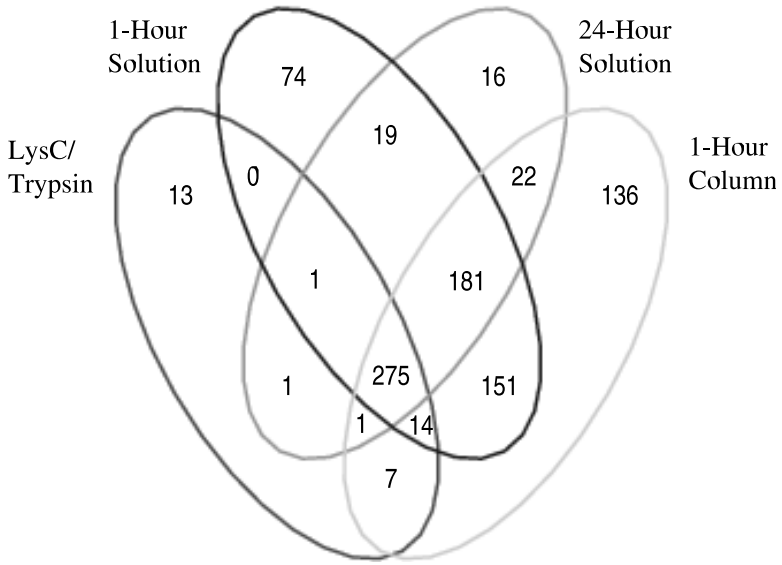

Figure 1: Overlap of protein identifications for four different digestion protocols.

Table 1: Overlap of protein identifications for four different digestion protocols.

Condition	No overlap	Lys-C	Column	Column and Lys-C
No overlap	—	13	136	7
1-H Soln.	74	0	151	14
24-H Soln.	16	1	22	1
1-H and 24-H Soln.	19	1	181	275

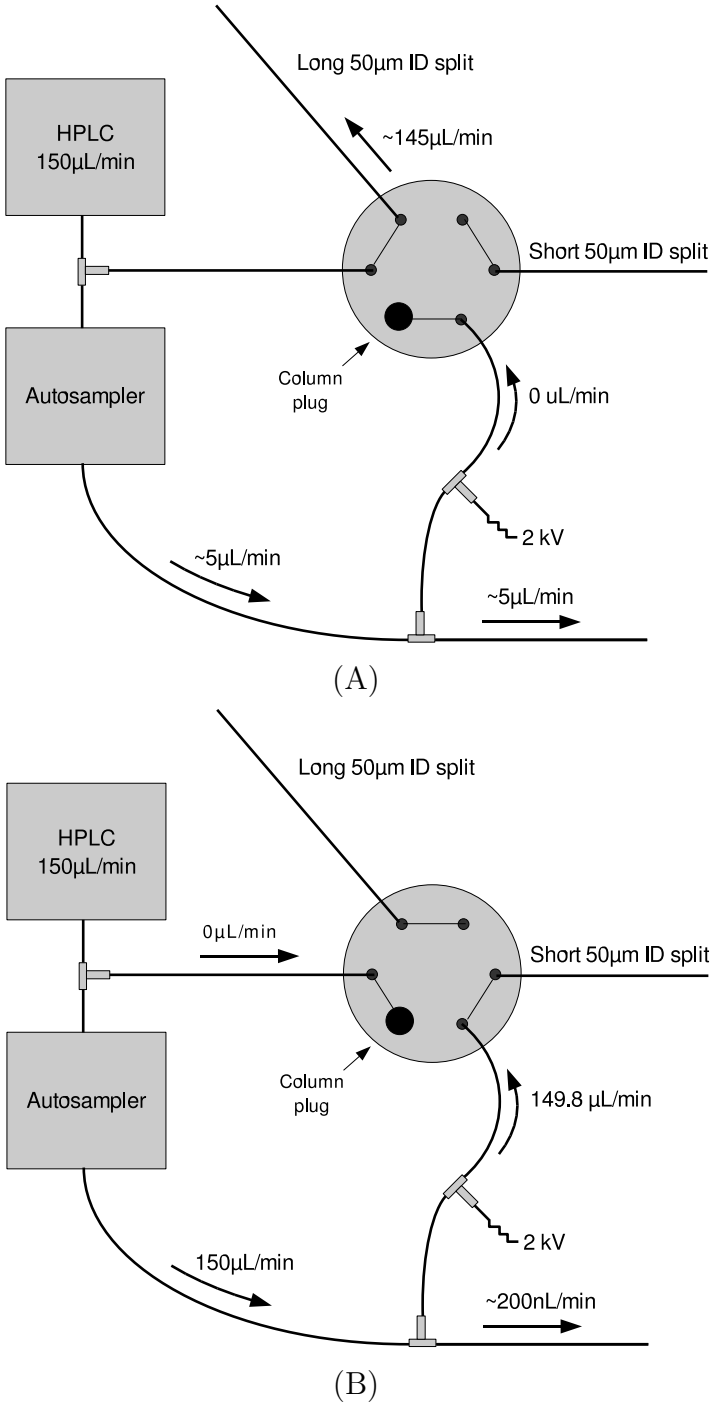

Figure 2: A unique autosampler configuration that comprises two distinct states: a sample loading configuration (A) and a sample running configuration (B). In both, the HPLC pump flows at 150μL/min, and flow rate to the electrospray tip is reduced through the use of a microcapillary split. The main difference between the two states is the position and length of this split. In the loading configuration (A), a relatively long split occurs between the HPLC pump and the autosampler, avoiding sample loss, while maintained output flow rate at 5μL/min (diverting 145μL/min to waste). In the running configuration (B), a relatively short split occurs after the autosampler, allowing further reduction in flow rate to 200nL/min (diverting 149.8μL/min to waste). This configuration allows rapid sample loading while allowing running at the nanoliter flow rate necessary for electrospray.

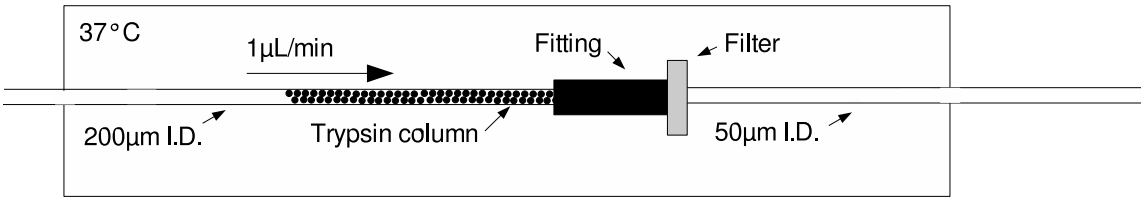

Figure 3: Immobilized-trypsin column. A reduced, alkylated and unfractionated protein mixture is pumped at 1μL/min into a 200μm ID microcapillary tube containing 7cm of immobilized trypsin beads. The beads are kept in place using a fitting and filter and held at a constant temperature of 37°C using a column heater. Sample exits the column at 1 μL/min through a 50μm I.D. microcappillary tube and is then subjected to mass spectrometric analysis.

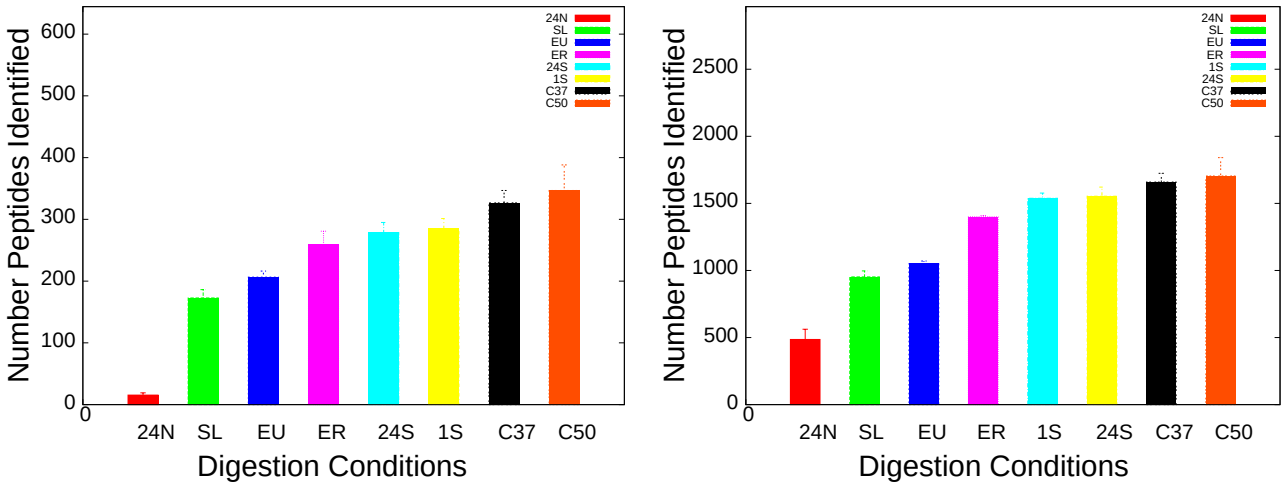

Figure 4: Number of true positive peptide identifications for several digestion conditions, including additional controls. Peptide samples were produced as described in the text with additional controls as noted below. Spectra were analyzed as described in the text, constraining peptides to have either tryptic-specificity only (A) or no specicity whatsoever (B). Digestion conditions are as follows: a 24-Hour-Null control (24N) identical to the 24-Hour-Solution digestion except without trypsin enzyme; a slurry control (SL) identical to the 1-Hour-Solution digestion except with a volume of trypsin bead slurry equal to that used in the 1-Hour-Column digestion trypsin column; the Lys-C/Tryp digestion (EU); a Lys-C/Tryp digestion with 50mM Rapigest instead of 8M urea (ER), but otherwise identical to EU; the 24-Hour-Solution digestion (24S); the 1-Hour-Solution digestion (1S); the 1-Hour-Column digestion (C37); finally, a 1-Hour-Column digestion at 50C instead of 37C (C50);

Table 2: Distributions of proteins identified with a given number of spectra for four different digestion conditions. The first value in each cell is the mean across four samples; the second is the standard deviation.

Peptide Count[1]	Lys-C	24-H Sol	1-H Col	1-H Sol
1	89.25 ± 7.80	144.00 ± 16.10	161.00 ± 12.68	185.50 ± 3.87
2	40.25 ± 6.65	64.00 ± 9.20	80.50 ± 12.97	78.75 ± 2.63
3	23.00 ± 2.71	36.25 ± 6.29	51.00 ± 8.76	52.25 ± 4.57
4	13.75 ± 3.50	28.25 ± 7.41	46.00 ± 8.64	39.50 ± 1.29
5	8.00 ± 2.45	19.00 ± 4.55	30.25 ± 3.59	24.75 ± 1.26
6	7.25 ± 1.50	12.75 ± 2.22	23.25 ± 4.92	14.50 ± 1.73
7	4.50 ± 1.29	11.50 ± 3.32	21.25 ± 9.54	14.25 ± 6.08
8	4.25 ± 2.50	10.25 ± 3.20	15.50 ± 4.51	12.50 ± 2.89
9	2.25 ± 0.50	8.25 ± 0.96	13.00 ± 2.71	14.50 ± 1.73
10	1.25 ± 1.50	4.00 ± 0.82	14.75 ± 0.96	7.50 ± 2.38
11	2.00 ± 1.83	5.00 ± 2.83	9.75 ± 2.87	9.25 ± 3.20
12	0.75 ± 0.96	5.00 ± 1.41	10.00 ± 3.16	7.25 ± 2.50
13	0.50 ± 0.58	3.75 ± 2.36	8.75 ± 2.22	4.75 ± 1.71
14	0.75 ± 0.50	3.75 ± 1.71	8.25 ± 1.71	4.75 ± 2.36
15	0.50 ± 0.58	2.00 ± 0.82	6.50 ± 3.70	4.75 ± 3.20
16	0.00 ± 0.00	1.75 ± 0.50	6.50 ± 3.42	4.50 ± 1.00
17	0.00 ± 0.00	0.75 ± 0.50	6.25 ± 3.20	5.00 ± 2.58
18	0.00 ± 0.00	0.25 ± 0.50	6.00 ± 2.94	4.00 ± 2.45
19	0.50 ± 1.00	0.50 ± 0.58	6.00 ± 1.83	3.25 ± 2.50
20	0.00 ± 0.00	0.75 ± 0.96	4.00 ± 1.41	3.25 ± 1.26
21-25	0.25 ± 0.50	1.75 ± 0.50	18.00 ± 4.97	7.25 ± 2.22
26-30	0.50 ± 0.58	0.25 ± 0.50	13.00 ± 2.16	4.50 ± 0.58
31-35	1.00 ± 0.82	0.50 ± 0.58	9.25 ± 2.22	1.75 ± 0.96
36-40	0.75 ± 0.96	0.50 ± 1.00	2.50 ± 3.11	1.25 ± 0.50
41-45	1.00 ± 0.82	0.50 ± 0.58	2.25 ± 2.63	0.25 ± 0.50
46-50	1.25 ± 0.96	2.00 ± 1.15	2.00 ± 1.15	0.50 ± 0.58
51-60	0.25 ± 0.50	1.50 ± 1.29	2.50 ± 0.58	1.00 ± 0.82
61-70	0.50 ± 0.58	1.75 ± 1.26	2.25 ± 0.50	1.75 ± 1.26
71-80	0.25 ± 0.50	0.75 ± 0.50	2.25 ± 0.50	1.75 ± 0.96
81-90	0.75 ± 0.50	0.00 ± 0.00	0.50 ± 0.58	2.25 ± 0.50
91-100	0.00 ± 0.00	0.75 ± 0.50	0.50 ± 1.00	0.75 ± 0.96
101-200	0.00 ± 0.00	0.00 ± 0.00	5.50 ± 0.58	1.25 ± 0.50
201-300	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00

[1]See text for detailed description of digestion conditions.