD (Bio101 system, Cat# 4001-032, Lot# 4001-032-101401)

Kanamycin Sulfate (Shelton Scientific Inc, Cat# IBU2120, Lot# 6I1150)

Trichloroacetic Acid (TCA) (VWR (100% W/V), Cat# 3372-2, Lot# 7022)

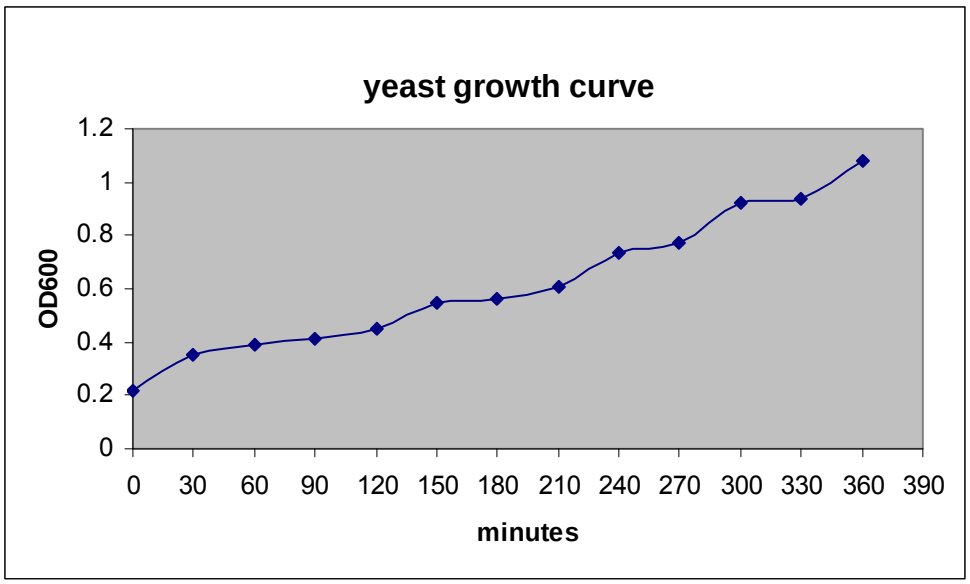
Acetone (Burdick & Jackson, Inc, Cat# AH010-4, Lot# 10071741)

Yeast stock BY4741 (MATa leu2Δ0 met15 Δ0 ura3 Δ0 his3 Δ1) (Open Biosystems, Cat# YSC1048-645440)

Protocol

All steps of this protocol were completed by Boston Biochem (Cambridge, MA), the commercial concern that produced the lysate.

- Yeast from glycerol stock were struck out onto a YPD plate and incubated overnight at 30˚C. The plate contained colonies of uniform shape, size and color.
- Inoculated 500 ml of YPD (supplemented with 25 µg/ml Kanamycin) with yeast from patch of cells (not an individual colony) and grown overnight at 30˚C with shaking.
- Diluted 100 ml of overnight culture into 10 L of YPDKan in a fermentor and grown at 30˚C. Initial OD600 = 0.21. Growth was done with aeration at 10K CC/min, agitation at 30 rpm.
- The OD600 was monitored every 30 minutes using a spectrophotometer.
- When the culture reached OD600 = 0.93 (~ 5 hours), the yeast was harvested using a Sharples continuous-flow centrifuge (over 30K rpm).
- Total pellet wet weight obtained = 5.4 g.
- The cell pellet was resuspended and washed 3 times in 150 ml ice-cold sterile water to remove residual media. The washes were monitored at OD600 until absorbance was at baseline.
- The washed cell pellet was then resuspended in 160 ml of ice-cold sterile water. The suspension was split into two sterile tubes and 80 ml of ice-cold 20% TCA was added to each (final TCA concentration is 10%).
- Each tube was incubated with agitation at 4˚C for 1 hour to facilitate cell lysis.
- The precipitate was collected by centrifugation at 18.3K rpm (50,000g) for 30 minutes.
- Each pellet was washed with 160 ml ice-cold 90% acetone. The pellet was recovered by centrifugation a at 18.3K rpm (50,000g) for 30 minutes.
- The acetone wash and centrifugation were repeated and all liquid removed.
- The pellet was flash-frozen in liquid nitrogen, transferred to a sterile 50 ml conical tube, and lyophilized.
- The lyophilized pellet was stored at -80˚C or dry ice (as required).



Yeast Growth Curve. The OD600 was monitored every 30 minutes using a spectrophotometer. The cells were harvested when the culture reached OD600 = 0.93 (~ 5 hours), corresponding to late log phase growth.

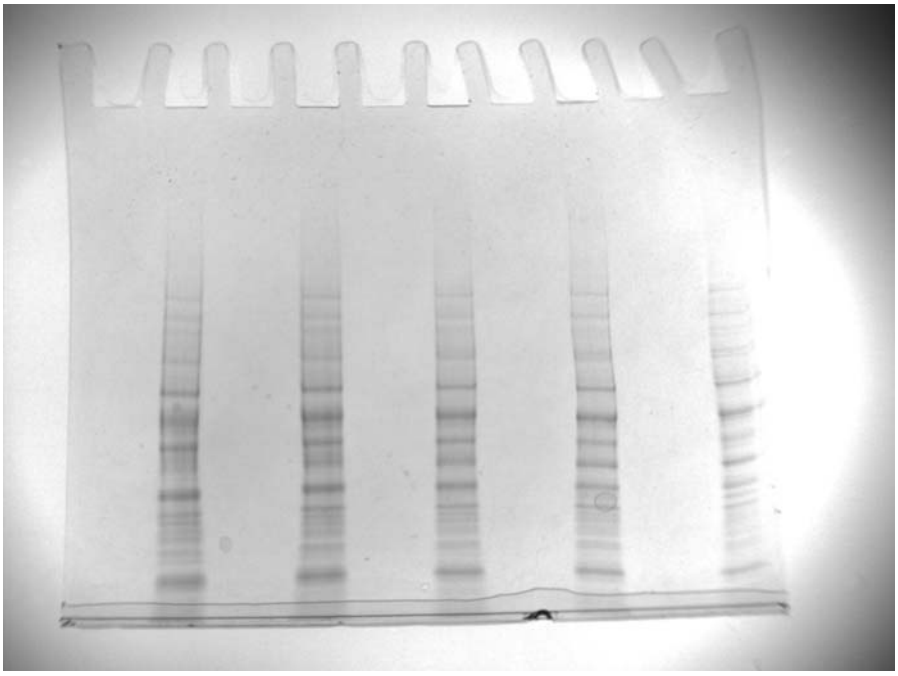
B. Quality Control studies of yeast protein lysate

To prepare the material for LC-MS analysis, the solubilized lysate was digested with trypsin using the procedure of Piening *et al.* (1). In brief, the yeast protein (approximately 1 mg/ml in 50 mM ammonium bicarbonate, 8 M urea at pH 8.0), was reduced and denatured at 60 °C for 1 h following the addition of dithiothreitol at a concentration of 15 mM. Next, the proteins were alkylated by adding iodoacetamide to a concentration of 25 mM. After quenching the reaction by the addition of dithiothreitol (DTT) to a concentration of 50 mM, the solution was diluted 8-fold with 50 mM aqueous ammonium bicarbonate. Digestion with trypsin was carried out at 37 °C for approximately 18 h. The digestion was halted by the addition of acetic acid to a concentration of 1% and the sample was stored at -80 °C prior to analysis. All reagents used, including trypsin, were from Sigma and used without further purification.

For LC-MS analysis, varying amounts of the trypsin digest, corresponding to approximately 5, 25 and 50 pmol of total yeast protein, were injected onto an Atlantis dC18 column (3 µm particle size, 300 µm x 150 mm; Waters, Milford, MA). Elution was accomplished with a 2 h gradient from 3 % to 40 % acetonitrile in an aqueous solution of 0.1 % formic acid using a flow rate of 15 µl/min. For the LC-MS analysis, a Waters nanoAcquity LC was coupled to a Waters QTof Premier mass spectrometer. Mass spectra were obtained from m/z 400 to m/z 1600 with a scan time of 1 s. A 300 µM solution of glu-fibrinopeptide in a 50 % acetonitrile, 50 % water was sprayed at 2 µl/min, orthogonally to the LC effluent spray, for lock-mass calibration.

Prior to data analysis, the Waters RAW file was converted to mzXML format using *massWolf* (http://tools.proteomecenter.org/wiki/index.php?title=Software:massWolf). For feature detection/analysis of the LC-MS TIC, *msInspect* (2) (build # 4603, http://proteomics.fhcrc.org/CPL/msinspect.html) was used. To count the total number of features, the output file generated by *msInspect* was imported into Excel and the total number of entries was used to determine the total number of features detected.

LC-MS/MS analysis of the digestion was performed on a Thermo LTQ using a 95 min gradient as described in the SOP (Supplemental Section C). The results were searched using the MyriMatch algorithm (3) and the results run through the IDPicker algorithm (4) to determine the total confidently identified spectra, peptides, proteins and most parsimonious protein groups.



1D-PAGE results from the analysis of the yeast lysate. The sample loading for the 5 lanes, from left to right, was approximately 12.5 µg, 10 µg, 7.5 µg, 5 µg, and 2.5 µg total protein.

Total number of LC-MS featured detected at various sample loads of the trypsin digest of the yeast lysate. The number of features was determined using *msInspect* (2).

Total Protein (pre-digestion) on Column	Number of Features Detected
5 pmol	303
25 pmol	3588
50 pmol	7924

C. Study 6 SOP

Sample	Description	[Total Yeast Protein]	[Sigma48]
Sample 6-QC1	Sigma48	0	20 fmol/uL
Sample 6-QC2	unspiked digested yeast	60 ng/µL	0
Sample 1-B	digested NCI-20 protein mix		
Sample 6A	0.25 fmol/uL Sigma48 spiked into yeast digest	60 ng/uL	0.25 fmol/uL
Sample 6B	0.74 fmol/uL Sigma48 spiked into yeast digest	60 ng/uL	0.74 fmol/uL
Sample 6C	2.2 fmol/uL Sigma48 spiked into yeast digest	60 ng/uL	2.2 fmol/uL
Sample 6D	6.7 fmol/uL Sigma48 spiked into yeast digest	60 ng/uL	6.7 fmol/uL
Sample 6E	20 fmol/uL Sigma48 spiked into yeast digest	60 ng/uL	20 fmol/uL

An equimolar mixture of 48 human proteins (Sigma UPS1) was prepared for Study 6 as follows:

TFE-assisted Digestion of Sigma 48

1. Resuspend contents of a single Sigma 48 vial in:
50 uL 100 mM ammonium bicarbonate pH 8
50 uL TFE (Acros Organics)

Incubate at 60°C for 45 minutes and vortex repeatedly. You'll see solid material "stuck" at the bottom of the vial; vortex to try to get as much of this into solution as possible.

2. Reduce (using 1/10 volume) with 10 uL of 50 mM DTT (prepared in water) and incubate at 55-66°C for 20 minutes.

3. Cool to room temperature and alkylate with 10 uL of 100 mM IAM (also prepared in water) and incubate in the dark for 20 minutes.

4. Dilute with enough volume of Ambic pH 8 (360 uL) so the final concentration of TFE is about ≤10 % prior to adding the trypsin.

5. Add trypsin (Promega) in 1:50 ratio. Trypsin concentration is 0.01 ug/uL in 100 mM Ambic, and based on the average protein concentration of a Sigma 48 vial (around 6.5 ug), add 20 uL of the 0.01 ug/uL trypsin stock.

6. Digest at 37° C overnight, dry down and reconstitute in 0.1 % formic acid for LC/MS/MS analysis. I reconstituted in 50 uL = 100 fmol/uL

(Note: DTT and IAM were from Sigma.)

For Study 6, 1 vial of digested Sigma48 (Sample 6-QC1), 1 vial of digested, unspiked yeast (Sample 6-QC2), 1 vial of digested NCI 20 protein mix (Sample 1B; see Supplemental Section H and Rudnick *et. al., Submitted*) and 5 vials with varying concentrations of digested Sigma48 spiked into digested yeast (Samples 6A-6E) were sent to each laboratory. Prior to analysis, **Sample 1B was diluted 150-fold with 0.1% formic acid in water**. All other samples were not diluted. 120 ng of the yeast proteome digest was loaded on column.

Below are checklists that outline the HPLC and mass spectrometry parameters of this SOP for both a Thermo LTQ and Thermo Orbitrap. The run order for the samples follows the checklists.

If possible, participants should pack their columns at 1000 PSI in methanol and then finish packing with the HPLC at 50:50 0.1% formic:acetonitrile on the HPLC for 30 min prior to cutting back the columns to their final dimensions. The column should also be conditioned prior to use with an injection of a concentrated protein digest (such as a BSA digest) followed by several injections of a less concentrated digest. Several blank runs should be performed after conditioning to eliminate carryover of the conditioning digest into the first blank of the study.

Study 6 LTQ SOP Checklist				
Source/APstack parameters				Notes
	☐	Capillary temperature	150 ± 25°C	
	☐	Capillary voltage	48 ± 20 V	
	☐	Tube Lens	100 ± 25 V	
	☐	Source voltage	2.0 ± 0.5 kV	
Tune File Parameters				Notes
	☐	Ion Trap Full Microscans	1	
	☐	Ion Trap MSn Microscans	1	
	☐	Ion Trap Full Max Ion Time	100	
	☐	Ion Trap MSn Max Ion Time	100	
	☐	Ion Trap Full AGC Target	30,000	
	☐	Ion Trap MSn AGC Target	10,000	
MS detector				Notes
	☐	Acquire time (min)	184	
	☐	Start delay (min)	15	
	☐	Segments	1	
	☐	Scan Events	9	
	☐	Scan Range for Event 1	m/z 300-2000	
	☐	Mass range for all events	Normal	
	☐	Scan Rate for all events	Normal	
	☐	Polarity for all events	Positive	
	☐	Data type for MS scan	Centroid	
	☐	Data Type for MSn Scan	Centroid	
	☐	Additional microscans	0	
Dynamic Exclusion				Notes
	☐	Repeat Count	1	
	☐	Repeat duration (s)	0	
	☐	Exclusion list size	150	
	☐	Exclusion duration (s)	60	
	☐	Exclusion mass width	By mass	
	☐	Exclusion mass width (low)	1.0	
	☐	Exclusion mass width (high)	3.5	
	☐	Early expiration is NOT		

		used		
Scan Event				Notes
	☐	Charge state screening and Rejection is NOT enabled		
	☐	Minimum MS signal Threshold for MS2 trigger	500	
	☐	Mass determined from scan event	1	
	☐	Activation type	CID	
	☐	Default charge state	4	
	☐	Isolation width (m/z)	2.0	
	☐	Normalized collision energy	28	
	☐	Activation Q	0.250	
	☐	Activation time (ms)	30.0	
	☐	Mass lists and global mass lists NOT used		
	☐	Wideband Activation NOT used		
	☐	For each Current Scan Event, the Nth most intense ion is set at 8-1 for scan events 2-9, respectively		
HPLC Parameters and chromatography				Notes
	☐	Autosampler temperature	10C	
	☐	Column dimensions	11-13 cm x 100μm*	
	☐	Precolumn dimensions	2-4 cm x 100 μm**	
	☐	Column packing material	Jupiter C18	
	☐	Column temperature	Room temperature	
	☐	Mobile phase A	0.1% (v/v) formic acid in water	
	☐	Mobile phase B	0.1% (v/v) formic acid in acetonitrile	
	☐	Injection volume	2.0 μL	
	☐	Injection loop (or needle) volume	8.0 μL***	
	☐	Flow rate for gradient	600 nL/min at column tip	
	☐	Flow rate for loading phase	1.2 uL/min	
	☐	184 min HPLC gradient for yeast samples	As per attached table	
	☐	95 min HPLC gradient for NCI20 and blanks samples	As per attached table	

* Column length may vary depending upon the individual nanospray set up, but inner diameter should remain the same.

** Precolumn is optional depending upon individual laboratory expertise, but should be used if at all possible. Column length may vary depending upon the individual nanospray set up, but inner diameter should remain the same.

*** If not possible to achieve, use same set up as dress rehearsal and make note of change.

184 min HPLC Gradient with "vented" column for yeast samples					
Time	%A	%B	Flow path	Flow rate	Notes
0	100	0	To Waste*	1.2 uL/min	
0.01	100	0	To Waste	1.2 uL/min	
10	100	0	To Waste	1.2 uL/min	
15	98	2	To Waste	1.2 uL/min	
15.01	98	2	To column (source)	600 nL/min	
135	60	40	To column (source)	600 nL/min	
150	10	90	To column (source)	600 nL/min	
155	10	90	To column (source)	600 nL/min	
159	10	90	To column (source)	600 nL/min	
164	95	5	To column (source)	600 nL/min	
169	100	0	To column (source)	600 nL/min	
184	100	0	To column (source)	600 nL/min	

*If using a precolumn, the flow should be directed to waste.

95 min HPLC Gradient with "vented" column for blanks and NCI-20					
Time	%A	%B	Flow	Flow rate	Notes
0	100	0	To Waste*	1.2 uL/min	
0.01	100	0	To Waste	1.2 uL/min	
10	100	0	To Waste	1.2 uL/min	
15	98	2	To Waste	1.2 uL/min	
15.01	98	2	To column (source)	600 nL/min	
50	75	25	To column (source)	600 nL/min	
65	10	90	To column (source)	600 nL/min	
70	10	90	To column (source)	600 nL/min	
74	10	90	To column (source)	600 nL/min	
75	95	5	To column (source)	600 nL/min	
80	100	0	To column (source)	600 nL/min	
95	100	0	To column (source)	600 nL/min	

*If using a precolumn, the flow should be directed to waste.

Run order:

This series of samples was run in **triplicate**, either back-to-back or as three separate replicates with other samples or maintenance in between. (If the series is run back-to-back, there is no need to run the Sample 1B (NCI20) two times in a row.)

Run number	Sample	Gradient
1	**Sample 1-B-NCI20**	95 min gradient
2	Wash*	
3	Blank	95 min gradient
4	**Sample 6-QC2 –Yeast only**	184 min gradient
5	Wash*	
6	Blank	95 min gradient
7	**Sample 6A - 0.25 fmol/uL Sigma 48 spiked yeast**	184 min gradient
8	Wash*	
9	Blank	95 min gradient
10	**Sample 6B - 0.74 fmol/uL Sigma 48 spiked yeast**	184 min gradient
11	Wash*	
12	Blank	95 min gradient
13	**Sample 6C - 2.2 fmol/uL Sigma 48 spiked yeast**	184 min gradient
14	Wash*	
15	Blank	95 min gradient
16	**Sample 6D - 6.7 fmol/uL Sigma 48 spiked yeast**	184 min gradient
17	Wash*	
18	Blank	95 min gradient
19	**Sample 6E - 20 fmol/uL Sigma 48 spiked yeast**	184 min gradient
20	Wash*	
21	Blank	95 min gradient
22	**Sample 6-QC1 - Sigma 48 only**	184 min gradient
23	Wash*	
24	Blank	95 min gradient
25	**Sample 1-B-NCI20**	95 min gradient

* As per individual laboratory protocol

Study 6 Orbitrap SOP Checklist				
Source/APstack parameters				Notes
	☐	Capillary temperature	150 ± 25°C	
	☐	Capillary voltage	48 ± 20 V	
	☐	Tube Lens	100 ± 25 V	
	☐	Source voltage	2.0 ± 0.5 kV	
Tune File Parameters				Notes
	☐	FTMS Full Microscans	1	
	☐	Ion Trap MSn Microscans	1	
	☐	FTMS Full Max Ion Time	1000	
	☐	Ion Trap MSn Max Ion Time	100	
	☐	FTMS Full AGC Target	1,000,000	
	☐	Ion Trap MSn AGC Target	10,000	
MS detector				Notes
	☐	Acquire time (min)	184	
	☐	Start delay (min)	15	
	☐	Segments	1	
	☐	Scan Events	9	
	☐	Scan Range for Event 1	m/z 300-2000	
	☐	Mass range for all events	Normal	
	☐	Scan Rate for all events	Normal	
	☐	Polarity for all events	Positive	
	☐	Data type for MS scan	Profile	
	☐	Data Type for MSn Scan	Centroid	
	☐	Additional microscans	0	
	☐	Resolution of MS scan	60,000	
	☐	Lock mass is NOT enabled		
Dynamic Exclusion				Notes
	☐	Repeat Count	1	
	☐	Repeat duration (s)	0	
	☐	Exclusion list size	150	
	☐	Exclusion duration (s)	60	
	☐	Exclusion mass width	By mass	
	☐	Exclusion mass width (low)	0.6	
	☐	Exclusion mass width (high)	0.6	
	☐	Early expiration is NOT used		
Global Data-Dependent Settings				Notes
	☐	Charge state screening enabled		
	☐	Monoisotopic precursor		

		selection enabled		
	☐	Non-peptide monoisotopic recognition enabled		
	☐	Charge state +1 rejected		
	☐	Unassigned charge state rejected		
Scan Event				Notes
	☐	Minimum MS signal Threshold for MS2 trigger	500	
	☐	Mass determined from scan event	1	
	☐	Activation type	CID	
	☐	Default charge state	4	
	☐	Isolation width (m/z)	2.0	
	☐	Normalized collision energy	28	
	☐	Activation Q	0.250	
	☐	Activation time (ms)	30.0	
	☐	Mass lists and global mass lists NOT used		
	☐	Wideband Activation NOT used		
	☐	For each Current Scan Event, the Nth most intense ion is set at 8-1 for scan events 2-9, respectively		
HPLC Parameters and chromatography				Notes
	☐	Autosampler temperature	10C	
	☐	Column dimensions	11-13 cm x 100μm*	
	☐	Precolumn dimensions	2-4 cm x 100 μm**	
	☐	Column packing material	Jupiter C18	
	☐	Column temperature	Room temperature	
	☐	Mobile phase A	0.1% (v/v) formic acid in water	
	☐	Mobile phase B	0.1% (v/v) formic acid in acetonitrile	
	☐	Injection volume	2.0 μL	
	☐	Injection loop (or needle) volume	8.0 μL***	
	☐	Flow rate for gradient	600 nL/min at column tip	
	☐	Flow rate for loading phase	1.2 uL/min	
	☐	184 min HPLC gradient for yeast samples	As per attached table	
	☐	95 min HPLC gradient for NCI20 and blanks samples	As per attached table	

* Column length may vary depending upon the individual nanospray set up, but inner diameter should remain the same.
** Precolumn is optional depending upon individual laboratory expertise, but should be used if at all possible. Column length may vary depending upon the individual nanospray set up, but inner diameter should remain the same.

***If not possible, use same set up as dress rehearsal and make note of change.

184 min HPLC Gradient with "vented" column for yeast samples					
Time	%A	%B	Flow path	Flow rate	Notes
0	100	0	To Waste*	1.2 uL/min	
0.01	100	0	To Waste	1.2 uL/min	
10	100	0	To Waste	1.2 uL/min	
15	98	2	To Waste	1.2 uL/min	
15.01	98	2	To column (source)	600 nL/min	
135	60	40	To column (source)	600 nL/min	
150	10	90	To column (source)	600 nL/min	
155	10	90	To column (source)	600 nL/min	
159	10	90	To column (source)	600 nL/min	
164	95	5	To column (source)	600 nL/min	
169	100	0	To column (source)	600 nL/min	
184	100	0	To column (source)	600 nL/min	

*If using a precolumn, the flow should be directed to waste.

95 min HPLC Gradient with "vented" column for blanks and NCI-20					
Time	%A	%B	Flow	Flow rate	Notes
0	100	0	To Waste*	1.2 uL/min	
0.01	100	0	To Waste	1.2 uL/min	
10	100	0	To Waste	1.2 uL/min	
15	98	2	To Waste	1.2 uL/min	
15.01	98	2	To column (source)	600 nL/min	
50	75	25	To column (source)	600 nL/min	
65	10	90	To column (source)	600 nL/min	
70	10	90	To column (source)	600 nL/min	
74	10	90	To column (source)	600 nL/min	
75	95	5	To column (source)	600 nL/min	
80	100	0	To column (source)	600 nL/min	
95	100	0	To column (source)	600 nL/min	

*If using a precolumn, the flow should be directed to waste.

Run order:

This series of samples was run in **triplicate**. You may run the triplicate runs back-to-back or as three separate replicates with other samples or maintenance in between. If the series is run back-to-back, there is no need to run the Sample 1B (NCI20) two times in a row.

Run number	Sample	Gradient
1	**Sample 1-B-NCI20**	95 min gradient
2	Wash*	
3	Blank	95 min gradient
4	**Sample 6-QC2 –Yeast only**	184 min gradient
5	Wash*	
6	Blank	95 min gradient
7	**Sample 6A - 0.25 fmol/uL Sigma 48 spiked yeast**	184 min gradient
8	Wash*	
9	Blank	95 min gradient
10	**Sample 6B - 0.74 fmol/uL Sigma 48 spiked yeast**	184 min gradient
11	Wash*	
12	Blank	95 min gradient
13	**Sample 6C - 2.2 fmol/uL Sigma 48 spiked yeast**	184 min gradient
14	Wash*	
15	Blank	95 min gradient
16	**Sample 6D - 6.7 fmol/uL Sigma 48 spiked yeast**	184 min gradient
17	Wash*	
18	Blank	95 min gradient
19	**Sample 6E - 20 fmol/uL Sigma 48 spiked yeast**	184 min gradient
20	Wash*	
21	Blank	95 min gradient
22	**Sample 6-QC1 - Sigma 48 only**	184 min gradient
23	Wash*	
24	Blank	95 min gradient
25	**Sample 1-B-NCI20**	95 min gradient

* As per individual laboratory protocol

D. Methods used by each laboratory in Study 8

Thermo LTQ parameters			
	LTQ@73	**LTQ@65**	**LTQ@95**
Source/APstack parameters			
Capillary temperature	160°C	160°C	150°C
Capillary voltage	46V	47 V	49V
Tube Lens	125V	110 V	120V
Source voltage	2.1kV	1.8 V	2.5kV
Tune File Parameters			
Ion Trap Full Microscans	1	1	1
Ion Trap MSn Microscans	1	1	1
Ion Trap Full Max Ion Time	100ms	50ms	100ms
Ion Trap MSn Max Ion Time	100ms	100ms	100ms
Ion Trap Full AGC Target	30,000	30,000	30,000
Ion Trap MSn AGC Target	10,000	10,000	1,000
MS detector			
Acquire time (min)	172	184	184
Start delay (min)	15	15	15
Segments	1	1	1
Scan Events	6	6	9
Scan Range for Event 1	300-1600 m/z	400-1600 m/z	300-2000 m/z
Mass range for all events	Normal	Normal	Normal
Scan Rate for all events	Normal	Normal	Normal
Polarity for all events	Positive	Positive	Positive
Data type for MS scan	Centroid	Centroid	Centroid
Data Type for MSn Scan	Centroid	Centroid	Centroid
Additional microscans	0	0	0
Dynamic Exclusion			
Repeat Count	1	1	1
Repeat duration (s)	30	1	0
Exclusion list size	500	150	150
Exclusion duration (s)	30	45	60
Exclusion mass width	By mass	By mass	By mass
Exclusion mass width (low)	0.5	1	1.0
Exclusion mass width (high)	2.5	2	3.5
Early expiration is NOT used	Not enabled	Not enabled	Not enabled

Scan Event			
Charge state screening and Rejection is NOT enabled	Not enabled	Not enabled	Not enabled
Minimum MS signal Threshold for MS2 trigger	1000	1000	500
Mass determined from scan event	1	1	1
Activation type	CID	CID	CID
Default charge state	4	4	4
Isolation width (m/z)	2	2	2
Normalized collision energy	30	30	28
Activation Q	0.25	0.25	0.25
Activation time (ms)	30	30	30
Mass lists and global mass lists NOT used	TRUE	TRUE	TRUE
Wideband Activation NOT used	TRUE	TRUE	TRUE
For each Current Scan Event, the Nth most intense ion is set at 8-1 for scan events 2-9, respectively	Nth most intense at 5-1 for scan events 2-6, respectively	Nth most intense at 1-5 for scan events 2-6, respectively	Nth most intense at 8–1 for scan events 2-9, respectively
HPLC Parameters and chromatography			
LC instrument make and model	Agilent 1100 nano	Eksigent NanoLC 1D Plus	Agilent G2226A Nanopump
Autosampler temperature	10°C	10°C	10°C
Column dimensions	15 cm x 100 um id	11 cm x 100 um id	12cm x 100um id
Precolumn dimensions	15 cm x 100 um	4 cm x 100 um	4 cm x 100 um
Column packing material	Chromolith CapRod Monolithic C18	Jupiter C18, 5 micron	Jupiter C18, 5 micron
Column temperature	Room temp	Room temp	Room temp
Mobile phase A	0.1% (v/v) formic acid in water	0.1% (v/v) formic acid in water	0.1% (v/v) formic acid in water
Mobile phase B	0.1% (v/v) formic acid in acetonitrile	0.1% (v/v) formic acid in acetonitrile	0.1% (v/v) formic acid in acetonitrile
Injection volume	2 uL	5 uL	2uL
Injection loop (or needle) volume	2 uL	10 uL	8uL
Flow rate for gradient	600 nL/min	600nL/min	600nL/min
Flow rate for loading phase	1.2 uL/min	1.2 uL/min	1.2 uL/min
HPLC gradient	As per attached table	As per attached table	As per attached table

LC Gradient

LTQ@73

184 min SOP Gradient				**120 min Deviation Gradient**				
Time	%A	%B	Flow rate	Time	%A	%B	Flow rate	Notes
0	100	0	1.2 uL/min	0				
0.01	100	0	1.2 uL/min					
10	100	0	1.2 uL/min					
15	98	2	1.2 uL/min	15				
15.01	98	2	600 nL/min					
135	60	40	600 nL/min					
150	10	90	600 nL/min		10	90		
155	10	90	600 nL/min		10	90		
159	10	90	600 nL/min					
164	95	5	600 nL/min					
169	100	0	600 nL/min					
184	100	0	600 nL/min					

LTQ@65

184 min SOPGradient				**184 min Deviation Gradient**				
Time	%A	%B	Flow rate	Time	%A	%B	Flow rate	Notes
0	100	0	1.2 uL/min				1.5 uL/min	
0.01	100	0	1.2 uL/min				1.5 uL/min	
10	100	0	1.2 uL/min				1.5 uL/min	
15	98	2	1.2 uL/min				1.5 uL/min	
15.01	98	2	600 nL/min					
135	60	40	600 nL/min					
150	10	90	600 nL/min					
155	10	90	600 nL/min					
159	10	90	600 nL/min					
164	95	5	600 nL/min					
169	100	0	600 nL/min					
184	100	0	600 nL/min					

LTQ@95

184 min HPLC Gradient with "vented" column for yeast samples					
Time	%A	%B	Flow path	Flow rate	Notes
0	97	3	To Waste*	5 uL/min	Second Pump to precolumn
15	97	3	To Waste	5 uL/min	Second Pump to precolumn
135	60	40	To column (source)	600 nL/min	Nano pump to pre & Analytical Column
150	10	90	To column (source)	600 nL/min	
155	10	90	To column (source)	600 nL/min	
159	10	90	To column (source)	600 nL/min	
164	95	5	To column (source)	600 nL/min	
169	97	3	To column (source)	600 nL/min	
184	97	3	To column (source)	600 nL/min	

Thermo Orbitrap parameters			
	Orbi@56	**Orbi@86**	**Orbi@65**
Source/APstack parameters			
Capillary temperature	150°C	160°C	160°C
Capillary voltage	48V	46V	43V
Tube Lens	100V	110V	100V
Source voltage	2.0V	1.85V	1.9V
Tune File Parameters			
FTMS Full Microscans	1	1	1
Ion Trap MSn Microscans	1	1	1
FTMS Full Max Ion Time	500	750	1000
Ion Trap MSn Max Ion Time	100	100	100
FTMS Full AGC Target	1,000,000	1,000,000	1,000,000
Ion Trap MSn AGC Target	10,000	10,000	10,000
MS detector			
Acquire time (min)	135	180	184
Start delay (min)	15	0	15
Segments	1	1	1
Scan Events	9	10	6
Scan Range for Event 1	350-1800 m/z	300-2000 m/z	350-1600 m/z
Mass range for all events	Normal	Normal	Normal
Scan Rate for all events	Normal	Normal	Normal
Polarity for all events	Positive	Positive	Positive
Data type for MS scan	Profile	Profile	Profile
Data Type for MSn Scan	Centroid	Centroid	Centroid
Additional microscans	0	0	0
Resolution of MS scan	60,000	60,000	60,000
Lock mass is NOT enabled	True	False	True
Dynamic Exclusion			
Repeat Count	1	1	1
Repeat duration (s)	0	30	0
Exclusion list size	500	500	150
Exclusion duration (s)	60	50	45
Exclusion mass width	Relative to reference mass (ppm)	Relative to ppm of parent mass	By mass
Exclusion mass width (low)	25 ppm	15 ppm	0.6 m/z
Exclusion mass width (high)	25 ppm	20 ppm	0.6 m/z

Early expiration is NOT used	True	True	True
Charge state screening and Rejection is enabled	True	True	True
Monoisotopic precursor selection enabled	True	True	True
Non-peptide monoisotopic recognition enabled	True	True	True
Charge state +1 rejected	True	True	True
Unassigned charge state rejected	True	True	True
Scan Event			
Minimum MS signal Threshold for MS2 trigger	500	5000	1000
Mass determined from scan event	1	1	1
Activation type	CID	CID	CID
Default charge state	4	3	4
Isolation width (m/z)	2.0	2.0	2.0
Normalized collision energy	28	28	30
Activation Q	.250	.250	.250
Activation time (ms)	30	30	30
Mass lists and global mass lists NOT used	True	True	True
Wideband Activation NOT used	True	True	True
For each Current Scan Event, the Nth most intense ion is set at 8-1 for scan events 2-9, respectively	True	Nth most intense ion set at 1-9 for scan events 2-10, respectively	Nth most intense ion set at 1-5 for scan events 2-6, respectively
HPLC Parameters and chromatography			
Autosampler temperature	10°C	10°C	10°C
Column dimensions	12 cm x 75um ID	15 cm x 75 um	11-13 cm x 100um
Precolumn dimensions	n/a	n/a	4 cm x 100 um
Column packing material	Reprosil C18AQ 3um	Reprosil C18AQ 3um	Jupiter C18 5um
Column temperature	Room temp	Room temp	Room temp
Mobile phase A	0.1% (v/v) formic acid in water	0.1% (v/v) formic acid in water	0.1% (v/v) formic acid in water
Mobile phase B	0.1% (v/v) formic acid in 90% acetonitrile	50% (v/v) acetonitrile, 0.1% formic acid	0.1% (v/v) formic acid in acetonitrile
Injection volume	2.0 uL	2.0 uL	2.0 uL

Injection loop (or needle) volume	8.0 uL	10 uL	10 uL
Flow rate for gradient	200 nL/min	400 nL/min	600 nL/min at column tip
Flow rate for loading phase	700 nL/min	1.0 uL/min	1.2 uL/min
HPLC gradient	As per attached table	As per attached table	As per attached table

LC Gradient for Thermo LTQ-Orbitraps

Orbi@56

184 min SOPGradient				**133 min Deviation Gradient**				
Time	%A	%B	Flow rate	Time	%A	%B	Flow rate	Notes
0	100	0	1.2 uL/min	0	97	3	700 nL/min	3.5x elution flow rate
0.01	100	0	1.2 uL/min	13	97	3	700 nL/min	
10	100	0	1.2 uL/min	14	95	5	700 nL/min	
15	98	2	1.2 uL/min	15	95	5	200 nL/min	Same load time as SOP
15.01	98	2	600 nL/min					
135	60	40	600 nL/min	105	65	35	200 nL/min	Same slope as SOP 0.33% B/min
150	10	90	600 nL/min	115	10	90	200 nL/min	
155	10	90	600 nL/min	124	10	90	200 nL/min	
159	10	90	600 nL/min					
164	95	5	600 nL/min	125	97	3	700 nL/min	3.5x elution flow rate
169	100	0	600 nL/min					
184	100	0	600 nL/min	133	97	3	700 nL/min	

Orbi@86

3hr HPLC Gradient with "vented" column for yeast samples					
Time	%A	%B	Flow path	Flow rate	Notes
0	50	50	To Waste*	1.0 uL/min	Channel 1 on (mobA=mob= 0.1%formic)
22	50	50	To Waste	1.0 uL/min	Channel 1 off, channel 2 on
0	99	1	To column (source)	400 nL/min	Time 0, MS trigger from channel 2
2	99	1	To column (source)	400 nL/min	
160	20	80	To column (source)	400 nL/min	
161	2	98	To column (source)	400 nL/min	
180	2	98	To column (source)	400 nL/min	

Wash Gradient					
Time	%A	%B	Flow path	Flow rate	Notes
0	50	50	To Waste*	1.0 uL/min	Channel 1 on, full loop inj 100% ACN
20	50	50	To Waste	1.0 uL/min	Channel 1 off, channel 2 on
0	90	10	To column (source)	400 nL/min	Time 0, MS trigger from channel 2
5	10	90	To column (source)	400 nL/min	
20	10	90	To column (source)	400 nL/min	

Equilibration Gradient					
Time	%A	%B	Flow path	Flow rate	Notes
0	50	50	To Waste*	1.0 uL/min	Channel 1 on, full loop inj 100% water
25	50	50	To Waste	1.0 uL/min	Channel 1 off, channel 2 on
0	99	1	To column (source)	400 nL/min	Time 0, MS trigger from channel 2
10	99	1	To column (source)	400 nL/min	

Orbi@65

184 min SOPGradient				**189 min Deviation Gradient**				
Time	%A	%B	Flow rate	Time	%A	%B	Flow rate	Notes
0	100	0	1.2 uL/min	0				
0.01	100	0	1.2 uL/min					
10	100	0	1.2 uL/min					
15	98	2	1.2 uL/min	20				Flow diverted to waste for only 1st 15 min.
15.01	98	2	600 nL/min	20.01				
135	60	40	600 nL/min	140				
150	10	90	600 nL/min	155				
155	10	90	600 nL/min	160				
159	10	90	600 nL/min	164				
164	95	5	600 nL/min	169				
169	100	0	600 nL/min	174				
184	100	0	600 nL/min	189				

E. Sigma UPS1 Proteins

(http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=UPS1|SIGMA&N5=Product%20No.|BRAND_KEY&F=SPEC)

1. Alpha-lactalbumin
2. Annexin A5
3. Antithrombin-III
4. Beta-2-microglobulin
5. BH3 interacting domain death agonist [BID]
6. Carbonic anhydrase 1
7. Carbonic anhydrase 2
8. Catalase
9. Cathepsin D
10. Cathepsin G
11. Complement C5 [Complement C5a]
12. C-reactive protein
13. Creatine kinase M-type [CK-MM]
14. Cytochrome b5
15. Cytochrome c [Apocytochrome c]
16. Epidermal growth factor
17. Fatty acid-binding protein
18. Gelsolin
19. Glutathione S-transferase A1 [GST A1-1]
20. Glutathione S-transferase P [GST]
21. GTPase HRas [Ras protein]
22. Hemoglobin alpha chain
23. Hemoglobin beta chain
24. Histidyl-tRNA synthetase [Jo-1]
25. Insulin-like growth factor II
26. Interleukin-8
27. Lactotransferrin
28. Leptin
29. Lysozyme C
30. Microtubule-associated protein tau [Tau protein]
31. Myoglobin
32. NAD(P)H dehydrogenase [quinone] 1 [DT Diaphorase]
33. Neddylin [Nedd8]
34. Peptidyl-prolyl cis-trans isomerase A [Cyclophilin A]
35. Peroxiredoxin 1
36. Platelet-derived growth factor B chain
37. Retinol-binding protein
38. Ribosyldihydronicotinamide dehydrogenase (quinone) [Quinone oxidoreductase 2 or NQO2]
39. Serotransferrin [Apotransferrin]
40. Serum albumin
41. Small ubiquitin-related modifier 1 [SUMO-1]
42. Superoxide dismutase [Cu-Zn]
43. Thioredoxin
44. Tumor necrosis factor [TNF-alpha]
45. Ubiquitin
46. Ubiquitin-conjugating enzyme E2 I [UbcH9]
47. Ubiquitin-conjugating enzyme E2 C [UbcH10]
48. Ubiquitin-conjugating enzyme E2 E1 [UbcH6]

F. NCI-20

This is a mixture of 20 proteins (shown below) assembled by NIST for LC-MS studies.

Protein	Commercial Source	Catalog Number
Albumin	New Century Pharmaceuticals	9801
Fibrinogen	Innovative Research	IFIB
Transferrin	Hyphen Biomed	PP002A
β-2-microglobulin	BioWorld	507410
C-reactive protein	CalBiochem	475828
Gastrin	CalBiochem	236608
transferrin receptor	Anaspec	20750
Vascular Endothelial Growth Factor	Research Diagnostics, Inc.	RDI-SCP185-3
Insulin-like growth factor I	R & D Systems	293-VE-050/CF
Prolactin	Affinity Bioreagents	ORP-16035
α-fetoprotein	Cell Sciences	CRB001B
Carcinoembryonic antigen	Cell Sciences	CRA010
Calcitonin	BioWorld	509225
Prostate specific antigen/α1-antichymotrypsin complex	Anaspec	20673
Thyroglobulin	Fitzgerald Industries	30-AP13
Granulocyte macrophage colony stimulating factor	Fitzgerald Industries	30-AT01
Tumor necrosis factor-α	Cell Sciences	CRG103B
Interleukin-2	Cell Sciences	CRT100B
Interleukin-6	Cell Sciences	CRI100B
Erythropoietin	Biomyx	I1005-200
	Cell Sciences	CRE600B

G. Performance Metric Methods for data in Tables 1 & 2

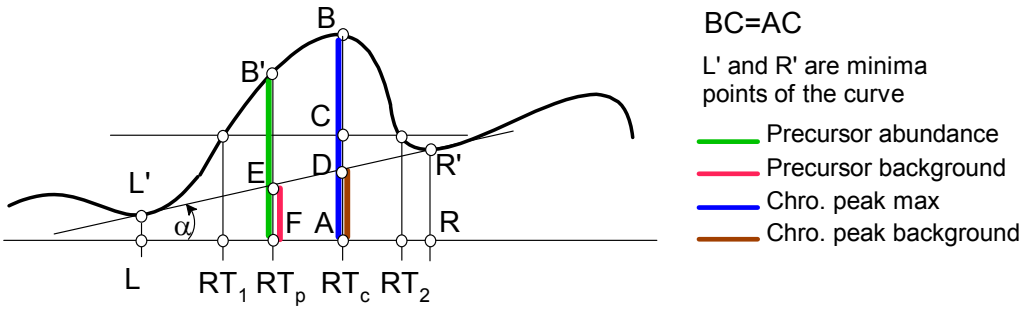
A full description of these performance metrics (as well as additional metrics) is presented in the accompanying manuscript by Rudnick *et al.* A brief description of the subset of metrics employed in this study is given here:

Middle 50% peptide retention period (C-2A)

The longer the time period over which peptides elute, the more time available to acquire MS2 spectra and the greater the number of peptides likely to be sampled and identified. C-2A is defined as the time over which the middle 50% of the identified peptides elute, which corresponds to the difference between their first and last retention quartiles (also called 'interquartile range').

Peak widths (C-3A)

Peak widths (FWHM) were calculated as RT2-RT1 (See Figure below).



The peak width in seconds was calculated for each identified peptide and the median value is reported. Narrow peak widths can generate higher signal intensities, increase the capacity of the column, and generally improve the efficiency of peptide identification.

Oversampling (DS-1, DS-2)

Oversampling can be monitored by calculating the ratios of peptides identified once/twice (DS-1) and twice/thrice (DS-2). Skews in these ratios typically indicates improper dynamic exclusion settings within the instrument control software or problems with chromatography (e.g. peak broadening).

MS1 and MS2 Scans of C-2A (DS-3A)

Large changes in the numbers of MS and MS2 spectra acquired between replicate runs indicates differences in chromatography and/or sampling. A reduced number of MS2 scans may also indicate reduced availability of signal from which to sample.

MS1 jumps/falls (IS-1A,IS-1B)

These two metrics screen for large (10X) differences in MS1 intensity between adjacent scans. Such large differences are an indication of electrospray instability, and result in a characteristic "saw tooth" appearance in the base peak chromatograms. Any value greater than 0 can indicate problems with the spray tip and/or source.

Precursor m/z (IS-2)

The median precursor m/z is calculated from all of the precursor m/z values for all of the identified peptides. These values are very stable for a typical tryptic digest, but vary in response to sample loading and source instability.

Ratio +3/+2 (IS-3B)

The relative ratio of +3/+2 peptide ions is also generally stable between runs. These ratios can be altered by differences in gradient pH or other causes affecting ionization of peptide analytes.

MS1 ion injection (MS1-1)

The MS1 ion injection time (ms) can be used as a measure of the available signal. The smaller the number the more signal is available. Time outs (typically 100ms) are reached if the total signal does not reach a threshold value. Therefore median MS1 injection times close to the maximum indicate below optimal signal.

MS1 S/N (MS1-2A)

MS1 signal-to-noise (S/N) is calculated by dividing the maximum peak intensity by the median peak intensity. MS1 S/N is a better indicator of signal strength than TIC because S/N values because absolute signal levels between instruments are not easily compared. A reduction in MS1 S/N would negatively affect the probability for peptide identification in general.

MS2 S/N (MS2-3)

MS2 S/N is calculated the same as MS1-2A. Since MS2 signal levels are generally 10X lower than MS1 signal levels, MS2 S/N is also a good indicator of signal strength.

Unique tryptic peptide IDs (P-2C)

The number of unique tryptic peptides is a key performance metric and is calculated by summing the total unique peptide sequences with scores above a 1% FDR threshold. Peptide identifications were made using SpectraST (Lam et al). FDRs were calculated using a peptide spectral library from an unrelated species. The yeast spectral library used in this work was generated from hundreds of repeat runs of the CPTAC yeast reference proteome and is available for download at http://peptide.nist.gov.

The software pipeline used to calculate these and the full set of QC metrics is available for download at http://peptide.nist.gov.

H. Interpretation and Calculation of CN50 for Studies 6 & 8

The CN50 for each run from each instrument in study is shown in the Table below.

	Study 6 (unspiked yeast)					Study 8 (120 ng)					Study 8 (600 ng)				
	run 1	run 2	run 3	mean	CV	run 1	run 2	run 3	mean	CV	run 1	run 2	run 3	mean	CV
LTQ@73	34147	36502	35773	35474	3.4%	27690	25976	25182	26282	4.9%	18369	17485	18263	18039	2.7%
LTQ2@95	42969	45309	43631	43969	2.7%	67762	56760	51446	58656	14.2%	28142	26730	27377	27416	2.6%
LTQ-XLx@65	38882	46758	38126	41256	11.6%	46734	44931	40807	44157	6.9%	21782	21566	23831	22393	5.6%
All LTQs				40233					43032					22616	
LTQ-OrbitrapO@65	17303	18142	19207	18217	5.2%	22893	22608	22343	22615	1.2%	17695	18265	17919	17959	1.6%
LTQ-XL-OrbitrapP@65	20370	23944	21016	21777	8.7%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
LTQ-Orbitrap@86	36117	26508	28433	30353	16.7%	18180	17813	18485	18159	1.9%	17903	17358	17951	17738	1.9%
LTQ-OrbitrapW@56	23620	25455	26109	25061	5.1%	15295	15312	15309	15305	0.1%	14064	13659	13245	13656	3.0%
All Orbitraps				23852					18693					16451	

The copy number at 50% detection (CN50) is a summary measure of the depth of sampling for the yeast proteome. This statistic makes use of the TAP copy number measurements(5) and a list of detected yeast proteins to estimate the copy number at which a randomly selected protein has 50% probability of detection in a single LC-MS/MS run. Smaller CN50 values denote more extensive sampling of the yeast proteome, and better performance. Many factors influence a specific protein's detection by LC-MS/MS; copy number is just one such factor. We consider CN50 to be a summary measure of the performance of an instrument platform. The following section describes calculation of CN50. In addition, example code for the R language (http://www.R-project.org) demonstrates this calculation.

CN50 is computed via logistic regression. Observations (the Y values) are binary responses for each yeast ORF denoting whether that protein is observed in an LC-MS/MS run. These binary observations are regressed against the logarithm (base 10) of the TAP copy numbers. The log transformation is used to improve model fit. The results of this regression are estimated intercept (*a*) and slope (*b*) coefficients. Given the coefficients and the logistic regression equation, one may set the probability of detection equal to 0.5, and algebraically solve for the corresponding copy number (log 10 scale). This solution is easily shown to be $-a/b$. Raising 10 to the power of this ratio ($10^{-a/b}$) results in an estimate of the CN50 on the copy number scale. Thus, CN50 summarizes the detection of yeast proteins, as a function of copy number, for each LC-MS/MS run. Note that since the logistic regression uses log 10 of copy number as the independent variable, the statistical properties of the slope and intercept coefficients (and subsequently their ratio) are better behaved on this scale. The antilog transformation is performed to aid interpretation of CN50. Finally, note that the TAP copy numbers(5) include measurement error. This uncertainty in a regressor is known as the "errors in variables" problem, and leads to a bias in estimation of the regression coefficients. In this use of logistic regression to construct the CN50, all LC-MS/MS runs are affected equally by this phenomenon. The bias is believed to be relatively small in this application, and does not significantly affect the comparison of platforms' performance.

The remainder of this section outlines computation of CN50 for an LC-MS/MS run using the R programming language. TAP copy number data are available in the supplementary material of Ghaemmaghami, *et al*(5). Lines beginning with hash marks (##) denote comments explaining the ensuing steps. The calculation assumes that the TAP copy number data have been merged with protein detection data with yeast ORF as the merge key. The variables used in the calculation are defined as follows:

N: the number of yeast proteins in the data set
Y: the *Nx1* vector of binary indicators of detection for yeast proteins, 0-non detected; 1-detected
copy: the *Nx1* vector of TAP copy numbers (copies per cell)

R code:

```
## The copy vector includes values of '-', '%,' and '#', as well as measured values of molecules per cell.
## '-' indicates no detected expression, '%' indicates a detected band that is below the limit of quantitation
## (50 copies /cell), and '#' indicates a detected band that was unquantifiable due to experimental problems
```

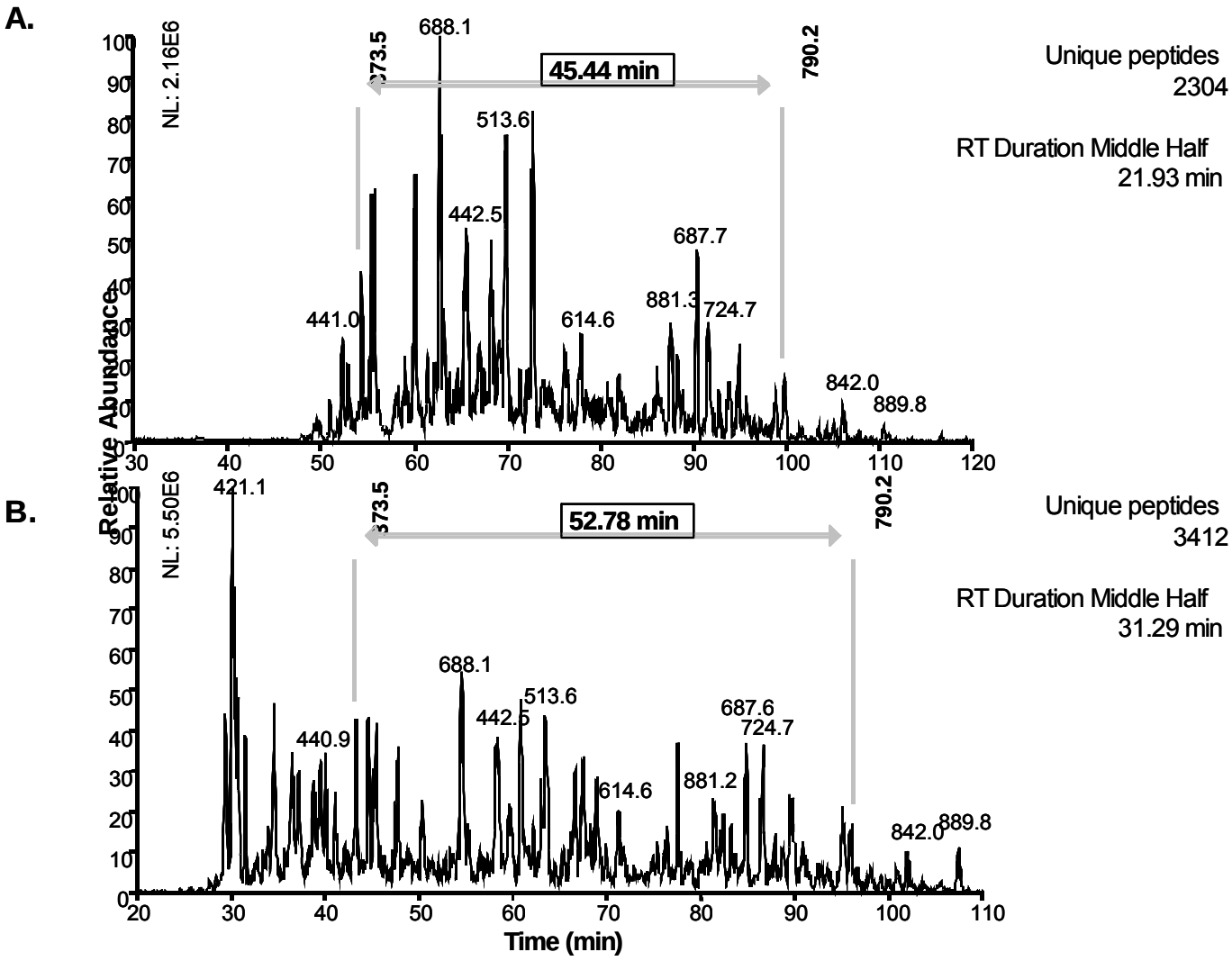
```
## with the Western blot.   These values were coded as -1 before reading into R.  Because these are
## unmeasured proteins (possibly not expressed), they are not useful in evaluating the relationship between
## copy number and protein detection.  In this analysis they are re-coded as missing.
copy[copy < 1] <- NA

## take the logarithm
copy.log10 <- log10(copy)

## compute the logistic regression and save the coefficients to an object named ‘fit.coef’
fit.coef <- glm( Y ~ copy.log10, family="binomial")$coef

## compute the CN50  on log10 scale, and on original copy number scale
CN50.log10 <- - fit.coef[1, 1] / fit.coef[1, 2]
CN50 <- 10^(CN50.log10)
```

I. Correction of chromatography issue diagnosed by Performance Metrics.



Example of the utility of the performance metrics to help troubleshoot a performance issue. A) Base peak chromatogram of yeast lysate digest. Note that the retention time difference between two peaks in the chromatogram, m/z 373.5 and m/z 790.2, is 45.4 minutes, which was significantly shorter compared to other instruments in the study. B) Base peak chromatogram of yeast lysates digest following repair of pump seal, replacement of pump check valve, manual recalibration of flow rate, and adjusting sample loading to lower flow rate. The retention time duration for the middle half of peptides increased along with the number of unique peptide identifications.

J. INSTRUMENT PARAMETERS FOR STUDIES 6 AND 8

Study

Study 6 'Yeast + Sigma48'

Site				65	65	56	86	65	73	95
Instrument				OrbitrapP	OrbitrapO	OrbitrapW	Orbitrap	LTQ-XLx	LTQ	LTQ2
Instrument model				LTQ-XL-Orbitrap	LTQ-Orbitrap	LTQ	LTQ-Orbitrap	LTQ-XL	LTQ	LTQ
Parameter	Units	Suggested Range	SOP Value							
Sample Handling										
Storage Temp.	C	-20 or -80		-20	-20	-80	-80	-20	-80	-80
Comments on samples										
Chromatography										
HPLC Manufacturer				Eksigent	Eksigent	Agilent	Eksigent	Eksigent	Agilent	Agilent
HPLC Model				NanoLC 1D plus	NanoLC 1D plus	1100 Nanopump G2226A	2D nano	NanoLC 1D plus	1100 nano	G2226A Nanopump
autosampler manufacturer				Eksigent	Eksigent	Agilent	Spark Holland	Eksigent	Spark Holland	Agilent
autosampler model				NanoLC-AS1	NanoLC-AS1	1100 MicroWPS G1377A	Endurance	NanoLC-AS1	Endurance	G1377A u-WPS
pre-column used	Y|N			Y	Y	N	N	Y	Y	Y
pre-column is vented	Y|N			Y	Y	N	N/A	Y	Y	Y
pre-column length	cm	2-4		4	4	N/A	N/A	4	4	4
pre-column ID	um	100		100	100	N/A	N/A	100	100	100
split-flow used	Y|N			N	N	N	N	N	N	N
loading flowrate	nl/min		1,200	1200	1200	1200	1200	1500*	1200	1,200
flow-rate from pump	nl/min		600	600	600	600	600	600	600	600
gradient per SOP	Y|N		Y	Y*	Y*	Y	Y	Y**	Y	Y
first 15 min diverted to waste	Y|N		Y	Y	Y	N	Y	Y	Y	Y
comments on chromatography				*note - Due to the design of the 1Dplus HPLC pump, we could not run a gradient from 100-98% Mobile Phase A from 10-15 min. Instead we switched from 100% to 98% at 15 min.	*note - Due to the design of the 1Dplus HPLC pump, we could not run a gradient from 100-98% Mobile Phase A from 10-15 min. Instead we switched from 100% to 98% at 15 min.			*note - Due to problems getting reproducible results with the 8uL loop, the loop was changed to a 10 uL loop and the flow rate for the loading phase was changed such that the loop was flushed with at least two volumes before the gradient was started **note - Due to the design of the 1Dplus HPLC pump, we could not run a gradient from 100-98% Mobile Phase A from 10-15 min. Instead we switched from 100% to 98% at 15 min.		
Wash runs										
short wash gradients run on column	Y|N			N	N	Y	Y	N	Y	Y
loop washed	Y|N			Y	Y	Y	Y	Y	Y	Y
comments on wash steps										
Columns										
manufacturer				Self made with Jupiter resin	Self made with Jupiter resin	self	N/A	Self made with Jupiter resin	N/A	
packing per SOP	Y|N			Y	Y	Y	Y	Y	Y	Y
packing finished on HPLC	Y|N			Y	Y	Y	Y	Y	Y	Y
analytical column length	cm	11-13		11	11	12	13	11	13	12
analytical column ID	um		100	100	100	100	100	100	100	100
other comments on columns										
Nano Spray/ AP Stack parameters										
source manufacturer				Thermo	Thermo	James A. Hill Instrument Services	Jamie Hill Instruments	Thermo	Thermo	In House
source model				NanoSpray	NanoSpray	LTQ nanoflow	N/A	NanoSpray	N/A	N/A
nano spray modifications				none	none	electrospray voltage supplied at T upstream of column.	N/A	none	N/A	Sheath Gas
Instrument Software										
Xcalibur version				2.0 SR2	2.07	2.0.5	2.0 SR2	2.07	2.0.7	2.0 SR2
FT Programs (Orbitrap)				2.0.2.0614	2.0.7 0703	2.0.1.0539	2.02.0614	2.0.7	N/A	N/A
LTQ MS or LTQ-Orbi MS				2.2 SP1	2.4 SP1	2.4	2.2	2.4	2.4	2.2
other instrument software				Eksigent	Eksigent		Eksigent	Eksigent	Eksigent Autosampler	
other instrument software version				2.08	2.08		v.2.08	2.08	v.2.08	
software comments									Agilent ChemStation v.A.10.02	

Study

Study 8 'Yeast'

Parameter	Units	Suggested Range	SOP Value						
Site				65	56	86	65	73	95
Instrument				OrbitrapO	OrbitrapW	Orbitrap	LTQ-XLx	LTQ	LTQ2
Instrument model				LTQ-Orbitrap	LTQ-Orbitrap	LTQ-Orbitrap	LTQ-XL	LTQ	LTQ
Sample Handling									
Storage Temp.	C	-20 or -80		-20	-80		-20	-80	-80
Chromatography									
HPLC Manufacturer				Eksigent	Agilent	Eksigent	Eksigent	Agilent	Agilent
HPLC Model				NanoLC 1D plus	1100 Nanopump G2226A	2D nano	NanoLC 1D plus	1100 nano	G2226A Nanopump
autosampler manufacturer				Eksigent	Agilent	Spark Holland	Eksigent	Spark Holland	Agilent
autosampler model				NanoLC-AS1	1100 MicroWPS G1377A	Endurance	NanoLC-AS1	Endurance	G1377A u-WPS
pre-column used	Y|N			Y	N	N	Y	Y	Y
pre-column is vented	Y|N			Y	N	N/A	Y	Y	Y
pre-column length	cm	2-4		4	2-4	N/A	4	15	4
pre-column ID	um	100		100	75	N/A	100	100	100
split-flow used	Y|N			N	N	N	N	N	N
loading flowrate	nl/min		1,200	1200	700	1000	1500	1200	1,200
flow-rate from pump	nl/min		600	600	200	400	600	600	600
gradient per SOP	Y|N		Y	Y	N	N	Y	Y	N
first 15 min diverted to waste	Y|N		Y	Y	N	Y	Y	Y	Y
comments on chromatography				*note - Due to the design of the 1Dplus HPLC pump, we could not run a gradient from 100-98% Mobile Phase A from 10-15 min. Instead we switched from 100% to 98% at 20 min.			**note - Due to the design of the 1Dplus HPLC pump, we could not run a gradient from 100-98% Mobile Phase A from 10-15 min. Instead we switched from 100% to 98% at 15 min.		
Wash runs									
short wash gradients run on column	Y|N			N	Y	Y	N	Y	Y
loop washed	Y|N			Y	Y	Y	Y	Y	Y
comments on wash steps									
Columns									
manufacturer				Self made with Jupiter resin	New Objective PicoFrit	New Objective PicoFrit Self Pack, 75um id, 10um tip id	Self made with Jupiter resin	Merck	
packing per SOP	Y|N			Y	Y	N/A	Y	Y	Y
packing finished on HPLC	Y|N			Y	Y	Y	Y	Y	Y
analytical column length	cm	11-13		11	12	15	11	15	12
analytical column ID	um		100	100	75 um id packed with Reprosil C18AQ 3 um particles	75	100	100um Chromolith CapRod Monolithic C18	100
other comments on columns									
Nano Spray									
source manufacturer				Thermo	James A. Hill Instrument Services	Jamie Hill Instruments	Thermo	Thermo	In House
source model				NanoSpray	LTQ nanoflow	N/A	NanoSpray	N/A	N/A
nano spray modifications				none	electrospray voltage supplied at T upstream of column.	N/A	none	N/A	Sheath Gas
Instrument Software									
Xcalibur version				2.07	2.0.5	2.0.7	2.07	2.0.7	2.0 SR2
FT Programs (Orbitrap)				2.0.7 0703	2.0.1.0539	2.0.7 0703	2.0.7	N/A	N/A
LTQ MS or LTQ-Orbi MS				2.4 SP1	2.5	2.4 SP1	2.4	2.4	2.2
other instrument software				Eksigent		Eksigent 2.08	Eksigent	Eksigent Autosampler	
other instrument software version				2.08			2.08	v.2.08	
software comments								Agilent ChemStation	
								v.A.10.02	

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