

## SUPPLEMENTARY INFORMATION

### **Middle-Down Approach: A choice to sequence and characterize proteins/proteomes by mass spectrometry**

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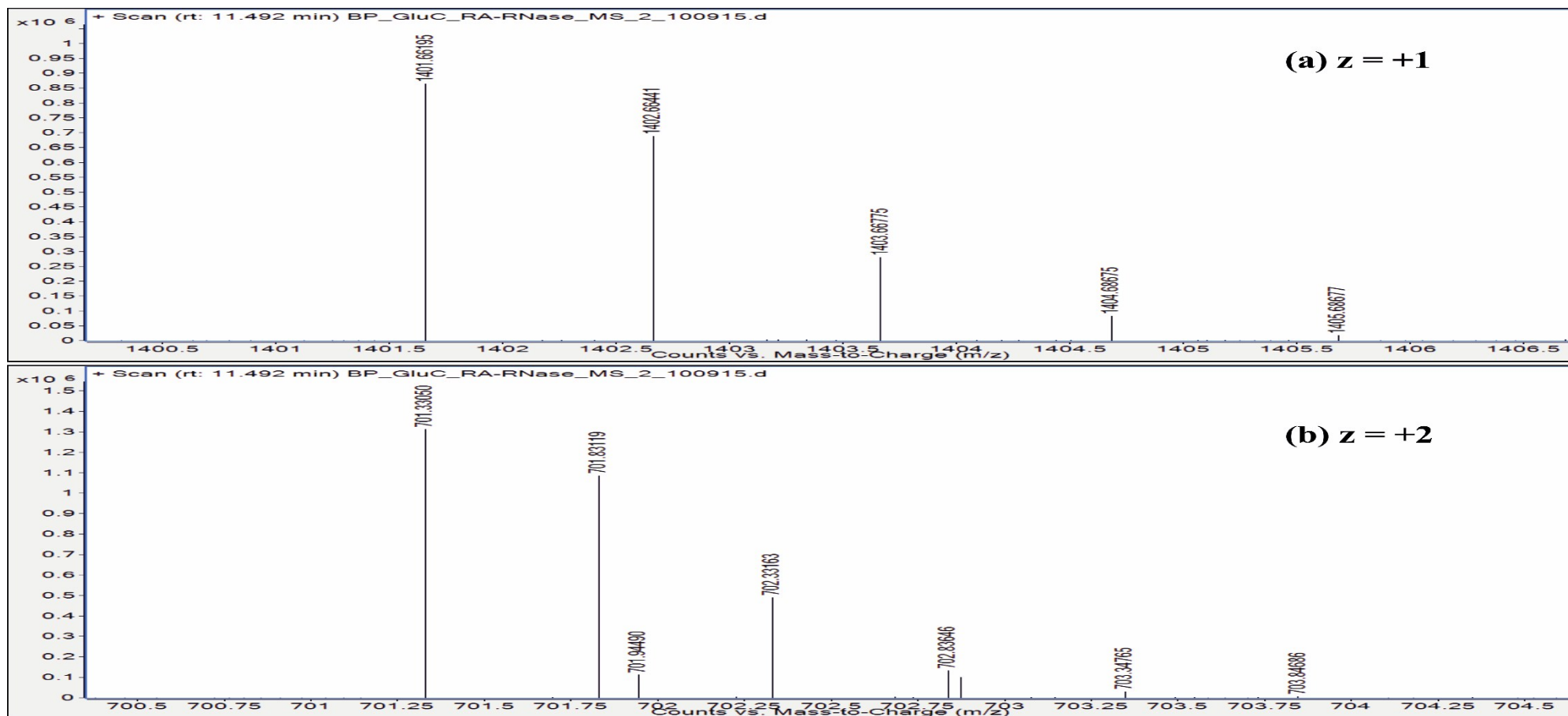
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**Table S1.** List of 15 proteins from each of five different species along with their respective Uniprot KB IDs selected for 0-missed cleavage (0-MC), 1-MC and 2-MC, in-silico proteolysis.

S. No	Enzymes	Human		Yeast <sup>^</sup>		<i>Escherichia coli</i>		<i>Arabidopsis thaliana</i>		Archaea <sup>†</sup>	
		Uniprot KB ID	Mol. mass (approx.) (kDa)	Uniprot KB ID	Mol. mass (approx.) (kDa)	Uniprot KB ID	Mol. mass (approx.) (kDa)	Uniprot KB ID	Mol. mass (approx.) (kDa)	Uniprot KB ID	Mol. mass (approx.) (kDa)
1	G3P dehydrogenase	P04406	36	P00358	36	P0A9B2	35	P25858	37	Q58546	38
2	Isocitrate dehydrogenase	P48735	47	P28834	38	P08200	46	P93032	37	Q58991	38
3	malate dehydrogenase	P40926	33	P22133	40	P61889	32	P93819	35	Q58130	36
4	Enolase	P06733	47	P00924	47	P0A6P9	45	Q9C9C4	47	Q60173	46
5	Adenylosuccinate synthetase	P30520	50	P80210	48	P0A7D4	47	Q96529	48	Q57981	38
6	Ribonuclease	O60930	32	Q02555	54	P0A7Y0	25	P42815	23	Q57599	26
7	NAD kinase	Q4G0N4	43	P21373	59	P0A7B3	32	Q9C5W3	102	Q58327	64
8	Citrate synthase	O75390	49	P00890	49	P0ABH7	48	P20115	51	Q57926	45
9	Glucokinase	P35557	55	P17709	55	P0A6V8	35	---	---	---	---
10	Adenylosuccinate lyase	P30566	50	Q05911	54	P0AB89	51	---	---	Q58339	52
11	Catalase	P04040	60	P06115	64	P13029	80	P25819	56	---	---
12	Hexokinase	P19367	102	P04807	54	---	---	P93834	55	---	---
13	Succinate dehydrogenase	P21912	29	---	---	---	---	---	---	---	---
14	Fumarate hydratase	P07954	50	---	---	---	---	P93033	50	---	---
15	MAP kinase	P28482	50	---	---	---	---	Q39024	43	---	---
16	D-lactate dehydrogenase	---	---	P39976	55	P06149	64	Q94AX4	56	---	---
17	Cyclin-dependent kinase	---	---	P00546	34	---	---	P24100	34	---	---
18	Fructokinase-like 1, chloroplastic	---	---	---	---	P23917	32	Q9M394	49	Q58999	53
19	Enoyl reductase	---	---	P38071	41	P0AEK4	28	---	---	---	---
20	Phosphate dehydrogenase	---	---	---	---	P0A9M8	77	---	---	---	---
22	Glucose-6-phosphate isomerase	---	---	---	---	---	---	---	---	Q59000	47
23	Acetyl-co-A decarboxylase/synthase	---	---	---	---	---	---	---	---	Q57620	53
24	Fructose-1,6 biphosphate	---	---	---	---	---	---	---	---	Q57573	28
25	Lactaldehyde dehydrogenase	---	---	---	---	---	---	---	---	Q58806	51
26	Pyruvate kinase	---	---	---	---	---	---	---	---	Q57572	50

<sup>^</sup>*Saccharomyces cerevisiae* ; <sup>†</sup>*Methanococcus janaschii*



**Figure S1.** LC-ESI mass spectrum of a GluC peptide (residue No. [112-124], 13 amino acid residues long) obtained from carbamidomethylated RNase A (Bovine pancreas). (a) Isotope peaks distribution for the charge state,  $z = +1$  and (b) Isotope peaks distribution for the charge state,  $z = +2$ . These data were acquired on an ESI - Q/TOF mass spectrometer (6540 Ultra High Definition Accurate-Mass Q-TOF LC/MS attached to 1290 Infinity LC; Agilent Technologies)