

## Two-Dimensional Reference Map of *Schizosaccharomyces pombe* Proteins (Update)

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**Abstract** Based on the first 2D reference map of the fission yeast *Schizosaccharomyces pombe* protein reported previously, we expanded and updated the map using narrower pI ranges. In this paper, 240 protein spots were identified on our reference map. In the pI 4–7 range, 144 spots corresponding to 86 different proteins were identified. In the pI 6–9 range, 43 spots corresponding to 35 different proteins were identified. Fifty-three new spots corresponding to 39 different proteins were further identified in the pI 5–6 range.

**Key words:** Proteome, matrix-assisted laser desorption/ionization, peptide fingerprinting, two-dimensional gel electrophoresis, *Schizosaccharomyces pombe*

*Schizosaccharomyces pombe* has been used as an excellent model for the studies of cell cycle control, meiosis and mitosis, mating type switching, and chromosome segregation [2, 5, 7, 9, 16, 19, 23, 26, 32, 34]. *S. pombe* is different from *Saccharomyces cerevisiae* that contains about 6,000 genes [12]. The 13.8-Mb genome of *S. pombe* has the smallest number at 4,824 of open reading frames (ORFs) among eukaryotes [35]. *S. pombe* also has functional genes regarding post-transcriptional gene silencing system, signalosome, and spliceosome [1, 20], differently from *S. cerevisiae*.

Two-dimensional gel electrophoresis (2D-GE) is one of the methodologies that can be used to determine the function of a new gene [18]. The complete sequencing of the yeast genome made it possible to identify proteins by the method based on mass spectrometry [30, 31]. Therefore, 2D-GE and mass spectrometry provide the opportunity of

obtaining a wide variety of information on a gene product, such as its regulation, its cellular location, or post-translational modifications.

The 2D-GE map of *S. cerevisiae*, considered as an important model system in the study of biological function along with *S. pombe*, was initially reported by Garrels *et al.* [10], identifying 90 protein spots in 1994, and updated continuously [4, 15, 27, 29]. Several other yeast protein maps are available [11, 24, 25], and some of them are available on Web sites [37–40].

The objective of this study was to update the proteome map of *S. pombe*. We report here the identification of 91 novel spots on the 2D protein map. Ninety-one novel spots included the identification of 53 new spots corresponding to 39 different proteins in the pI 5–6 range.

## MATERIALS AND METHODS

### Strain and Culture Condition

The *S. pombe* strain used in this study was a nonsporulating fission yeast haploid ED665 (*h-*, *ade 6-M210/leu 1-32/ura 4-D18*). The yeast cell was grown in yeast extract-peptone-dextrose (YEPD) (peptone, 0.5%; yeast extract, 0.5%; glucose, 3%) medium (Difco Laboratories, Detroit, MI, U.S.A.) at 32°C.

### Cell Lysis

Yeast cells were grown to 0.8 of OD<sub>590</sub> in YEPD broth and harvested by centrifugation. The supernatant was discarded and collected cells were washed three times with ice-cold water in 30 sec. The pellet was suspended in phosphate-buffered saline (PBS) buffer (sodium chloride, 137 mM; potassium chloride, 2.7 mM; sodium phosphate dibasic,

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4.3 mM; potassium phosphate monobasic, 1.8 mM) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 2% NP40. The cells were then disrupted for 30 s at 46,000 rpm (5 cycles with 1 min cooling in ice between each cycle) in a mini-bead beater (Biospec Products, Barlesville, OK, U.S.A.) using 0.5-mm diameter glass beads and centrifuged at 13,200  $\times g$  for 20 min. The supernatant was pooled and precipitated by trichloroacetic acid (TCA)/acetone solution [8, 13]. After centrifugation, the supernatant was discarded and the pellet was washed more than three times with cold acetone containing 0.007%  $\beta$ -mercaptoethanol. Finally, the pellet was lyophilized using a vacuum dryer and stored at  $-20^{\circ}\text{C}$ .

### Two-Dimensional Electrophoresis

The equipment for the first-dimension isoelectric focusing (IEF), Ettan IPGphor system, and nonlinear immobilized IPG strips (18 cm; pH range 3–10, pH 4–7, and pH 6–9, respectively) were purchased from Amersham Biosciences (Uppsala, Sweden). The first-dimension IEF was performed according to the manual of Amersham Biosciences [3]. Samples were suspended in sample buffer (8 M urea, 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonia]-1-propanesulfonate (CHAPS), 2% (v/v) IPG buffer, 0.002% (w/v) bromophenol blue) and centrifuged. After centrifugation, the supernatant was placed to a strip holder without trapping air bubbles and covered with drystrip cover fluid (Amersham Biosciences, Uppsala, Sweden). Rehydration was performed for 12 h at room temperature. The first dimension separation was carried out at  $20^{\circ}\text{C}$  using the following programs: 500 V for 1 h, 1,000 V for 1 h, 4,000 V for 4 h, and 8,000 V for 4 h. After the first IEF, IPG strips were equilibrated for 15 min in sodium dodecyl sulfate (SDS) equilibrium buffer (Tris, 50 mM; urea, 8 M; glycerol, 30%; SDS, 2%; bromophenol blue, 0.002%) containing 65 mM of dithiothreitol (DTT), and re-equilibrated in SDS equilibrium buffer containing 135 mM of idoacetamide instead of DTT. The gels were then prepared according to the method of Laemmli [21] without a stacking gel. The equilibrated strips were then loaded on the top of a vertical SDS-PAGE gel (12.5%) for the second-dimension separation (20 cm  $\times$  20 cm  $\times$  1 mm, PROTEAN II 2D Cell System, Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.). The strips on the gel were fixed with 0.5% low melting agarose dissolved in running buffer. Electrophoresis was performed at 1 W per gel for the initial 30 min and then 250 V was applied. The gels were then visualized with a silver staining kit (No. 17-1150-01; Amersham Biosciences, Uppsala, Sweden) based on the acidic/silver nitrate method. Silver staining followed the procedure recommended by the supplier except for glutardialdehyde treatment. Gels were scanned with a Image Scanner (Amersham Biosciences, Uppsala, Sweden) and gel images were analyzed using PHORETIX 2D (Non-Linear Dynamics, Durham, NC, U.S.A.).

### In-Gel Digestion

Protein spots on the gel were excised, and gel pieces were destained according to the method of Guerreiro *et al.* [14]. As briefly described, 30  $\mu\text{l}$  to 50  $\mu\text{l}$  of working solution having 30 mM of potassium ferricyanide and 100 mM sodium thiosulfate (1:1, v/v) were added to the gel pieces, and occasionally vortexed until the brownish color of the gel pieces disappeared or changed to yellow. Gel pieces were then washed a few times with distilled water and 100  $\mu\text{l}$  of 200 mM ammonium bicarbonate was added and incubated for 20 min. After centrifugation, the supernatant was discarded and the gel pieces were dehydrated repeatedly with acetonitrile until the color of the gel pieces turned into opaque white. After destaining, the gel pieces were dried in a vacuum to remove solvent and 30–50  $\mu\text{l}$  of 50 mM ammonium bicarbonate was added for swelling. Then, samples were digested with 3–5  $\mu\text{l}$  of trypsin (0.1  $\mu\text{l}/\mu\text{l}$ , Promega Co., Madison, WI, U.S.A.) in digestion buffer (50 mM Tris with 1 mM  $\text{CaCl}_2$ ) at  $37^{\circ}\text{C}$  for 12 h. After in-gel digestion, 100  $\mu\text{l}$  of 50 mM ammonium bicarbonate was added and incubated for 1 h. After centrifugation, the supernatant was pooled and the above step was repeated. The pooled peptide solution was lyophilized and redissolved in 0.1% trifluoroacetic acid (TFA) and eluted by the  $\text{C}_{18}$  Zip tip (Millipore Co., Bedford, MA, U.S.A.) with 50% ACN in 0.1% TFA.

### Mass Spectrometry and Database Search

Tryptic peptides were applied to the sample target and air-dried. Saturated solution of  $\alpha$ -cyano-4-hydroxycinnamic acid was prepared separately in acetone and diluted by 5:1 and spread on the sample plate. A sample mixture of the  $\alpha$ -cyano-4-hydroxycinnamic acid in TA (50% ACN in 0.1% TFA) was added on the plate as a double layer. Mass spectra were calibrated externally using a known peptide mixture: Bradykinin 1-7 ( $[\text{M}+\text{H}]^+$ , monoisotopic mass; 757.399), Angiotensin I (1,046.541), Angiotensin I (1,296.684), Substance P (1,347.735), Bombesin (1,619.822), Renin Substrate (1,758.93), ACTH clip 1-17 (2,093.086), ACTH clip 18-39 (2,465.198), and Somatostatin 28 (3,147.471). Peptides from the auto-digestion of trypsin were used for the internal calibration. The dried sample plate was then analyzed using an Ultraflex-TOF/TOF (Bruker Daltonics, Germany). The spectrometer was run in a positive ion reflection delayed extraction mode with an accelerating voltage of 25 kV and a delay time of 60 ns. For MALDI-TOF/TOF, LIFT mode with accelerating voltage of 8 kV was used and argon was used as the collision gas. Identification of a protein from the MALDI-TOF peptide mass fingerprint was performed using the Mascot search engine at <http://www.matrixscience.com> and the National Center for Biotechnology Information (NCBI) database of *S. pombe*. Monoisotopic peptide masses and 50 ppm of mass tolerance were used. If the matched proteins were not found, mass tolerance was extended to 100 ppm. Oxidation of methionine

and carbamidomethylation modification of cysteine were considered.

## RESULTS AND DISCUSSION

### 2D Gel Profile

Most protein spots were located in the pI range of 4.0–7.5 and the molecular weight of 10–100 kDa. Using the immobililine IPG strip with pH range 4–7 and 6–9, about 520 protein spots (Fig. 1) and 330 protein spots (Fig. 2) were resolved, respectively. To further analyze the protein spots reported previously [33], the pH gradient was narrowed down to pI 5–6. We selected pH range 5–6 since more spots could

be detected in pH 5–6 than pH 4–7. The advantage of an overlapping narrow pH range is the gain in higher resolution by stretching the protein pattern in the first dimension [36]. The 2D-GE profile using IPG strip pI 5–6 showed distinctive 360 or so protein spots (Fig. 3). About seventy percent of the proteins produced in the pI 4–7 range was resolved in pI 5–6. A similar result was observed for 2D analysis of *Staphylococcus aureus*, where the use of narrow-range IPG strips increased the number of resolved protein spots by 45% [6].

### Protein Identification

Silver-stained protein spots on 2D-GE were sequentially numbered, excised, and destained. We identified 240 protein

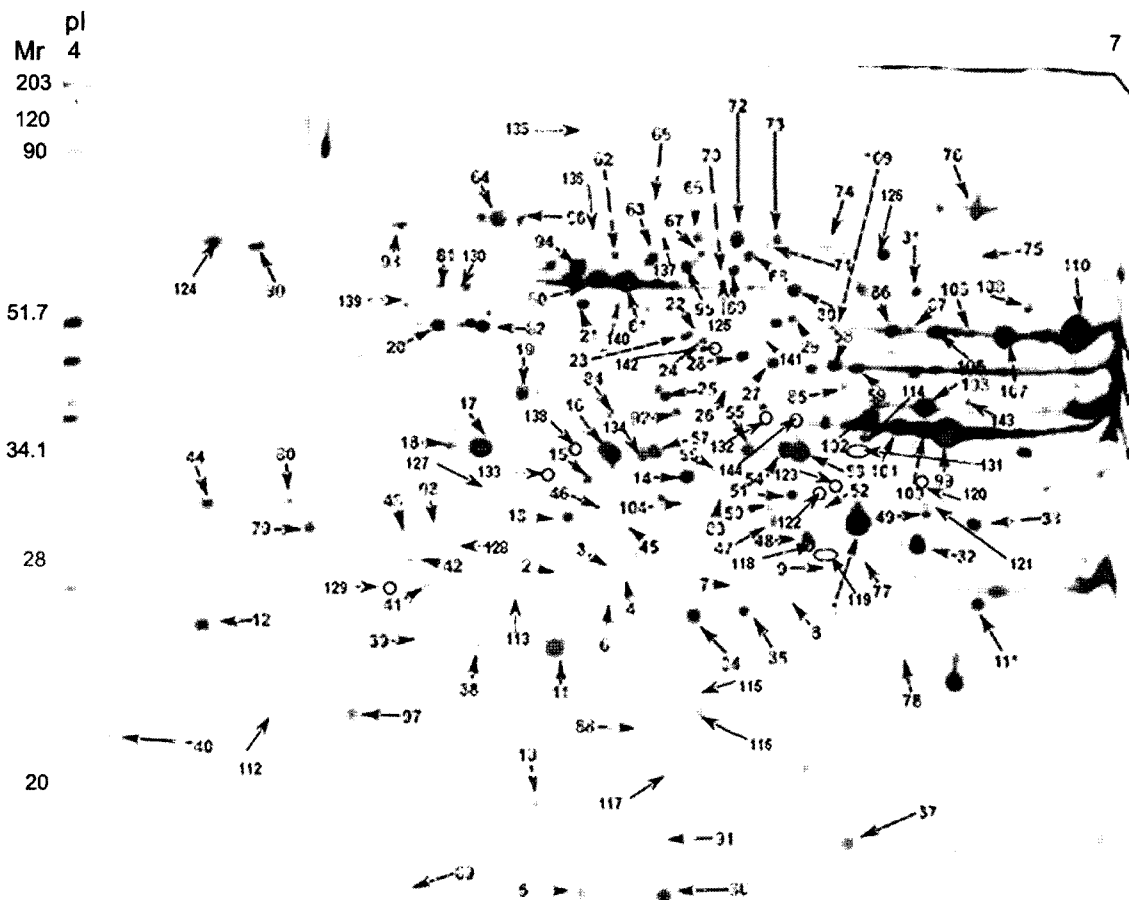
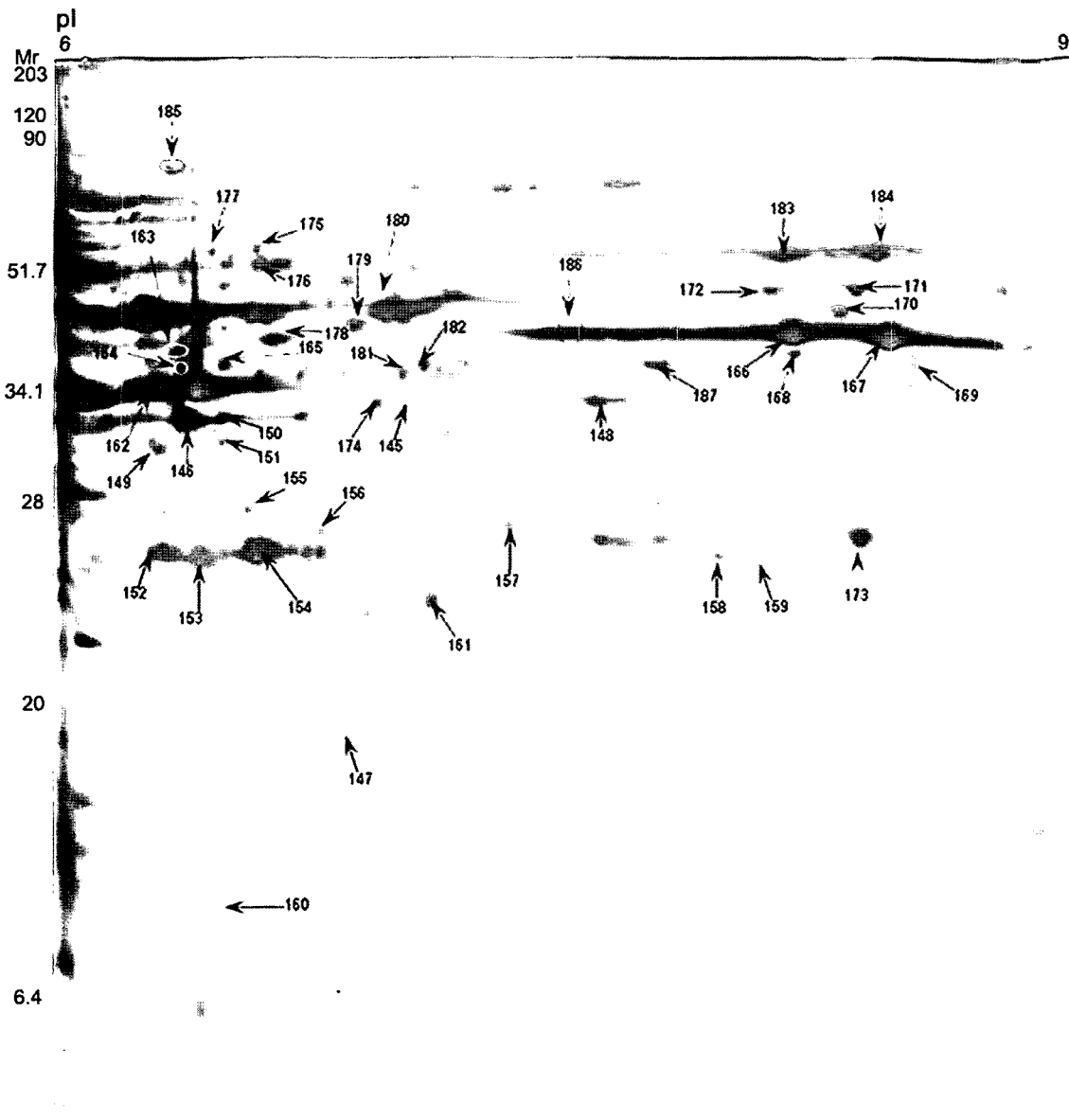


Fig. 1. A proteome map of *S. pombe* ED665 using IPG strip pH 4–7.



**Fig. 2.** A proteome map of *S. pombe* ED665 using IPG strip pH 6–9.

spots, among which 149 spots had been previously reported [33]. Two-hundred-forty protein spots corresponded to 160 different proteins. Protein spots that could not be characterized were either numerous peptides generated by trypsin or insufficient concentration for analysis, and the majority of these proteins were of low abundance and low  $M_r$  [22]. In general, 2DE-GE has a limit in separating very high and low molecular proteins and/or hydrophobic proteins [17]. The identified spots are shown in Figs. 1–3. Two-hundred-forty identified spots are also classified (supplementary material).

In the range of pI 4–7, 86 proteins corresponding to 144 spots were identified. In the range of pI 6–9, 35 different proteins corresponding to 43 spots were identified. Fifty

three spots corresponding to 39 proteins on IPG strip pH 5–6 were also identified. Thirty-seven proteins were expressed by more than one spot. Some proteins such as Glucose-6-phosphate 1-dehydrogenase (Genome ID: 19075349), Pyruvate decarboxylase (Genome ID: 19113708), Adenosyl homocysteinase (Genome ID: 2950505), Heat, shock protein 70 family (Genome ID: 19075921), Transaldolase (Genome ID: 19075453), Pyrimidine precursor biosynthesis enzyme thi3 (Genome ID: 19075847), and Phosphoglycerate kinase (Genome ID: 19113522) were identified in range pI 5–6.

It has been reported that many yeast proteins are resolved into multiple spots on 2D gels [10, 17]. Regarding multiple spots, there are two categories: multiple spots

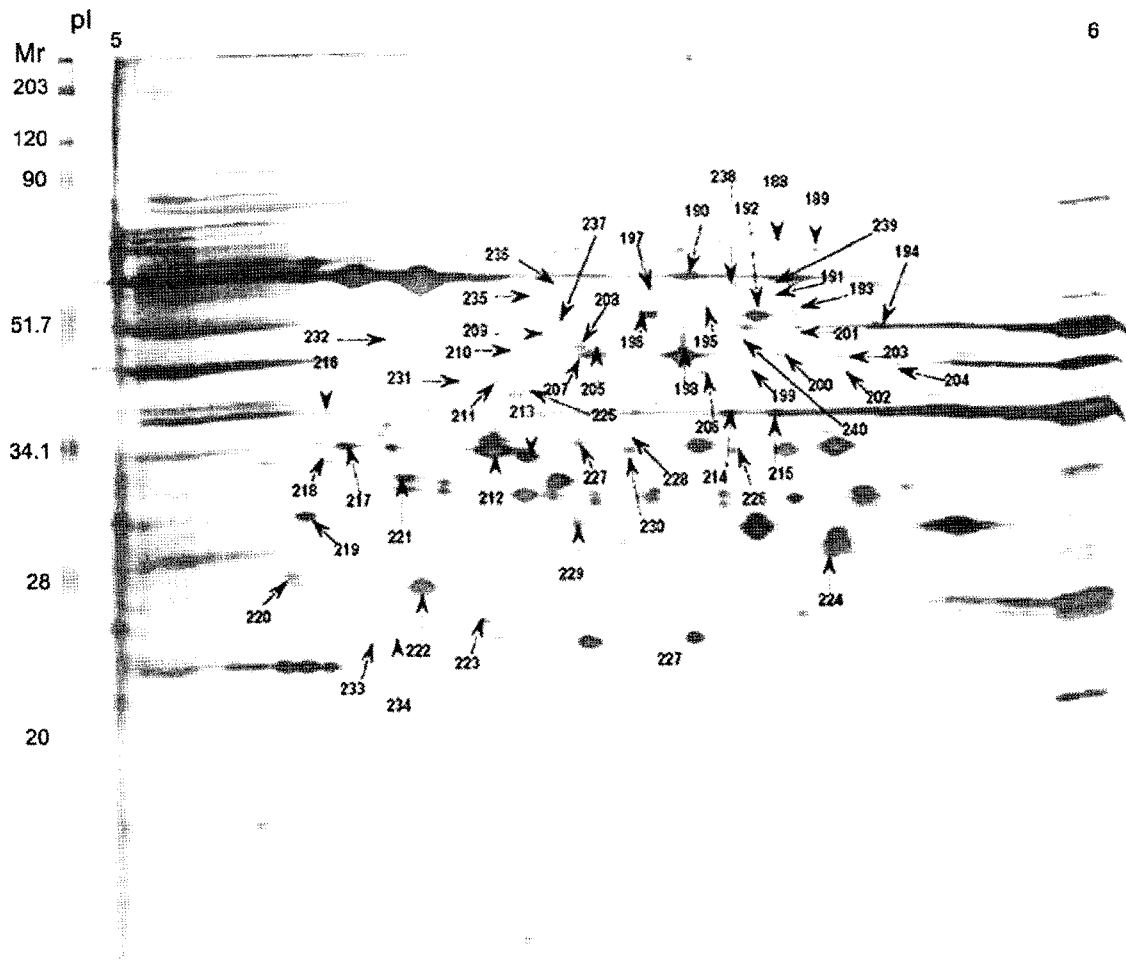


Fig. 3. A proteome map of *S. pombe* ED665 using IPG strip pH 5–6.

having the same molecular weights and different pI values, and multiple spots having different molecular weights and different pI values. These spots were considered to be due to post-translational modifications. Heat-shock proteins (Genome ID: 19112230) were the most abundant proteins, representing 13 spots with different apparent molecular weights and different pI values. Enolase (Genome ID: 19112695) and heat-shock protein 70 family (Genome ID: 19075921) were also abundant, found in 8 spots.

#### Functions of the Identified Proteins

The identified proteins were classified according to their biological functions using the NCBI database ([http://](http://www.ncbi.nlm.nih.gov)

[www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). More than 56% of the identified spots are involved in transport and metabolism of amino acid, carbohydrate, coenzyme, inorganic ion, nucleotide, and lipid as well as energy production and conversion (Table 1). In particular, 41 proteins corresponding to 77 spots are involved in amino acid and carbohydrate transport and metabolism. The identified 22 proteins corresponding to 49 spots are also involved in cellular processing and signaling such as post-translational modification, protein turnover, intracellular trafficking, and chaperones, whereas 21 proteins are of unknown function and 10 proteins corresponding to 13 spots have been poorly characterized. According to the protein map of *S. cerevisiae* [28], most of the identified proteins were categorized into three m:

**Table 1.** Cellular function categories.

Categories	Number of spots with identified proteins	Number of independent proteins
Amino acid transport and metabolism	25	22
Carbohydrate transport and metabolism	52	19
Coenzyme transport and metabolism	22	7
Inorganic ion transport and metabolism	7	4
Nucleotide transport and metabolism	5	5
Lipid transport and metabolism	1	1
Energy production and conversion	22	14
Secondary metabolite biosynthesis, transport, and metabolism	2	2
Translation	12	9
Transcription	5	3
Cellular processing and signaling (post-translational modification, protein turnover, and chaperones)	49	22
General function prediction	17	11
Unknown	21	11

classes: carbon metabolism (20%), biosynthesis of small molecules such as amino acids and nucleotides (25%), and protein metabolism (30%).

In summary, we report here the identification of 53 new spots corresponding to 39 different proteins in the pI 5–6 range.

**Table 2.** Identification of *S. pombe* ed665 proteins by MALDI-TOF MS analysis

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
1	Thiazole biosynthetic enzyme	35.368/5.91	36	19113434	Coenzyme transport and metabolism
2	Phosphomannomutase	23.319/5.26	65	19115237	Carbohydrate transport and metabolism
3	Hypothetical protein	30.428/4.94	33	7490740	Unknown
4	Hypothetical protein	28.163/5.53	50	19112112	Unknown
5	Cofilin	15.724/5.6	42	19115653	Unknown
6	20s Proteasome component (beta7)	29.259/5.04	50	19112100	Cellular processes and signaling
7	Vip1 protein	27.500/5.54	33	19114169	Unknown
8	Probable proteasome subunit alpha type 4	28.136/5.88	53	1172601	Cellular processes and signaling
9	Hypothetical protein	24.986/5.77	73	19113728	Unknown
10	Peroxisomal membrane protein, pmp 20	16.722/5.18	81	19075206	Cellular processes and signaling
11	Thioredoxin peroxidase	21.292/5.37	56	19075930	Cellular processes and signaling
12	Translationally controlled tumor protein homolog	19.093/4.36	44	19115240	Unknown
13	Putative Lon protease homolog, mitochondrial precursor	118.97/6.76	53	19113947	Cellular processes and signaling
14	4-Nitrophenylphosphatase	33.058/5.58	36	19113047	Carbohydrate transport and metabolism
15	Thioredoxin reductase	34.768/5.19	59	2500128	Nucleotide transport and metabolism
16	Conserved hypothetical protein	34.065/5.51	41	19115384	General function prediction only
17	Inorganic pyrophosphatase	32.675/5.20	45	19114548	Energy production and conversion
18	Inorganic pyrophosphatase	32.675/5.20	43	19114548	Energy production and conversion
19	Putative adenosine kinase	37.142/5.26	53	19075654	Carbohydrate transport and metabolism
20	ATP Synthase beta chain, mitochondrial precursor	56.898/5.72	52	19114063	Energy production and conversion
21	Hypothetical protein	52.833/5.27	42	19111869	Amino acid transport and metabolism

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
22	Putative AICAR transformylase/IMP cyclohydrolase	64.596/6.25	37	19075527	Nucleotide transport and metabolism
23	Heat-shock protein	67.449/5.82	18	19112230	Cellular processes and signaling
24	Elongation initiation factor subunit	62.826/6.59	32	19115537	Transcription
25	Halotolerance protein homolog	39.008/5.48	43	19075730	Inorganic ion transport and metabolism
26	Heat-shock protein 70 family	70.472/5.13	30	19075921	Cellular processes and signaling
27	S-Adenosylmethionine synthetase	42.091/5.70	57	19113523	Coenzyme transport and metabolism
28	Putative adenosylhomocysteinase	47.866/5.61	39	19112372	Coenzyme transport and metabolism
29	Pyruvate decarboxylase	65.316/5.71	19	19113708	Carbohydrate transport and metabolism
30	Malate oxidoreductase [nad]	63.066/5.68	47	19075260	Energy production and conversion
31	Probable thiamine biosynthetic bifunctional enzyme	56.101/5.99	37	19114308	Coenzyme transport and metabolism
32	Putative stress-induced protein	31.611/5.92	29	19115894	Coenzyme transport and metabolism
33	Malate oxidoreductase [nad]	63.066/5.68	47	19075260	Energy production and conversion
34	Hypothetical protein	17.760/9.41	54	19113225	Translation
35	Putative ribulose phosphate 3-epimerase	25.417/5.53	58	19114917	Carbohydrate transport and metabolism
36	Hypothetical protein	17.760/9.41	67	19113225	Translation
37	Cu, Zn-Superoxide dismutase	16.017/5.80	77	19114075	Inorganic ion transport and metabolism
38	Thioredoxin peroxidase	21.292/5.37	45	19075930	Cellular processes and signaling
39	Thioredoxin peroxidase	21.292/5.37	45	19075930	Cellular processes and signaling
40	Hypothetical nuclear protein	23.145/5.85	61	6474201	Unknown
41	20s Proteasome component C3	26.502/5.0	47	19075820	Cellular processes and signaling metabolism
42	Putative protein disulfide isomerase	40.894/5.54	38	19114496	Cellular processes and signaling
43	Proteasome component PUP2 homolog	27.67/4.96	45	19115284	Cellular processes and signaling
44	Elongation factor 1 beta	23.47/4.38	57	19075803	Translation
45	mRNA export factor crp 79	79.469/8.98	17	34395978	General function prediction only
46	Putative GTP-binding protein	69.689/9.09	25	19114860	General function prediction only
47	Thiazole biosynthetic enzyme	35.366/5.91	30	19113434	Coenzyme transport and metabolism
48	Putative stress-induced protein	31.611/5.92	43	19115894	Coenzyme transport and metabolism
49	Heat-shock protein 70 family	70.475/5.13	32	19075921	Cellular processes and signaling
50	Putative stress-induced protein	31.611/5.92	38	19115894	Coenzyme transport and metabolism
51	Phosphoribosylamidoimidazole-succinocarboxamide synthase	33.95/5.80	61	19112252	Nucleotide transport and metabolism
52	Putative tyrosine kinase	36.929/6.09	28	19075949	Unknown
53	Pyrimidine precursor biosynthesis enzyme thi 3	39.511/5.82	36	19075847	Inorganic ion transport and metabolism
54	Elongation factor 2	93.714/5.95	18	2641944	Translation
55	Heat-shock protein 70 family	70.475/5.13	35	19075921	Cellular processes and signaling
56	Phosphoglycerate kinase	44.278/8.33	32	19113522	Carbohydrate transport and metabolism
57	Pyrimidine precursor biosynthesis enzyme thi 3	39.511/5.82	42	19075847	Inorganic ion transport and metabolism
58	S-Adenosylmethionine synthetase	42.091/5.70	34	19113523	Coenzyme transport and metabolism
59	Putative 2-hydroxyacid dehydrogenase	37.020/5.81	58	19114880	Energy production and conversion
60	Pyruvate decarboxylase	65.316/5.71	39	19113708	Carbohydrate transport and metabolism
61	Pyruvate decarboxylase	65.316/5.71	42	19113708	Carbohydrate transport and metabolism

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
62	Heat-shock protein	67.449/5.82	36	19112230	Cellular processes and signaling
63	Heat-shock protein	67.449/5.82	42	19112230	Cellular processes and signaling
64	Heat-shock protein 70 family	70.475/5.13	35	19075921	Cellular processes and signaling
65	Hypothetical protein stil+	66.047/5.42	28	25461543	General function prediction only
66	Mitochondrial heat-shock protein	69.597/5.73	26	173408	Cellular processes and signaling
67	Heat-shock protein	67.449/5.82	34	19112230	Cellular processes and signaling
68	Heat-shock protein	67.449/5.82	37	19112230	Cellular processes and signaling
69	Heat-shock protein	67.449/5.82	28	19112230	Cellular processes and signaling
70	Heat-shock protein	67.449/5.82	37	19112230	Cellular processes and signaling
71	Heat-shock protein	67.449/5.82	29	19112230	Cellular processes and signaling
72	Mitochondrial heat-shock 70 kd protein precursor	73.160/6.73	31	19114371	Cellular processes and signaling
73	Mitochondrial heat-shock protein	69.597/5.73	54	173408	Cellular processes and signaling
74	Heat-shock protein	67.449/5.82	38	19112230	Cellular processes and signaling
75	5-Methyltetrahydropteroyltri-glutamate-homocysteine methyltransferase	85.685/5.99	49	19114264	Amino acid transport and metabolism
76	3-Isopropylmalate dehydratase	83.415/5.75	30	19115488	Amino acid transport and metabolism
77	Putative short chain dehydrogenase	28.043/6.00	50	19075770	Secondary metabolites biosynthesis, transport, and catabolism
78	Proteasome component precursor	24.776/6.08	53	19113087	Cellular processes and signaling
79	Heat-shock protein 70 family	70.475/5.13	29	19075921	Cellular processes and signaling
80	Heat-shock protein 70 family	70.475/5.13	23	19075921	Cellular processes and signaling
81	Hexokinase I	52.248/5.02	27	19113860	Carbohydrate transport and metabolism
82	ATP Synthase beta chain, mitochondrial precursor	56.898/5.72	31	19114063	Energy production and conversion
83	4-Nitrophenylphosphatase	33.058/5.58	31	19113047	Carbohydrate transport and metabolism
84	Pyruvate dehydrogenase e1 beta subunit, mitochondrial precursor	39.776/6.04	33	19113064	Energy production and conversion
85	Hypothetical coiled-coil protein	63.745/4.61	23	19112576	Unknown
86	Enolase	47.577/6.23	32	19112695	Carbohydrate transport and metabolism
87	Ornithine aminotransferase	48.673/5.94	38	19113380	Amino acid transport and metabolism
88	Putative GTP-binding protein	69.689/9.09	19	19114860	General function prediction only
89	Cell cycle protein cwf16	31.861/8.40	39	20138042	Transcription
90	Putative protein disulfide isomerase precursor	55.244/4.50	33	19118783	Cellular processes and signaling
91	Heat-shock protein 16	15.958/5.72	55	19112883	Cellular processes and signaling
92	Carbamoyl-phosphate synthase	128.08/6.01	16	19113477	Amino acid transport and metabolism
93	78 kd Glucose-regulated protein homolog precursor	78.239/4.83	36	9114157	Cellular processes and signaling
94	Heat-shock protein 60 precursor	62.414/5.76	62	19113806	Cellular processes and signaling
95	Heat-shock protein	67.449/5.82	30	19112230	Cellular processes and signaling
96	Heat-shock protein 70 family	70.475/5.13	40	19075921	Cellular processes and signaling
97	Thioredoxin peroxidase	21.292/5.37	45	19075930	Cellular processes and signaling
98	Proteasome component PUP 2 homolog	27.670/4.96	40	19115284	Cellular processes and signaling
99	Fructose-bisphosphate aldolase	39.716/5.92	44	19112484	Carbohydrate transport and metabolism
100	Fructose-bisphosphate aldolase	39.716/5.92	44	19112484	Carbohydrate transport and metabolism



**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
101	Fructose-bisphosphate aldolase	39.716/5.92	38	19112484	Carbohydrate transport and metabolism
102	Carbamoyl-phosphate synthase	128.08/6.10	16	19113477	Amino acid transport and metabolism
103	Iron-containing alcohol dehydrogenase	46.011/6.60	31	19113731	Energy production and conversion
104	Elongation factor 2	90.439/5.95	23	19114887	Translation
105	Enolase	47.577/6.23	44	19112695	Carbohydrate transport and metabolism
106	Enolase	47.577/6.23	55	19112695	Carbohydrate transport and metabolism
107	Enolase	47.577/6.23	49	19112695	Carbohydrate transport and metabolism
108	Aldehyde dehydrogenase	55.475/6.29	59	19115494	Energy production and conversion
109	Enolase	47.577/6.23	29	19112695	Carbohydrate transport and metabolism
110	Enolase	47.577/6.23	51	19112695	Carbohydrate transport and metabolism
111	Adenylate kinase	24.417/6.14	36	19114597	Nucleotide transport and metabolism
112*	WD Repeat protein	59.052/5.78	17	19114336	General function prediction only
113*	Hypothetical protein	30.428/4.94	33	7490740	Unknown
114*	Homoserine dehydrogenase	40.013/6.03	29	19113110	Amino acid transport and metabolism
115*	Fructose-bisphosphate aldolase	39.716/5.92	33	19112484	Carbohydrate transport and metabolism
116*	Cell cycle control protein cwf16	31.861/8.44	31	19112485	Carbohydrate transport and metabolism
117*	Yeast chaperonin hsp78 homolog	90.270/7.16	19	19112909	Cellular processes and signaling
118*	Putative stress-induced protein	31.611/5.92	33	19115894	Coenzyme transport and metabolism
119*	Probable pyridoxamine <sup>5</sup> '-phosphate oxidase	26.998/5.94	49	19115562	Coenzyme transport and metabolism
120*	Thiazole biosynthetic enzyme	35.366/5.91	57	19113434	Coenzyme transport and metabolism
121*	Glycerol-3-phosphate dehydrogenase	42.229/5.31	37	4952	Energy production and conversion
122*	Putative stress-induced protein	31.611/5.92	34	19115894	Coenzyme transport and metabolism
123*	Thiazole biosynthetic enzyme	35.366/5.91	57	19113434	Coenzyme transport and metabolism
124*	Calnexin homolog precursor	63.768/4.41	26	19114524	Amino acid transport and metabolism
125*	Heat-shock protein	67.449/5.82	37	19112230	Cellular processes and signaling
126*	mRNA export factor crp79	79.469/8.98	19	34395978	General function prediction only
127*	SCF complex protein cul-1-fission yeast	89.965/6.27	17	11359308	Amino acid transport and metabolism
128*	Hypothetical protein	52.112/9	24	19115421	Translation
129*	Very hypothetical protein	12.512/7.57	31	19075635	Unknown
130*	Hexokinase 1	54.248/5.02	42	19113860	Carbohydrate transport and metabolism
131*	Elongation factor 2	93.714/5.95	19	2641946	Translation
132*	Hypothetical protein	35.844/9.56	23	19112711	Unknown
133*	Thioredoxin reductase	34.768/5.19	42	19075813	Carbohydrate transport and metabolism
134*	Transaldolase	35.330/5.67	33	19075453	Carbohydrate transport and metabolism
135*	Hypothetical protein	28.163/5.53	35	19112112	Unknown
136*	Conserved hypothetical protein	66.844/6.33	18	19075813	Cellular processes and signaling
137*	Mitochondrial heat-shock 70 kd protein precursor	73.160/6.73	24	19114371	Cellular processes and signaling
138*	Hypothetical protein	72.989/8.74	20	26401547	General function prediction only

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
139*	Hexokinase I	54.248/5.02	24	19113860	Carbohydrate transport and metabolism
140*	Putative GTP-binding protein	69.689/9.09	21	19114860	General function prediction only
141*	Heat-shock protein	67.450/5.82	22	19112230	Cellular processes and signaling
142*	Argininosuccinate synthase	46.568/5.41	39	19111975	Amino acid transport and metabolism
143*	Similarity to Drosophila crooked neck protein	93.035/5.82	20	19113404	Transcription
144*	Fructose-bisphosphate aldolase (EC 4.1.2.13)	39.716/5.92	30	19112484	Carbohydrate transport and metabolism
145	Probable phospho-2-dehydro-3-deoxyheptonate aldolase	40.955/6.76	45	19115858	Amino acid transport and metabolism
146	Alcohol dehydrogenase (EC 1.1.1.1)	38.031/6.46	35	65898	General function prediction only
147	Fructose-bisphosphate aldolase	39.716/5.92	36	19112484	Carbohydrate transport and metabolism
144	Probable oxidoreductase	36.227/7.78	73	19115800	General function prediction only
149	ATP phosphoribosyltransferase	34.236/6.55	53	19115437	Amino acid transport and metabolism
150	Putative quinine oxidoreductase; zinc-binding oxidoreductase	38.505/6.71	39	19113579	Energy production and conversion
151	Actin-interacting protein 3 homolog	154.23/5.18	14	12344049	Unknown
152	Triosephosphate isomerase	27.324/6.54	71	19075524	Carbohydrate transport and metabolism
153	Phosphoglycerate mutase	23.808/6.92	69	19115801	Carbohydrate transport and metabolism
154	Triosephosphate isomerase	27.324/6.54	44	19075524	Carbohydrate transport and metabolism
155	Similar to putative GTP-binding protein	44.533/7.6	27	19115315	Translation
156	Putative proteasome component c5	25.238/7.01	40	19115641	Cellular processes and signaling
157	Outer mitochondrial membrane protein porin; voltage-dependent anion-selective channel	29.692/7.1	39	19115573	Unknown
158	Acetylmithine aminotransferase precursor	47.936/9.21	45	19075455	Amino acid transport and metabolism
159	Probable serine/ threonine-protein kinase	94.853/9.24	21	1170605	Unknown
160	Glyceraldehyde 3-phosphate dehydrogenase I	36.018/6.24	24	19112946	Carbohydrate transport and metabolism
161*	Probable superoxide dismutase [mn] precursor	24.332/9.12	53	19115001	Inorganic ion transport and metabolism
162	Fructose-bisphosphate aldolase	39.716/5.92	54	19112484	Carbohydrate transport and metabolism
163	Enolase	47.577/6.23	44	19112695	Carbohydrate transport and metabolism
164	Hypothetical protein	40.146/6.14	40	11279920	Unknown
165	Probable aspartate-semialdehyde dehydrogenase	38.723/6.51	58	7447645	Amino acid transport and metabolism
166	Phosphoglycerate kinase	44.278/8.33	58	19113522	Carbohydrate transport and metabolism
167	Phosphoglycerate kinase	44.278/8.33	48	19113522	Carbohydrate transport and metabolism
168	Cytochrome C reductase core protein II	45.794/9.12	25	19075198	General function prediction only
169	Mitochondrial heat-shock protein	69.597/5.73	30	173408	Cellular processes and signaling

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
170	Putative aminomethyltransferase precursor	42.61/8.85	59	19114927	Amino acid transport and metabolism
171*	Hypothetical protein with possible coiled-coil region	51.843/6.6	26	1907552	Unknown
172	Dihydrolipoamide dehydrogenase, mitochondrial precursor	55.267/8.78	32	19114408	Energy production and conversion
173	Translation elongation factor 1-alpha	41.243/8.36	32	32563418	Translation
174	Probable oxidoreductase	36.227/7.78	47	19115800	General function prediction only
175*	Probable succinate dehydrogenase flavoprotein subunit precursor (EC 1.3.5.1)	71.049/8.57	41	1911523	Energy production and conversion
176	Delta-1-pyrroline-5-carboxylate dehydrogenase precursor	60.466/6.88	51	19112750	Energy production and conversion
177*	Probable succinate dehydrogenase flavoprotein subunit precursor (EC 1.3.5.1)	71.049/8.57	29	1911523	Energy production and conversion
178	Phosphoglycerate kinase	71.049/8.57	43	19113522	Carbohydrate transport and metabolism
179	Similar to <i>Saccharomyces cerevisiae</i> NADP-specific glutamate dehydrogenase	47.864/7.64	60	1749514	Amino acid transport and metabolism
180	6-Phosphogluconate dehydrogenase, decarboxylating 1	54.216/6.73	43	19111887	Carbohydrate transport and metabolism
181	Probable phospho-2-dehydro-3-deoxyheptonate aldolase	40.955/6.76	44	19115858	Amino acid transport and metabolism
182	Putative aspartate aminotransferase	46.396/7.24	37	19114176	Amino acid transport and metabolism
183	Pyruvate kinase	55.479/8.18	55	19115258	Carbohydrate transport and metabolism
184	Pyruvate kinase	55.479/8.18	62	19115258	Carbohydrate transport and metabolism
185	Putative glycine dehydrogenase	113.55/6.81	16	19113744	Amino acid transport and metabolism
186*	Phosphoglycerate kinase	44.278/8.33	46	19113522	Carbohydrate transport and metabolism
187	Branched-chain amino acid aminotransferase, mitochondrial precursor (EC2.6.1.42).	42.9/7.29	32	1911197	Amino acid transport and metabolism
188*	V-type ATPase; vacuolar ATP synthase catalytic subunit A	69.219/5.80	34	19114337	Energy production and conversion
189*	Hypothetical protein	23.575/7.22	44	6474320	Unknown
190*	Malate oxidoreductase [nad]	63.075/5.68	41	19075260	Energy production and conversion
191*	Chain a, crystal structure of the actin-crosslinking core of pombe	57.635/5.52	47	50513408	Cellular processes and signaling (cytoskeleton)
192*	Pyruvate decarboxylase	65.316/5.71	25	19113708	Carbohydrate transport and metabolism
193*	Glucose-6-phosphate 1-dehydrogenase	53.762/5.65	56	19075249	Carbohydrate transport and metabolism
194*	Enolase	47.577/6.23	37	19112695	Carbohydrate transport and metabolism
195*	Glucose-6-phosphate 1-dehydrogenase	53.762/5.65	52	19075349	Carbohydrate transport and metabolism
196*	Pyruvate decarboxylase	65.316/5.71	33	19113708	Carbohydrate transport and metabolism
197*	Malate oxidoreductase [nad]	63.066/5.68	33	19075260	Energy production and conversion
198*	Adenosylhomocysteinase (predicted)	47.866/5.61	41	2950505	Coenzyme transport and metabolism

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
199*	S-Adenosylmethionine synthetase	42.091/5.70	67	19113523	Coenzyme transport and metabolism
200*	Adenosylhomocylhomocysteinase (predicted)	47.866/5.61	32	2950505	Coenzyme transport and metabolism
201*	Probable secretory pathway GDP dissociation inhibitor	49.867/5.83	37	19113450	Cellular processes and signaling
202*	Heat-shock protein 70 family	70.475/5.13	33	19075921	Cellular processes and signaling
203*	Hexokinase (EC 2.7.1.1)	51.230/5.89	33	19114777	Carbohydrate transport and metabolism
204*	S-Adenosylmethionine synthetase	42.091/5.70	54	19113523	Coenzyme transport and metabolism
205*	Adenosylhomocysteinase (predicted)	47.866/5.61	44	2950505	Coenzyme transport and metabolism
206*	Adenosylhomocysteinase	47.866/5.61	30	2950505	Coenzyme transport and metabolism
207*	Argininosuccinate synthase	46.568/5.41	40	19111975	Coenzyme transport and metabolism
208*	Elongation initiation factor subunit	62.826/6.59	31	19115537	Transcription
209*	Heat-shock protein	67.449/5.82	25	19112230	Cellular processes and signaling
210*	Adenosylhomocysteinase	47.866/5.61	53	2950505	Coenzyme transport and metabolism
211*	Diphosphomevalonate decarboxylase	43.128/5.43	50	19114939	Lipid transport and metabolism
212*	Transaldolase	35.330/5.67	47	19075453	Carbohydrate transport and metabolism
213*	WD Repeat protein; RACK1 family	35.228/5.43	77	2440194	General function prediction only
214*	Carbamoyl-phosphate synthase	128.084/6.10	17	19113477	Amino acid transport and metabolism
215*	Probable 5-methyltetrahydropteroyltriglutamate-homocysteine s-methyltransferase	51.186/5.90	30	11256627	Amino acid transport and metabolism
216*	Putative delta-sterol c-methyltransferase	43.125/5.56	35	19112579	Secondary metabolites biosynthesis, transport, and catabolism
217*	Transaldolase	35.330/5.67	53	19075453	Carbohydrate transport and metabolism
218*	SPBC3F6.03	34.768/5.19	40	2924500	Nucleotide transport and metabolism
219*	Glutathione synthetase	56.373/6.01	27	17646699	Amino acid transport and metabolism
220*	Phosphomannomutase	29.188/5.26	57	1813335	General function prediction only
221*	4-Nitrophenylphosphatase	33.058/5.58	37	19113047	Carbohydrate transport and metabolism
222*	Hypothetical protein	28.163/5.53	70	19112112	Unknown
223*	Vacuolar ATP synthase subunit	25.783/5.50	42	19115844	Energy production and conversion
224*	Putative isocitrate dehydrogenase	38.905/7.71	12	19115309	Amino acid transport and metabolism
225*	Probable tRNA methyltransferase	48.451/9.10	26	19114314	Translation
226*	Conserved hypothetical protein	36.878/5.75	37	19115396	Unknown
227*	Pyrimidine precursor biosynthesis enzyme thi3	38.941/5.82	34	19075847	Inorganic ion transport and metabolism
228*	Pyrimidine precursor biosynthesis enzyme thi3	39.511/5.82	26	19075847	Inorganic ion transport and metabolism
229*	Cell cycle control protein cwf16	31.861/8.44	48	20138042	Transcription
230*	Elongation factor2	93.714/5.95	22	2641946	Translation
231*	60s Ribosomal protein L32	14.563/11.18	45	19115094	Translation
232*	Putative GTP-binding protein	69.689/9.09	15	19114860	General function prediction only
233*	Phosphoglycerate kinase	44.278/8.33	24	19113522	Carbohydrate transport and metabolism
234*	Phosphoglycerate kinase	44.278/8.33	32	19113522	Carbohydrate transport and metabolism
235*	Asparagine synthetase	63.771/5.98	35	19112083	Amino acid transport and metabolism

**Table 2.** Continued.

Protein No.	Description	Theoretical M <sub>r</sub> (kDa)/pI	Sequence coverage (%)	Genome ID	Function
236*	Malate oxidoreductase [nad]	63.066/5.68	33	19075260	Energy production and conversion
237*	Putative GTP-binding protein	69.689/9.09	21	19114860	General function prediction only
238*	Pyruvate decarboxylase	65.316/5.71	52	19113708	Carbohydrate transport and metabolism
239*	Malate oxidoreductase [nad]	63.066/5.68	40	19075260	Energy production and conversion
240*	GCS1/GLO3/SPS18 family zinc finger protein; ArfGap GTP-ase activating protein	53.253/5.96	21	19115755	Cellular processes and signaling

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