Published in final edited form as:

Rapid Commun Mass Spectrom. 2013 January 15; 27(1): 157–162. doi:10.1002/rcm.6437.

Comparison of LTQ-Orbitrap Velos and Q-Exactive for proteomic analysis of 1-1000 ng RAW 264.7 cell lysate digests

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Abstract

Rational—There is interest in extending bottom-up proteomics to the smallest possible sample size. We investigated the performance of two modern instruments for the analysis of samples ranging from 1 ng to 1 µg of RAW 264.7 cell lysate digests.

Methods—A UPLC system coupled with either an LTQ-Orbitrap Velos or a Q-Exactive mass spectrometers was used for peptide separation and identification.

Results—For 1–1,000 ng RAW 264.7 cell lysate digests, the Q-Exactive generated 10–83% more protein groups and 11–109% more peptides than the LTQ-Orbitrap Velos (higher-energy collisional dissociation, HCD) with MASCOT database searching, due to faster scan rate and higher resolution. In addition, HCD and collision induced dissociation (CID) modes of the LTQ-Orbitrap Velos were compared. HCD produced higher peptide and protein group IDs than CID for 1–1,000 ng RAW 264.7 cell lysate digests with MASCOT database searching. Database searching results from SEQUEST and MASCOT were also compared and comparable protein group IDs were obtained from the two search engines.

Conclusions—These results indicated that Q-Exactive outperformed LTQ-Orbitrap Velos for shotgun proteomics analysis of 1 to 1,000 ng RAW 264.7 cell lysate digests in terms of obtained peptide and protein group IDs.

Keywords

Shotgun proteomics; LTQ-Orbitrap Velos; Q-Exactive; CID; HCD

Introduction

Shotgun proteomics has made great advances due to the improved sensitivity, resolution, and speed of recent generations of mass spectrometers [1, 2]. The LTQ-Orbitrap Velos [3] and Q-Exactive [4] are two widely used mass spectrometers for large-scale proteomic analysis. For the LTQ-Orbitrap Velos, the mass spectrum is acquired with an Orbitrap, and tandem mass spectrometry (MS/MS) can be acquired with ion trap (collision induced dissociation, CID) or Orbitrap (higher-energy collisional dissociation, HCD). For the Q-Exactive, the Orbitrap is used for acquiring both MS and MS/MS spectra. Mann *et al.* coupled a 50 cm long reversed phase capillary column with an LTQ-Orbitrap Velos for deep and highly sensitive proteome analysis without pre-fractionation [5]. After triplicate single-run analysis, 2,990 yeast proteins and 5,376 human embryonic kidney cell proteins were identified with about one day of measurement time. They further coupled the long column to a Q-Exactive and identified more than 3,900 yeast proteins with single 4 h gradient run [6].

Recently, Olsen *et al.* optimized the instrument setting of a Q-Exactive for different sample conditions, and set up “Fast” and “Sensitive” methods for shotgun proteomics with this instrument [7]. With the “Fast” method, more than 2,000 yeast proteins could be identified from 1 h of analysis time when the sample loading amount was above 125 ng. For the more complex tryptic HeLa digest, more than 4,000 proteins could be identified with the “Sensitive” method from a 1 µg sample with only a 3 h LC gradient.

Although the LTQ-Orbitrap Velos and the Q-Exactive are widely used, only a few reports have compared the two instruments for large-scale proteomic analysis. One report stated that the Q-Exactive produced about 28% more proteins and 13% more unique peptides than the LTQ-Orbitrap Velos (HCD) for tryptic digest of mammalian cell lysate [4]. Another study found that the Q-Exactive produced about 140% more unique peptides than the LTQ-Orbitrap Velos (HCD) for yeast peptides [7], most likely due to the faster scan rate and higher resolution of the Q-Exactive. However, these two reports only used high sample loading to perform comparisons. The loading amount might affect the results, so this manuscript compares the LTQ-Orbitrap Velos and the Q-Exactive across a three order of magnitude range in protein content (1–1,000 ng) of a tryptic digest of RAW 264.7 cell lysate. We observed that the Q-Exactive outperformed the LTQ-Orbitrap Velos (HCD) for shotgun proteomics analysis of 1 to 1,000 ng RAW 264.7 cell lysate digests in terms of peptide and protein group IDs. In addition, HCD and CID modes of LTQ-Orbitrap Velos were compared using 1–1,000 ng tryptic digest of RAW 264.7 cell lysate, and the results demonstrated that HCD always yielded more peptide and protein identifications than CID with MASCOT database searching.

Experimental section

Chemicals and reagents

Bovine pancreas TPCK-treated trypsin, urea, ammonium bicarbonate (NH_4HCO_3), dithiothreitol (DTT), and iodoacetamide (IAA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and formic acid (FA) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was deionized by a Nano Pure system from Thermo Scientific (Marietta, OH, USA). ZipTip C18 (ZTC18S096) was purchased from Millipore (Bedford, MA, USA).

Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine and fetal bovine serum (FBS) were purchased from ATCC (Manassas, VA, USA). Mammalian Cell-PE LB™ buffer for cell lysis was purchased from G-Biosciences (St. Louis, MO, USA). Complete, mini protease inhibitor cocktail (provided in EASY packs) was purchased from Roche (Indianapolis, IN, USA).

Sample preparation

The procedure for RAW 264.7 cell lysate preparation was same as references [8, 9]. RAW 264.7 cells were cultured in a T25 flask at 37 °C and 5% CO_2 in DMEM with L-glutamine and 10% FBS. After washing with cold PBS buffer twice, 1 mL mammalian cell-PE LB™ buffer (pH 7.5) supplemented with complete protease inhibitor was added to the flask, and the flask was shaken gently for 10 min on ice. The cell lysate was transferred to a 1.5 mL Eppendorf tube and incubated on ice for 15 min. Subsequently, the cell lysate was centrifuged at 18,000 g for 15 min, and the supernatant was collected for measurement of protein concentration with the bicinchoninic acid (BCA) method [10]. After that, 300 µL cell lysate (~54 µg proteins) was denatured at 90 °C for 20 min, followed by reduction with DTT (3.3 mM) at 65 °C for 1 h and alkylation with IAA (8.3 mM) at room temperature for 30 min in dark. Then, 1.2 mL cold acetone was added to the protein solution and incubated at –20 °C for 12 h, followed by centrifugation at 18,000 g for 15 min. The protein pellet was

washed with cold acetone again, and dried at room temperature. Finally, the protein pellet was redissolved in 100 μ L 1 M urea and 100 mM NH_4HCO_3 buffer (pH 8.0). Tryptic digestion was performed at 37 °C for 12 h with trypsin and protein mass ratio of 1/30. After digestion, the digest was acidified with FA, and desalted with Ziptip C18. The dried digest was redissolved in 0.1% FA to obtain 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 mg/mL solution, followed by UPLC-ESI-MS/MS analysis.

UPLC-ESI-MS/MS analysis

A nanoACQUITY UltraPerformance LC[®] (UPLC[®]) system (Waters, Milford, MA, USA) was used for separation of the protein digests. Buffer A (0.1% FA in water) and buffer B (0.1% FA in ACN) were used as mobile phases for gradient separation. Protein digests were automatically loaded onto a commercial C18 reversed phase column (Waters, 100 μ m $\times$ 100 mm, 1.7 μ m particle, BEH130C18, column temperature 40 °C) with 2% buffer B for 12 min at a flow rate of 0.7 μ L/min, followed by 3-step gradient separation. The 60-min gradient was 2 min from 2 % B to 10% B, 20 min to 40% B, 1 min to 85% B, and maintained for 10 min. The 100-min gradient was 2 min from 2% B to 10% B, 60 min to 40% B, 1 min to 85% B, and maintained for 10 min. The 150-min gradient was 2 min from 2 % B to 10% B, 110 min to 40% B, 1 min to 85% B, and maintained for 10 min. The column was equilibrated for 14 min with 2% buffer B prior to the next sample analysis. The eluted peptides from the C18 column were pumped through a capillary tip for electrospray, and analyzed by LTQ-Orbitrap Velos or Q-Exactive instruments (Thermo Fisher Scientific). The electrospray voltage was 1.8 kV, and the ion transfer tube temperature was 300 °C for LTQ-Orbitrap Velos and 280 °C for Q-Exactive. For each run, 1 μ L digest was loaded, and each sample was analyzed in duplicate.

For LTQ-Orbitrap Velos (CID mode), a top 20 method was used. Full MS scans were acquired in the Orbitrap mass analyzer over the m/z 350–1800 range with resolution 60,000 (m/z 400). The target value was 5.00E+05. The twenty most intense peaks with charge state 2 were selected for sequencing and fragmented in the ion trap with normalized collision energy of 35%, activation $q = 0.25$, activation time of 10 ms, and one microscan. The target value was 1.00E+04. The ion selection threshold was 500 counts, and the maximum allowed ion accumulation times were 500 ms for full scans and 100 ms for CID.

For LTQ-Orbitrap Velos (HCD mode), a top 10 method was used. Full MS scans were acquired in the Orbitrap mass analyzer over m/z 350–1800 range with resolution 30,000 (m/z 400). The target value was 1.00E+06. Ten most intense peaks with charge state 2 were fragmented in HCD collision cell with normalized collision energy of 40%, and tandem mass spectrum was acquired in the Orbitrap mass analyzer with resolution 7 500. The target value was 5.00E+04. The ion selection threshold was 5,000 counts, and the maximum allowed ion accumulation times were 500 ms for full scans and 250 ms for HCD.

For Q-Exactive, the method was based on reference [7] with slightly modifications. A top 12 method was used. Full MS scans were acquired in the Orbitrap mass analyzer over m/z 350–1800 range with resolution 70,000 (m/z 200). The target value was 1.00E+06. Twelve most intense peaks with charge state 2 were fragmented in the HCD collision cell with normalized collision energy of 30%, and tandem mass spectrum was acquired in the Orbitrap mass analyzer with resolution 35,000 at m/z 200. The target value was 1.00E+06. The ion selection threshold was 1.00E+05 counts, and the maximum allowed ion accumulation times were 250 ms for full MS scans and 120 ms for tandem mass spectrum. For 1 ng and 5 ng cell lysate digest analysis, ion selection threshold 1.00E+04 counts and maximum allowed ion accumulation time 250 ms for tandem mass spectrum were also applied.

For all the experiments, dynamic exclusion was set to 15 s.

Data Analysis

Database searching of all .raw files was performed in Proteome Discoverer 1.3 (Thermo Fisher Scientific). MASCOT 2.2.4 and SEQUEST were used for database searching against Uniprot_mouse database (updated on 09/05/2012, 59 375 sequences, downloaded from FTP directory /pub/databases/uniprot/current_release/knowledgebase/peptides/ at ftp.uniprot.org), respectively. Database searching against the corresponding reversed database was also performed to evaluate the false discovery rate (FDR) of peptide identification. The database searching parameters included up to two missed cleavages allowed for full tryptic digestion, precursor mass tolerance 10 ppm (for LTQ-Orbitrap Velos) and 6 ppm (for Q-Exactive), product ions mass tolerance 1.0 Da (for LTQ-Orbitrap Velos (CID mode)), 0.05 Da (for LTQ-Orbitrap Velos (HCD mode)), and 0.02 Da (for Q-Exactive), cysteine carbamidomethylation as a fixed modification, and methionine oxidation and deamidated (NQ) as variable modifications.

The result from each run was filtered with peptide confidence value as high to obtain FDR less than 1% on peptide level. On protein level, minimum number of peptide 1 for each protein, count only rank 1 peptides and count peptide only in Top scored proteins were applied for all data filtration. In addition, protein grouping was enabled, and strict maximum parsimony principle was applied. Therefore, if multiple proteins were identified from same peptides, these proteins were grouped into one protein group, and each protein group has at least one unique peptides. The protein groups and peptides identified by duplicate UPLC-ESI-MS/MS analysis from 1–1,000 ng RAW 264.7 cell lysate digest with MASCOT database searching are listed in supporting material I.

Results and discussions

Comparison of LTQ-Orbitrap Velos (HCD) with Q-Exactive

In order to compare LTQ-Orbitrap Velos (HCD) and Q-Exactive for proteomic analysis of 1–1000 ng RAW 264.7 cell lysate digest, the parameter setup for acquiring MS and MS/MS spectra was referred to references [3] and [7], Table 1. For the Q-Exactive, two methods were used in the manuscript. The main differences between the two methods were maximum injection time for MS/MS (120 ms vs. 250 ms) and intensity threshold (1.00E+05 counts vs. 1.00E+04 counts). In order to reduce the maximum cycle time of the “250 ms and 1.00E+04 counts” method, a Top 8 method was applied. The “120 ms and 1.00E+05 counts” method was applied for 1–1 000 ng cell lysate digest analysis, and “250 ms and 1.00E+04 counts” method was only applied for 1 ng and 5 ng cell lysate digest analysis. The cycle times for “120 ms and 1.00E+05 counts” and “250 ms and 1.00E+04 counts” methods were around 2.2 s and 2.6 s according to reference [7]. For the LTQ-Orbitrap Velos (HCD), consistent parameters were used for different sample loading amounts, and the cycle time was about 2.6 s according to reference [3]. Therefore, the cycle times of the Q-Exactive and LTQ-Orbitrap Velos (HCD) methods were comparable. In order to maximize the identifications, 150 min LC gradient was used for 500 and 1,000 ng digest, 100 min for 50 and 100 ng digest, and 60 min for 1, 5 and 10 ng digests.

After MASCOT database searching, the results were filtered with peptide confidence value as high to produce peptide identification FDR less than 1%, as shown in Table 2. When the sample loading amount was 1,000 ng, about 10% more protein groups, peptides and peptide spectrum matches (PSMs) were obtained with the Q-Exactive compared with the LTQ-Orbitrap Velos. In addition, the Q-Exactive yielded a slightly higher identification rate (41% vs. 37%). When the sample loading amounts ranged from 10 ng to 500 ng, the Q-Exactive

produced 31%–74% more protein groups, 32%–88% more peptides, 46%–109% more PSMs and 36%–78% more MS/MS spectra than the LTQ-Orbitrap Velos. The results were due to two reasons. First, the scan rate of Q-Exactive was faster than LTQ-Orbitrap Velos due to a smaller maximum injection time for MS/MS (120 ms vs. 250 ms), resulting in more MS/MS spectra. Secondly, a higher MS resolution (70,000 (m/z 200) vs. 30,000 (m/z 400)) and MS/MS resolution (35,000 (m/z 200) vs. 7,500 (m/z 400)) were used for the Q-Exactive. Higher resolution results in a lower mass error of precursor and product ions, leading to smaller number of peptides matched to the reversed database. Therefore, a lower MASCOT significance threshold (0.018 vs. 0.003 for 500 ng sample, 0.016 vs. 0.0035 for 100 ng sample, 0.0093 vs. 0.0020 for 50 ng sample, 0.0070 vs. 0.0010 for 10 ng sample) at peptide identification FDR less than 1% was obtained for Q-Exactive data, as shown in Table 2.

When only 1 ng cell lysate digest was loaded on the LC column and the “120 ms and 1.00E+05 counts” method was applied for Q-Exactive, we observed that the LTQ-Orbitrap Velos produced more protein groups, peptides, PSMs and MS/MS spectra than the Q-Exactive, Table 2. Interestingly, when the “250 ms and 1.00E+04 counts” method was applied for the Q-Exactive data, the number of protein groups, peptides, PSMs and MS/MS spectra was dramatically increased compared with that from the “120 ms and 1.00E+05 counts” method, and was also higher than that from LTQ-Orbitrap Velos, Table 2. We manually checked the intensity of peptides from “250 ms and 1.00E+04 counts” method, and found that the intensity of many peptides was lower than 1.00E+05. When both “120 ms and 1.00E+05 counts” and “250 ms and 1.00E+04 counts” methods were applied for 5 ng cell lysate digest analysis “250 ms and 1.00E+04 counts” method generated slightly more identifications than “120 ms and 1.00E+05 counts” method, and the number of identifications from these two methods were higher than that from LTQ-Orbitrap Velos. The results from 1 and 5 ng cell lysate digest indicated that for very small amount cell lysate digest (around 5 ng or less) analysis with Q-Exactive, it is necessary to set the intensity threshold to 1.00E+04 counts or lower, and also increase the maximum injection time for MS/MS (i.e. 250 ms) to obtain good MS/MS spectra.

We further evaluated the relationship between cell lysate digest loading amount and identifications (IDs), Figure 1. The number of IDs was roughly described by

$$\text{IDs} = \text{IDs}_{\infty} (1 - 2^{-a/a_{1/2}}) \quad (\text{eq 1})$$

where IDs_{∞} is the asymptotic number of IDs for large sample loadings, a is the protein amount, and $a_{1/2}$ is the amount of protein required to reach half the asymptotic value. The asymptotic value of protein and peptide identifications from Q-Exactive is higher than that from LTQ-Orbitrap Velos. The asymptotic values of protein identifications from MASCOT and SEQUEST database searching are comparable. In addition, combination of SEQUEST and MASCOT database searching results slightly increased the protein and peptide identification numbers.

We determined the overlap of IDs between duplicate runs of 1–1,000 ng cell lysate digest, S-Figure 1 in supporting material II. Q-Exactive and LTQ-Orbitrap Velos generated comparable overlap of protein groups and peptides for 1–1,000 ng cell lysate digest, and the overlap ranged from around 50% to 80% for protein groups, and ranged from around 40% to 70% for peptides. The overlaps from 1 ng and 5 ng cell lysate digest were lower than that from higher sample loading amount (10–1 000 ng). We further determined the relationship of peptide and protein group IDs between duplicate UPLC-ESI-MS/MS analysis of 1–1000 ng cell lysate digest, S-Figure 2 in supporting material II. The peptide and protein group IDs obtained from the 1st and 2nd runs of 1–1000 ng cell lysate digest were reasonably reproducible.

We also chose one peptide (TPEELSAIK) from a relatively high abundant protein (Annexin A5), and evaluated the relationship between the peptide intensity and cell lysate digest loading amount, S-Figure 3 in the supporting material II. The peptide intensity increased when the loaded total peptide amount increased from 1 ng to 1 000 ng for LTQ-Orbitrap Velos and Q-Exactive. The relationship was far away from linear, most likely due to the different LC gradient used for 1 ng–10 ng (60 min gradient), 50–100 ng (100 min gradient), and 500–1000 ng (150 min gradient) cell lysate digest analysis. Interestingly, the peptide intensity from Q-Exactive was always significantly higher than that from LTQ-Orbitrap Velos, which agreed with the result in reference [7], and the different internal software scaling of signal intensities between the two generations of Orbitrap mass spectrometers is most likely the main reason for the observed difference [7].

Comparison of CID and HCD

For LTQ-Orbitrap Velos, two kinds of dissociation methods, CID and HCD, can be used for peptide analysis. CID mode provides a faster scan rate and lower MS/MS resolution than HCD. Several reports made the comparison of CID and HCD for large-scale proteome [3, 11], phosphoproteome [12, 13] and peptidome [14, 15] analysis. Interestingly, the conclusions from those reports are not the same, which might be due to the different samples and sample preparation processes. In addition, most of these reports did not include the effect of different sample loading amounts. Therefore, in this work, we compared CID and HCD for 1–1,000 ng RAW 264.7 cell lysate digest analysis.

For 1–1 000 ng amounts of RAW 264.7 cell lysate digest analysis, HCD outperformed CID in terms of the number of peptides and protein groups with MASCOT database searching, S-Table 1 in supporting material II. HCD produced 47%–500% more peptides and 20%–267% more protein groups than CID. These results demonstrated that higher MS/MS resolution of HCD was very valuable for characterizing this complex sample.

We generated the curves based on the number of protein groups and number of peptides verse sample loading amount, Figure 2. The curves were also fit to the exponential function of Equation 1. The asymptotic numbers of protein and peptide IDs for HCD was roughly twice the values for CID. The asymptotic numbers of protein and peptide IDs from MASCOT and SEQUEST database searching were comparable for both CID and HCD, and combination of SEQUEST and MASCOT database searching results could slightly increase the protein and peptide identifications.

We further evaluated the overlap of IDs between duplicates of 1–1,000 ng cell lysate digest, S-Figure 1 in supporting material II. HCD yielded higher overlap of protein groups and peptides than CID, which meant that HCD could yield more reproducible identifications than CID. We also evaluate the relationship between the peptide intensity and cell lysate digest loading amount, S-Figure 3 in the supporting material II. The peptide intensity from CID and HCD was increased when the sample loading amount increased from 1 ng to 1 000 ng, and the peptide intensity from CID and HCD agreed well.

Conclusions

The LTQ-Orbitrap Velos and Q-Exactive mass spectrometers were compared for shotgun proteomic analysis of 1 to 1,000 ng RAW 264.7 cell lysate digests. Q-Exactive outperformed LTQ-Orbitrap Velos (HCD) in terms of number of peptide and protein groups, due to faster scan rate and higher resolution. In addition, LTQ-Orbitrap Velos (HCD) produced better identifications than LTQ-Orbitrap Velos (CID).

Because multiple kinds of dissociation methods (i.e. HCD, CID, Electron-transfer dissociation (ETD)) are available for LTQ-Orbitrap instruments, and combination of these dissociation methods is valuable for large-scale proteomics analysis, especially for protein posttranslational modifications analysis, the results obtained in this work do not mean that Q-Exactive could completely replace LTQ-Orbitrap Velos for proteomics analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the assistance of Dr. Bill Boggess of mass spectrometry & proteomics facility at the University of Notre Dame for this project. This work was funded by the National Institutes of Health (R01GM096767).

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Figure 1.

Calibration curve based on the number of protein groups (A) and number of peptides (B) vs. sample loading amount analyzed by UPLC-ESI-MS/MS. The total identifications from duplicate runs were used to generate the figure. The Q-Exactive data was obtained with “120 ms and 1.00E+05 counts” method.

Figure 2.

Calibration curves based on number of protein groups (A) and number of peptides (B) vs. sample loading amount analyzed by UPLC-ESI-MS/MS. The total identifications from duplicate runs were used to generate the figure.

Table 1

MS and MS/MS parameters of LTQ-Orbitrap Velos and Q-Exactive for analysis of RAW 264.7 cell lysate digest

	MS resolution	Target value	MS/MS resolution	Maximum injection time [ms]	Target value	Isolation window	Top N#	Intensity threshold
LTQ-Orbitrap Velos (CID)	60,000 (m/z 400)	5.00E+05	normal	100	1.00E+04	2	20	500
LTQ-Orbitrap Velos (HCD)	30,000 (m/z 400)	1.00E+06	7,500 (m/z 400)	250	5.00E+04	2	10	5000
Q Exactive ^a	70,000 (m/z 200)	1.00E+06	35,000 (m/z 200)	120	1.00E+06	2	12	1.00E+05
Q Exactive ^b	70,000 (m/z 200)	1.00E+06	35,000 (m/z 200)	250	1.00E+06	2	8	1.00E+04

^a parameters used for analysis of 1–1 000 ng Raw 264.7 cell lysate digests;

^b parameters used for analysis of 1 and 5 ng Raw 264.7 cell lysate digests.

[#] N most intense peaks with charge state 2 were chosen according to the previous full MS scan and fragmented, followed by MS/MS spectra acquisition.

Table 2

Identification results of 1–1,000 ng RAW 264.7 cell lysate digest after analyzed with Q-Exactive and LTQ-Orbitrap Velos (HCD) in duplicate runs*

Q-Exactive							
	Protein groups	Peptides	Peptide spectrum matches (PSMs)	MS/MS	Identification rate (%)	MASCOT significance threshold (FDR<1%#)	
	1000 ng	1382	5570	13895	33797	41.11	0.017
	500 ng	1310	5471	13347	32001	41.71	0.018
	100 ng	810	2975	6883	17341	39.69	0.016
	50 ng	663	2111	4049	11017	36.75	0.0093
	10 ng	344	970	1830	5233	34.97	0.0070
	5 ng ^a	200/242	426/588	755/992	2550/3952	29.61/25.10	0.0019/0.0059
	1 ng ^a	22/81	48/147	79/247	457/1497	17.29/16.50	0.020/0.020
LTQ-Orbitrap Velos (HCD)							
	1000 ng	1255	5023	12493	33695	37.08	0.0050
	500 ng	913	3651	9148	23469	38.98	0.0030
	100 ng	617	2251	4602	11384	40.43	0.0035
	50 ng	382	1184	2324	7505	30.97	0.0020
	10 ng	219	515	877	2942	29.81	0.0010
	5 ng	132	282	480	2133	22.50	0.0010
	1 ng	48	77	116	672	17.26	0.0030

* Results were from MASCOT database searching.

^a Two kinds of instrument methods (shown in Table 1) were used for analysis of 1 ng and 5 ng cell lysate digests. The first number was from maximum injection time for MS/MS 120 ms and intensity threshold 1.00E+05, and the second number was from maximum injection time for MS/MS 250 ms and intensity threshold 1.00E+04.

[#] FDR ranged from around 0.7% to 0.9% for 5–1 000 ng cell lysate digest data. For 1 ng cell lysate digest data, the FDR was 0.