

SUPPLEMENTAL INFORMATION:

Supplemental Table 1: A list of unique peptides with the percent sequence coverage using a MALDI 4700 TOF/TOF mass spectrometer. The peptides identified for heart and liver mitochondria correspond to Figure 1. Additionally, a list of references for each previously identified phosphoprotein is included.

Supplemental Table 2: A list of single-peptide based protein identifications with Mascot scores and E-values for each single-peptide.

Supplemental Figure 1: Single-peptide MS/MS labeled fragmentation patterns for the identifications in Supplemental Table 2. Panel A corresponds to the spectra for protein #23, Panel B to protein #41, Panel C to protein #46, Panel D to protein #52, Panel E to protein #59, Panel F to protein #62, and Panel G to protein #63, in Figure 1.

Supplemental Table 1

Supplemental Table 1. Sequence coverage of Phos-Tag stained protein from heart and liver mitochondria, corresponding to Figure 1.

Functional Category	Spot #	Protein Name	NCBI Accession Number	Unique MS/MS Peptides (%coverage) Heart	Unique MS/MS Peptides (%coverage) Liver
OXIDATIVE PHOSPHORYLATION					
Complex I	2	NADH dehydrogenase (ubiquinone) Fe-S protein 1 (75kDa) [11]	51858651	9 (15%)	
	13	NADH dehydrogenase (ubiquinone) Fe-S protein 2 (49kDa) [19]	116242673	9 (23%)	
	17	NADH dehydrogenase (ubiquinone) 1 α subcomplex 10 (42kDa) [16,17,22]	464254	4 (10%)	
	32	NADH dehydrogenase (ubiquinone) Fe-S protein 3 (30kDa)	6166589	10 (21%)	4 (18%)
	37	NADH dehydrogenase (ubiquinone) flavoprotein 2, mitochondrial precursor	128865	5 (22%)	
	35	NADH dehydrogenase (ubiquinone) Fe-S protein 8 (23kDa) [3]	62267022	6 (27%)	
	44	NADH dehydrogenase (ubiquinone) iron-sulfur protein 5 (15kDa)	3914138	2 (17%)	
	5	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial precursor [21]	75070503	7 (16%)	7 (16%)
	61	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial precursor	20455488		4 (16%)
	10	Ubiquinol-cytochrome c reductase complex core protein I, mitochondrial precursor [19,24]	10720406	4 (15%)	2 (6%)
Complex III	33	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur protein precursor [19]	52001457	5 (18%)	
	45	Ubiquinol-cytochrome c reductase complex, 14 kDa protein	136717	4 (44%)	
	41	Cytochrome b-c1 Complex, subunit 6 [15]	109940045	1 (7%)	
Complex IV	42	Cytochrome c oxidase polypeptide Va, mitochondrial precursor [9]	117099	2 (17%)	2 (17%)
	43	Cytochrome c oxidase polypeptide Vb, mitochondrial precursor [6]	75042739	5 (32%)	
Complex V (FoF1-ATPase)	8	ATP synthase, mitochondrial F1 complex, α subunit [18,24]	15030240	18 (41%)	13 (32%)
	9	ATP synthase, mitochondrial F1 complex, β subunit [12,18]	32189394	15 (35%)	15 (37%)
	38	ATP synthase d-chain, mitochondrial precursor	114686	2 (19%)	2 (19%)
	31	ATP synthase γ -chain, mitochondrial precursor [4,18,24]	543874	2 (14%)	
40	ATP synthase, oligomycin sensitivity-conferring protein [19]	143811365	3 (9%)		
INTERMEDIARY METABOLISM					
Krebs Cycle	1	Aconitase hydratase, mitochondrial precursor [7,18,21]	113159	18 (32%)	
	21	Citrate synthase, mitochondrial precursor [19]	116470	6 (14%)	
	12	**Pyruvate dehydrogenase complex, E1 α subunit [7,18,19,24]	448580	8 (24%)	5 (10%)
	26	Pyruvate dehydrogenase complex, E1 β subunit	116242689	5 (18%)	
	4	Pyruvate dehydrogenase complex, E2 subunit	3915777	3 (7%)	
	46	Phosphoenolpyruvate carboxykinase (GTP), mitochondrial precursor [24]	52783203		1 (1%)
	23	Isocitrate dehydrogenase (NADP-dependent) [8,19]	462384	13 (32%)	1 (1%)
	15	Isocitrate dehydrogenase (NAD) subunit alpha, mitochondrial precursor	68565369	9 (29%)	
	27	Malate dehydrogenase, mitochondrial precursor [7,18,20]	2506849	14 (52%)	11 (44%)
	16	Branched-chain alpha-keto acid dehydrogenase E1 component α chain [12,18]	129030	5 (16%)	
	25	Succinyl-CoA ligase (GDP-forming) alpha-chain, mitochondrial precursor [18]	8134728	5 (24%)	
	53	Succinyl-CoA ligase (GDP-forming) beta-chain, mitochondrial precursor	21264506		7 (17%)
	14	Succinyl-CoA ligase (ADP-forming) beta-chain, mitochondrial precursor [7,24]	21263966	9 (23%)	
	11	Dihydropyridine succinyltransferase, 2-oxoglutarate dehydrogenase complex	18203301	10 (22%)	
	Cysteine Metabolism	24	Aspartate aminotransferase, mitochondrial precursor [8,19]	112985	9 (31%)
57		Thiosulfate sulfurtransferase, mitochondrial precursor [3]	1174694		6 (19%)
Fatty Acid Oxidation	52	Trifunctional enzyme subunit beta, mitochondrial precursor [23]	6015048		1 (1%)
	67	Trifunctional enzyme subunit alpha, mitochondrial precursor	7387634		5 (10%)
	54	Short-branched chain specific acyl-CoA dehydrogenase (SBCAD) [19]	75060971		2 (4%)
	20	Long-chain specific acyl-CoA dehydrogenase (LCAD) [24]	2829676	11 (32%)	12 (34%)
	55	Medium-chain specific acyl-CoA dehydrogenase (MCAD)	148872486		6 (20%)
	19	Short-chain specific acyl-CoA dehydrogenase (SCAD)	13878316	6 (16%)	3 (10%)
	60	Short chain enoyl-CoA hydratase	119119		1 (4%)
	59	2,4-dienoyl-CoA reductase, mitochondrial precursor [19]	3913456		1 (2%)
	62	3-hydroxyacyl-CoA dehydrogenase type-2	3183024		2 (11%)
	58	D-beta-hydroxybutyrate dehydrogenase, mitochondrial precursor	25108876		2 (5%)
Urea Cycle	56	Ornithine carbamoyltransferase, mitochondrial precursor	3183093		5 (14%)
	65	Carbamoyl-phosphate synthase I, mitochondrial precursor [24]	117492		
Amino Acid Metabolism	34	Electron transfer flavoprotein, β subunit [24]	75053043	8 (38%)	21 (18%)
	49	Glutamate dehydrogenase 1, mitochondrial precursor [24]	118541		13 (29%)
	68	3-hydroxyisobutyrate dehydrogenase, mitochondrial precursor	122135732		3 (13%)
ANTIOXIDANT					
	39	Mn superoxide dismutase [18,24]	134677	4 (21%)	4 (21%)
	36	Thioredoxin-dependent peroxide reductase, mitochondrial precursor	2507170	4 (17%)	3 (14%)
TRANSPORT					
	30	Voltage-dependent anion channel 1 [1,5,8,12,13,14,18,19,25,26]	10720225	8 (37%)	
	29	Voltage-dependent anion channel 2 [5,18]	75050405	6 (26%)	
	63	Mitochondrial import inner membrane translocase, subunit Tim8A [24]	90101777		1 (11%)
OTHER	6	60 kDa heat shock protein, mitochondrial precursor [2,3,8,18,19,20,24]	51702252	12 (23%)	13(30%)
	3	70kDa heat shock protein, mitochondrial precursor [10,18,19]	14917005	13 (23%)	12 (25%)
	64	10 kDa heat shock protein, mitochondrial [8,19]	47606335		2 (18%)
	22	Creatine kinase, sarcomeric mitochondrial precursor	68052065	10 (28%)	
	51	Glycine amidinotransferase [24]	1730202		4 (8%)
	28	Prohibitin [19]	464371	11 (49%)	10 (51%)
	47	Aldehyde dehydrogenase class 2, mitochondrial precursor [19]	118502		9 (37%)
	48	Aldehyde dehydrogenase family 7, member A1 [18]	109940193		9 (25%)
	50	Serine hydroxymethyltransferase, mitochondrial precursor	6226865		4 (9%)
	18	Elongation factor Tu, mitochondrial precursor	1352352	10 (26%)	
7	Dihydropolyl dehydrogenase, mitochondrial precursor	118675	10 (24%)		
66	Propionyl-CoA carboxylase alpha chain, mitochondrial precursor [24]	6174892		6 (11%)	

Supplemental Table 1 Reference List

1. Beausoleil, S. A.; Jedrychowski, M.; Schwartz, D.; Elias, J. E.; Villen, J.; Li, J.; Cohn, M. A.; Cantley, L. C.; Gygi, S. P. Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl. Acad. Sci. U. S. A* **2004**, *101* (33), 12130-12135.
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Supplemental Table 2:

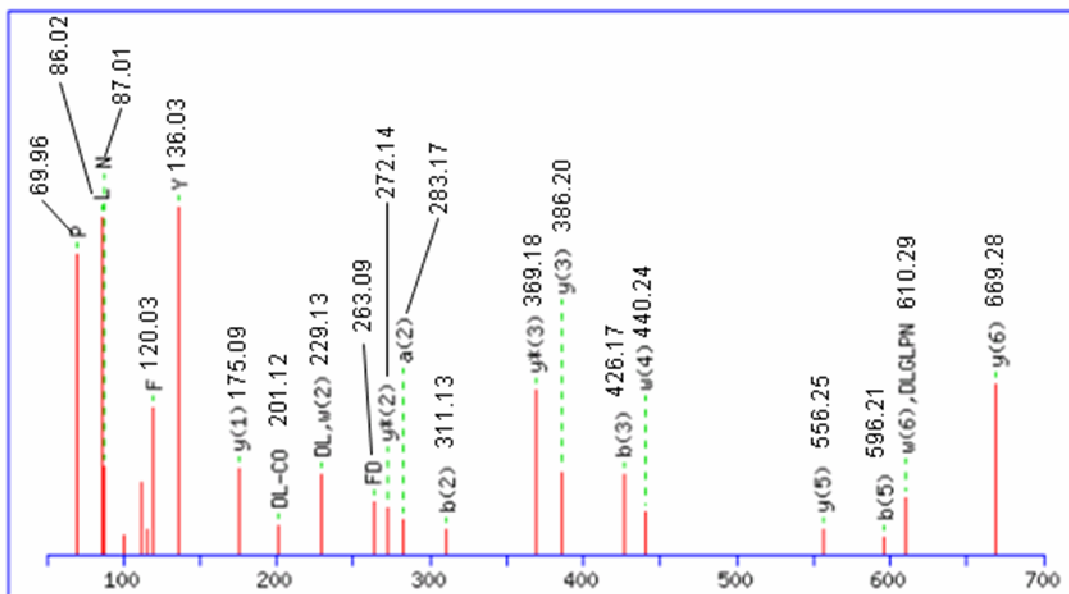
Supplemental Table 2. Single-peptide-based protein identifications using 4700 MALDI TOF/TOF mass spectrometer.

Spot #	Protein Name	NCBI Accession Number	Peptide sequence	Precursor m/z (+1)	Mascot score	Mascot E-value
40	Cytochrome b-c1 complex subunit 6	109940045	LELCDKR	933.44	25	0.49
45	Phosphoenolpyruvate carboxykinase (GTP), mitochondrial precursor	52783203	EVLAELEALEGR	1328.71	46	0.0025
22	Isocitrate dehydrogenase (NADP-dependent)	462384	YFDLGLPNR	1094.56	48	0.0017
52	Trifunctional enzyme subunit beta, mitochondrial precursor	6015048	NIVVVDGVR	970.56	37	0.024
61	Short chain enoyl-CoA hydratase	119119	NSSVGLIQLNRPK	1425.81	48	0.0016
60	2,4-dienoyl-CoA reductase, mitochondrial precursor	3913456	FNVIQPGPIK	1112.64	47	0.0021
64	Mitochondrial import inner membrane translocase, subunit Tim8A	90101777	FIDTSQFILNR	1353.69	28	0.16

Supplemental Figure 1:

MS/MS Fragmentation of **YFDLGLPNR**
Isocitrate dehydrogenase [NADP], mitochondrial precursor (EC 1.1.1.42)

A



Monoisotopic mass of neutral peptide Mr(calc): 1093.5556

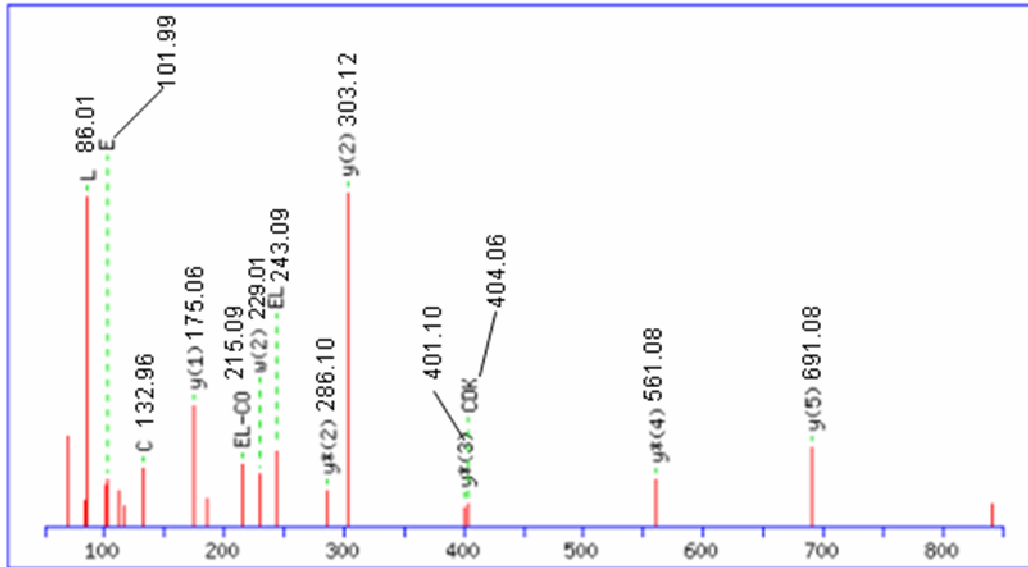
Fixed modifications: Carbamidomethyl (C)

Ions Score: 48 **Expect:** 0.0017

Matches (Bold Red): 24/110 fragment ions using 20 most intense peaks

MS/MS Fragmentation of **LELCDKR**
Cytochrome b-c1 complex subunit 6, mitochondrial precursor

D



Monoisotopic mass of neutral peptide Mr(calc): 932.4749

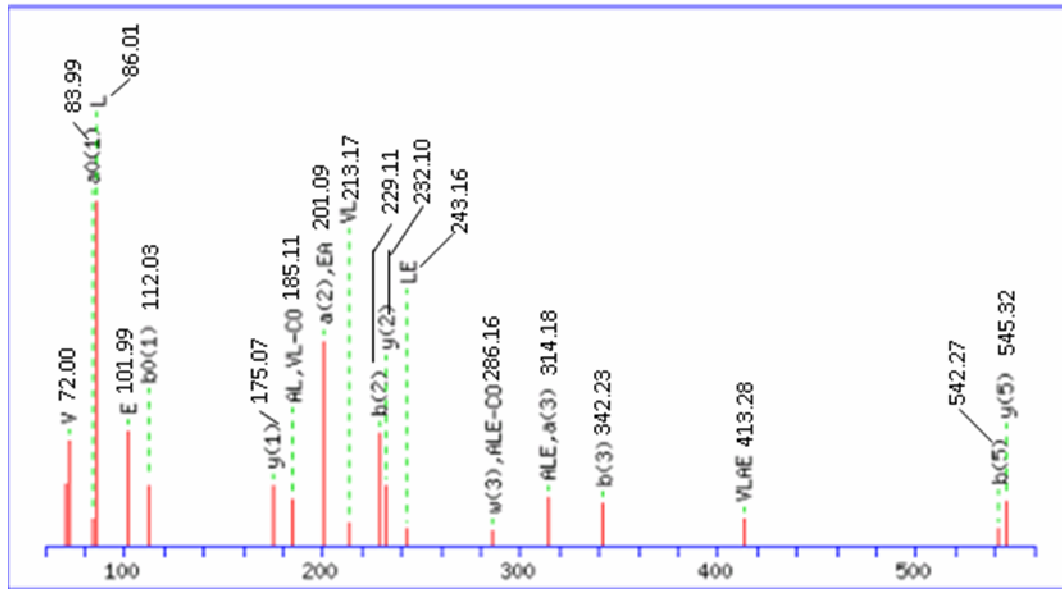
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Ions Score: 25 **Expect:** 0.49

Matches (Bold Red): 17/79 fragment ions using 15 most intense peaks

MS/MS Fragmentation of **EVLAELEALEGR**
 Phosphoenolpyruvate carboxykinase [GIP], mitochondrial precursor

C



Monoisotopic mass of neutral peptide Mr(calc): 1327.6983

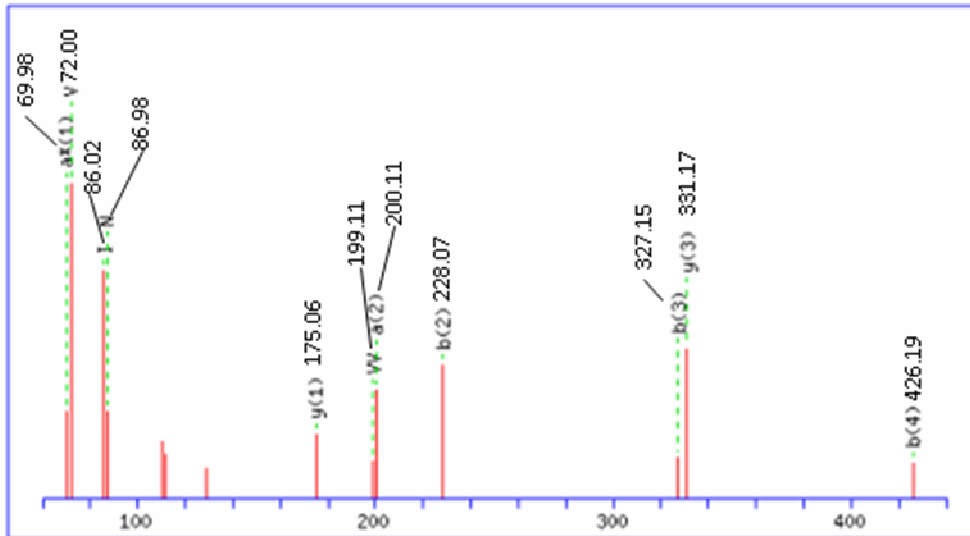
Fixed modifications: Carbamidomethyl (C)

Ions Score: 46 **Expect:** 0.0025

Matches (Bold Red): 41/176 fragment ions using 19 most intense peaks

MS/MS Fragmentation of **NIVVVDGVR**
Trifunctional enzyme subunit beta, mitochondrial precursor (TP-beta)

D



Monoisotopic mass of neutral peptide Mr(calc): 969.5607

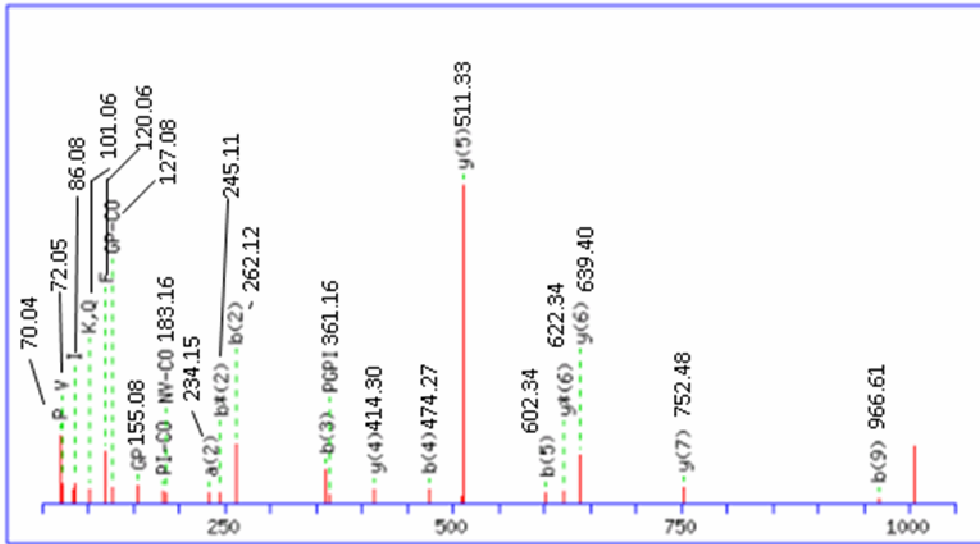
Fixed modifications: Carbamidomethyl (C)

Ions Score: 37 **Expect:** 0.024

Matches (Bold Red): 16/125 fragment ions using 11 most intense peaks

MS/MS Fragmentation of **FNVIOPGPIK**
 dienoyl-CoA reductase [NADPH] (4-enoyl-CoA reductase [NADPH]).

7



Monoisotopic mass of neutral peptide Mr(calc): 1111.6390

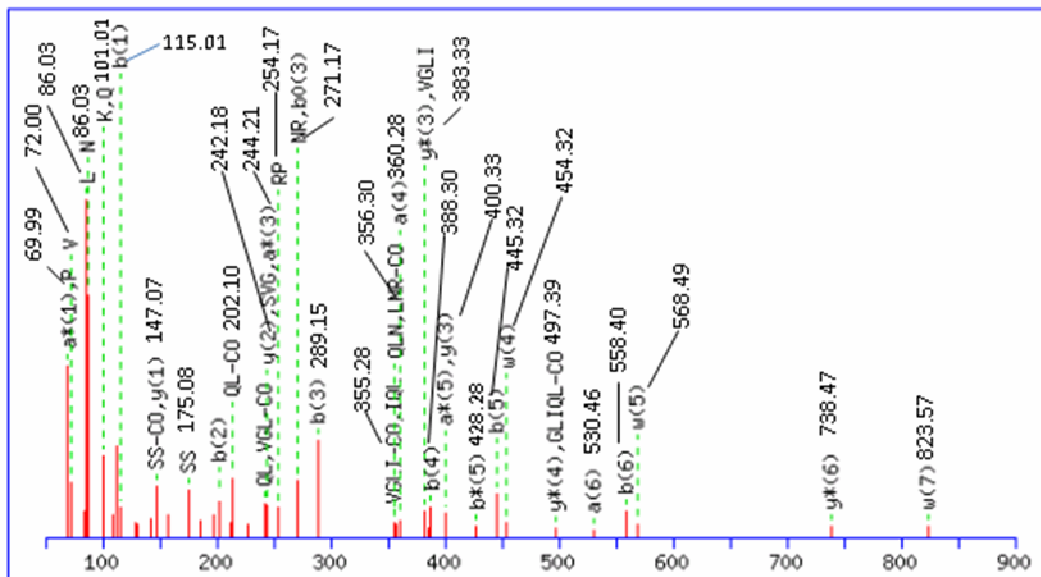
Fixed modifications: Carbamidomethyl (C)

Ions Score: 47 **Expect:** 0.0021

Matches (Bold Red): 28/114 fragment ions using 24 most intense peaks

MS/MS Fragmentation of **NSSVGLIQLNRPK**
 Enoyl-CoA hydratase, mitochondrial precursor (EC 4.2.1.17) (Short

T



Monoisotopic mass of neutral peptide Mr(calc): 1424.8099

Fixed modifications: Carbamidomethyl (C)

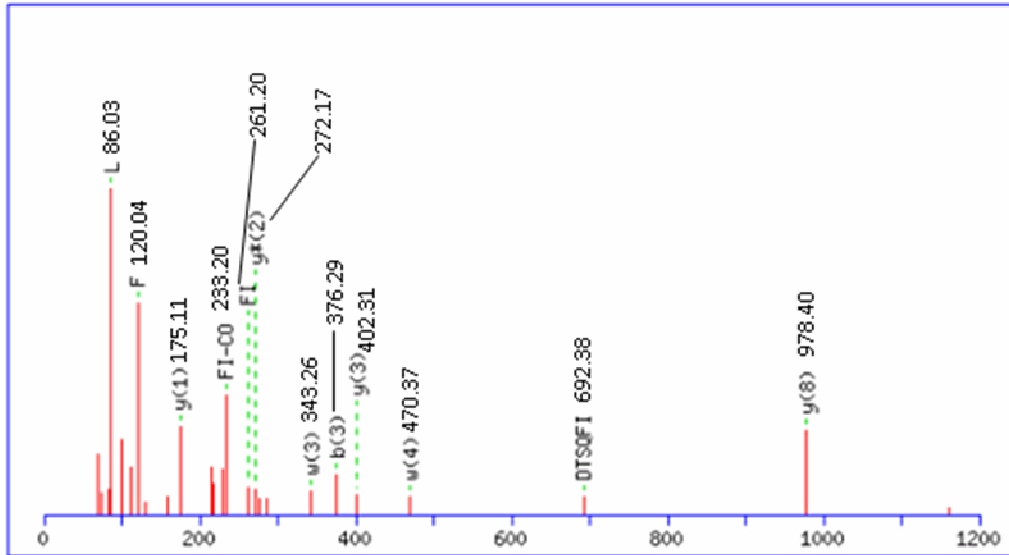
Ions Score: 48 **Expect:** 0.0016

Matches (Bold Red): 49/210 fragment ions using 37 most intense peaks

MS/MS Fragmentation of **FIDTSQFILNR**

Putative mitochondrial import inner membrane translocase subunit Tim8

G



Monoisotopic mass of neutral peptide Mr(calc): 1352.7088

Fixed modifications: Carbamidomethyl (C)

Ions Score: 28 **Expect:** 0.16

Matches (Bold Red): 20/162 fragment ions using 13 most intense peaks