

Electronic Supplementary Information

Rapid Fabrication of Glass/PDMS Hybrid μ IMER for High Throughput Membrane Proteomics.

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EXPERIMENTAL PROCEDURE

ELISA assay

Channels were exposed to different enzyme concentrations (0, 0.5, 5, 50, 100, 500, 1000, and 5000 $\mu\text{g}\cdot\text{ml}^{-1}$) using standard trypsin immobilisation strategy. To quantify the immobilised trypsin, a standard ELISA protocol was modified for direct detection within microfluidic channels. After trypsin immobilisation, surfaces were rinsed in PBS and 100 μL of PBSTM blocking solution [skimmed milk (5%) and Tween²⁰ (0.05%) in PBS] was flowed into the channel at 5 $\mu\text{L}\cdot\text{min}^{-1}$. This was followed by 100 μL of anti-trypsin HRP conjugate in blocking solution at 5 $\mu\text{L}\cdot\text{min}^{-1}$. The channels were then rinsed with PBS before 100 μL of ABTS substrate solution (8 mg ABTS and 20 μl of 30% H_2O_2 in 24 mL of 0.1 M citrate buffer at pH4) was flowed into the channel at 5 $\mu\text{L}\cdot\text{min}^{-1}$. Each solution was held in the filled channel for a 6 min incubation period. The sample was then collected and the optical densities were read using a microplate reader at a wavelength of 450 nm. Each analysis was carried out in triplicate.

In-solution tryptic digestion of protein standards

A conventional in-solution protein tryptic digestion was carried out with a trypsin to substrate ratio of 1:500 at 37 °C and for an incubation time of 4 or 20 hours. Different solvent conditions were evaluated for the protein digestions. The reduced and alkylated samples were diluted 10-fold with PBS buffer or 80% ACN in PBS buffer. Each digestion was carried out with 100 μL of the samples, containing 10 μg BSA (150 pmol) or 150 pmol for each of the six proteins. The solution was acidified by adding 0.1 μL TFA to stop digestion. The samples were dried in a vacuum concentrator, and stored at -20°C before MS analysis.

Cell culture, protein extraction and purification

Synechocystis sp. PCC 6803 cells were grown and proteins extracted following a previously reported protocol.¹ Cells were grown at 25°C in BG-11 medium under a white light for 24 h, then were harvested at mid-exponential phase (OD730 of 3.0) by centrifugation. Frozen cells were resuspended for 30 min at room temperature in Tris buffer before protein extraction with liquid nitrogen and mechanical cracking. The proteins were centrifuged at 21,000 x g at 4°C for 30 min to separate soluble proteins from insoluble proteins. The purification procedure was based on,² where a combination of ultracentrifugation and sodium carbonate wash was carried out. The solubilization and denaturation of the hydrophobic protein fraction without the use of surfactants and via thermal denaturing was carried out according to Blonder *et al.*³ The total protein concentration of the purified insoluble fraction was measured using the RC DC Protein Quantification Assay (Bio-Rad, UK).

Two-staged in-solution digestion of total membrane fraction

The digestion followed a previously reported protocol.⁴ Fifty µg isolated membrane proteins were digested overnight with sequencing grade trypsin (Promega, UK) (1:100 weight ratio) in 25 mM ammonium bicarbonate (pH 8.6) at 37°C. Next day the insoluble fraction was sedimented by centrifugation at 21,000 x g at 4°C for 1 hour. The supernatant, including the soluble undigested proteins and peptide mixture, was collected in a new tube, dried in a vacuum concentrator and stored at -20°C for later MS analysis. The pellet was resuspended with 500 µL of ice-cold 100 mM ammonium carbonate (pH 11.0) to isolate the insoluble remaining proteins. After centrifugation at 21,000 x g at 4°C for 1 hour, the pellet was gently washed with ice-cold deionised water. Finally the sample was resuspended in 60% methanol (in 25 mM ammonium bicarbonate) before adding sequencing grade trypsin (1:50 weight ratio) and chymotrypsin (Roche, UK) (1:50 weight ratio). Proteolysis was carried out overnight at 37°C. After cleavage, the insoluble proteins membranes were sedimented by centrifugation at 21,000 x g at 4°C for 1 hour. The peptide-containing supernatant was dried in a vacuum concentrator and stored at -20°C for later MS analysis.

Protein identification and annotation from tandem MS analysis

MS/MS data was submitted to automated database searching using Mascot (version 2.2 Matrix Science Inc.) search engine for protein identification, giving the number of unique peptides identified, peptide coverage percentage, exponentially modified Protein Abundance Index (emPAI) value,⁵ and Mowse score.⁶ For the soluble proteins, the entire NCBI non-redundant (NR) database (March 2004) was used. For the in-soluble proteins, *Synechocystis* sp. PCC 6803 sequences were obtained from the ftp site (<ftp://ftp.ncbi.nih.gov/>) of NCBI databases (downloaded 26th August 2008) and a FASTA file

was created and merged with a database containing the random sequences and sequences of 33 common contaminants (including trypsin and keratin). Using a decoy database (random database in this study) a good estimate of the number of false positives that are present in the results from the real database can be found, as described previously.⁷ The peptide tolerance was set to 100 ppm, the MS/MS tolerance was set to 0.6 Da, +2 and +3 charged ions were considered, and carbamidomethyl (C) and oxidation (M) were also set as fixed and variable modifications respectively. The enzyme was set to trypsin (soluble proteins) and trypsin-chymotrypsin (in-soluble proteins). ESI-QUAD-TOF was the set instrument. Several searches were carried out changing the number of missed cleavages, from zero to 2. Throughout the study, only confidently identified proteins are reported, that is proteins with a confident Mowse score ($P > 0.95$) above the threshold,⁸ and with two or more peptides identified.

The identifications of the in-soluble fraction were then annotated and analyzed using different *in silico* predictors to predict protein localization and physicochemical characteristics. The tools used were: (1) Uniprot (Universal Protein Resource) database (<http://www.uniprot.org/>), which is a catalogue of information on proteins; (2) PSORTb v.2.0, a computational method for bacterial protein sub-cellular localization prediction⁹ (<http://psort.nibb.ac.jp/>); (3) Molecular weight, theoretical pI, and grand average of hydropathicity (GRAVY)¹⁰ was determined using ProtParam (<http://www.expasy.ch/tools/protparam.html>); (4) TMHMM to calculate the predicted number of transmembrane helices in the protein sequences (<http://www.cbs.dtu.dk/services/TMHMM/>); and (5) LipoP¹¹ for the prediction of lipoproteins (<http://www.cbs.dtu.dk/services/LipoP/>).

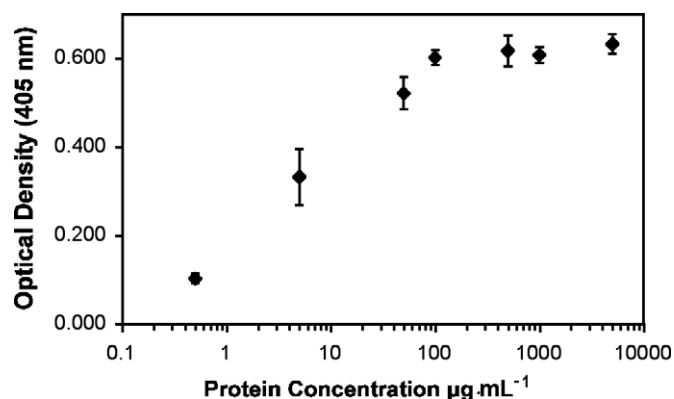
Supplementary Table 1. Summary of in-chip digestion evaluated, all triplicate digestions with lyophilized trypsin, PBS buffer (pH 7.6) and at 37°C in water bath. NC: negative control.

Protein Sample	PDMS Chip surface modification and enzyme	Flow rate (digestion time)
Single	No modification + trypsin	5 $\mu\text{L}\cdot\text{min}^{-1}$ (20 min)
	ppAAc + EDC/NHS + trypsin	5 $\mu\text{L}\cdot\text{min}^{-1}$ (20 min)
		200 $\mu\text{L}\cdot\text{min}^{-1}$ (30 sec)
	NC – ppAAc + EDC/NHS, no trypsin	5 $\mu\text{L}\cdot\text{min}^{-1}$ (20 min)
Six protein Mixture	ppAAc + EDC/NHS + trypsin	5 $\mu\text{L}\cdot\text{min}^{-1}$ (20 min)
		50 $\mu\text{L}\cdot\text{min}^{-1}$ (2 min)
		100 $\mu\text{L}\cdot\text{min}^{-1}$ (1 min)
		200 $\mu\text{L}\cdot\text{min}^{-1}$ (30 sec)

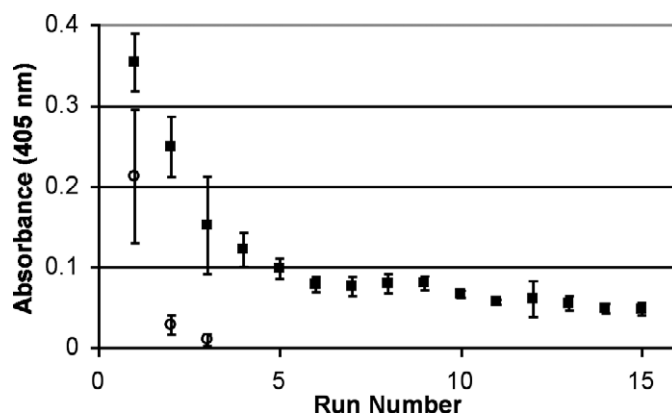
REFERENCES

1. C. S. Gan, K. F. Reardon and P. C. Wright, *Proteomics*, 2005, 5, 2468-2478.
2. Y. Fujiki, A. L. Hubbard, S. Fowler and P. B. Lazarow, *J Cell Biol*, 1982, 93, 97-102.
3. J. Blonder, M. B. Goshe, R. J. Moore, L. Pasa-Tolic, C. D. Masselon, M. S. Lipton and R. D. Smith, *J Proteome Res*, 2002, 1, 351-360.
4. F. Fischer, D. Wolters, M. Rogner and A. Poetsch, *Mol Cell Proteomics*, 2006, 5, 444-453.
5. Y. Ishihama, Y. Oda, T. Tabata, T. Sato, T. Nagasu, J. Rappsilber and M. Mann, *Mol Cell Proteomics*, 2005, 4, 1265-1272.
6. D. J. Pappin, P. Hojrup and A. J. Bleasby, *Curr Biol*, 1993, 3, 327-332.
7. J. E. Elias and S. P. Gygi, *Nat Methods*, 2007, 4, 207-214.
8. D. N. Perkins, D. J. C. Pappin, D. M. Creasy and J. S. Cottrell, *Electrophoresis*, 1999, 20, 3551-3567.
9. J. L. Gardy, M. R. Laird, F. Chen, S. Rey, C. J. Walsh, M. Ester and F. S. Brinkman, *Bioinformatics*, 2005, 21, 617-623.
10. J. Kyte and R. F. Doolittle, *J Mol Biol*, 1982, 157, 105-132.
11. A. S. Juncker, H. Willenbrock, G. Von Heijne, S. Brunak, H. Nielsen and A. Krogh, *Protein Sci*, 2003, 12, 1652-1662.

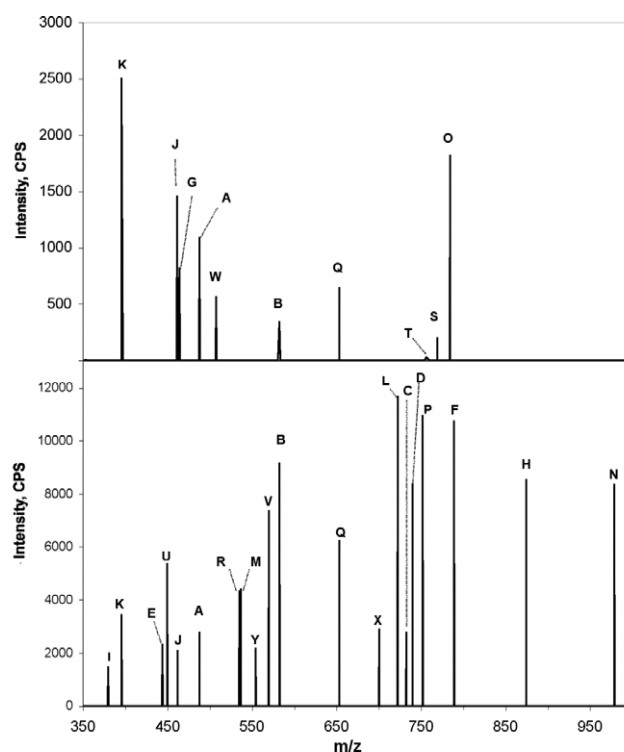
RESULTS



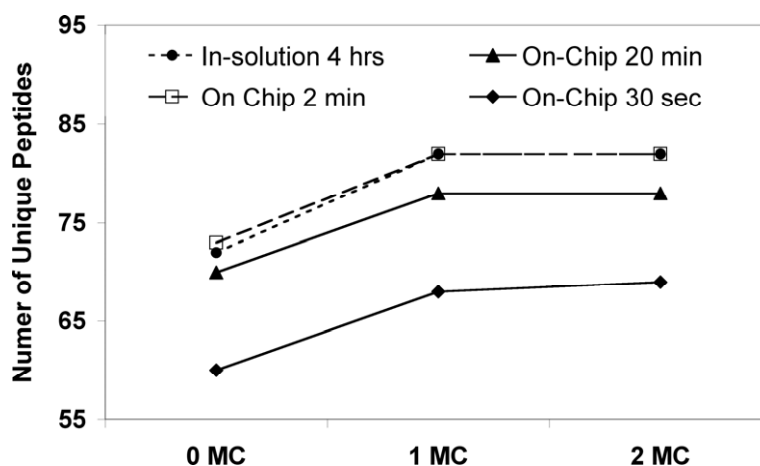
Supplementary Figure 1. Variations in optical densities of the ELISA substrate from different trypsin solution concentrations used to immobilise in the μIMERs (0, 0.5, 5, 50, 100, 500, 1000, 5000 $\mu\text{g}\cdot\text{mL}^{-1}$). The enzyme was covalently bound to the modified ppAAC plus EDC/NHS surface for 30 min at room temperature. Error bars represent one standard deviation of triplicate analyses. Note: background signal has been subtracted from the values reported in figure.



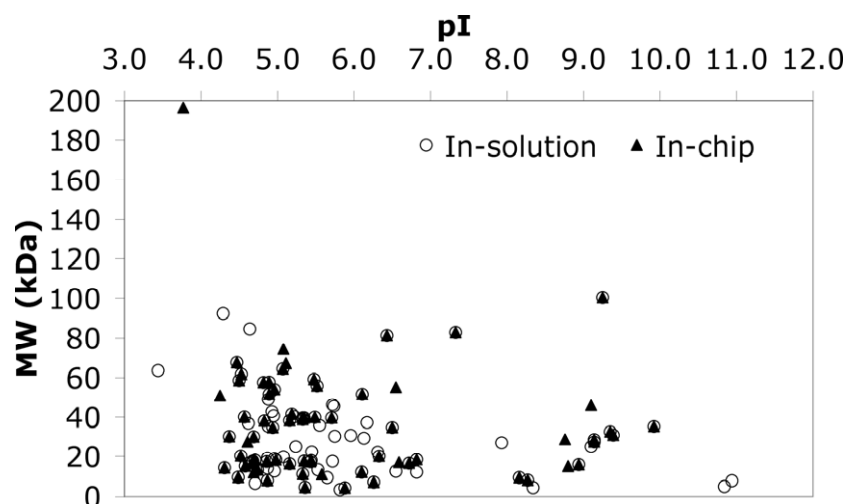
Supplementary Figure 2. Trypsin activity on L-BAPA substrate of 15 consecutive assays with intermediate washes using the same μIMER : (■) trypsin immobilized on PDMS surface modified with ppAAC + EDC/NHS and (○) trypsin physically adsorbed on untreated PDMS. After three washes negligible amount of unbound trypsin could be washed from the surface, as indicated by the L-BAPA assay (data not shown). Error bars represent one standard deviation of triplicate analyses. Note: background signal has been subtracted from the values reported in figure.



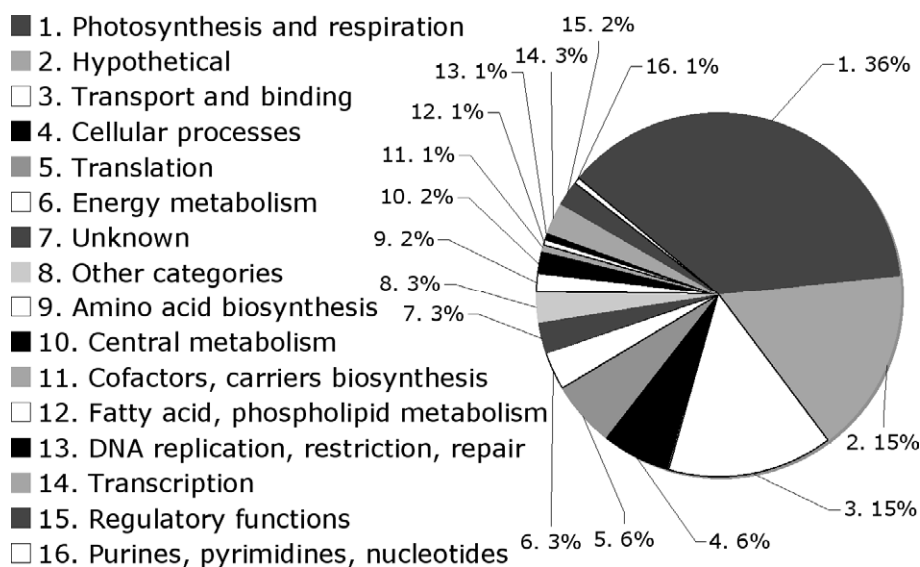
Supplementary Figure 3. Peptides identified after tryptic digestion of 10 μg of BSA in PBS (pH 7.6) (A) in-solution for 4 h at 37°C and (B) on-chip for 30 sec at 37°C, enzyme covalently bound on a ppAAc coated microchannel. Intensities reported are the CPS (counts per second) of each ion when it was detected for identification, based on each extracted ion chromatogram (XIC) of each peptide. HPLC-MS/MS analyses of 5 μL of 500 $\text{fmol}\cdot\mu\text{L}^{-1}$ BSA carried out using a 30 min gradient (3-55%). No miss cleavages were allowed. Labels indicate different peptides identified, refer to Table 2, for example ion A is peptide DLGEEHFK.



Supplementary Figure 4. Total number of peptides used to identify all six proteins after search in Mascot defining 0, 1, and 2 missed cleavages (MC).



Supplementary Figure 5. Virtual 2D gel showing the arrangement of proteins identified from the total membrane fraction sample, after in-solution and in-chip workflows, with membrane localization annotation, membrane localization prediction, 1 or more transmembrane helices, highly hydrophobic, or predicted lipoproteins. Note: visualized by JVirGel; www.jvirgel.de



Supplementary Figure 6. Functional classification of all the proteins identified from both samples and using both methods (according to CyanoBase <http://genome.kazusa.or.jp/cyanobase/Synechocystis/>).

Supplementary Table 2

List of identified proteins from the total membrane fraction sample after performing the two-staged in-solution and in-chip digestion workflows. Only proteins with reported Mowse score and values under each workflow have been identified. The values reported are the Mowse score, and inside the brackets the number of unique peptides over the number of total identified peptides, protein coverage, and emPAI values. Notes: TMH – Transmembrane helices; SpI or II - signal peptide (signal peptidase I or II); NS - No signal peptide.

No	Swiss-Prot /TrEMBL accession number	Protein name	MW (amu)	pI	Subcellular Location Uniprot	PSORTB	GRAVY	TMH	LipoP (Spl or SpII)	Total number of proteins identified per experiment	
										A In-solution Workflow	B In-chip Workflow
										158	105
										714	346
										139	92
										72	19
										4.2	5.4
No	Swiss-Prot /TrEMBL accession number	Protein name	MW (amu)	pI	Subcellular Location Uniprot	PSORTB	GRAVY	TMH	LipoP (Spl or SpII)	A	B
1	Q54714	phycocyanin beta subunit	18143	4.98	thylakoid, phycobilisome membrane	Cytoplasmic	0.094	0	NS	11437 (11/272, 75%, 9.96)	507 (6/12, 44%, 2.31)
	Q54714	phycocyanin b subunit	18115	4.98	C-phycocyanin b subunit	Unknown	0.094	0	NS		
2	Q54715	phycocyanin a subunit	17576	5.35	thylakoid, phycobilisome membrane	Unknown	-0.305	0	NS	7984 (14/162, 79%, 13.05)	405 (7/10, 52%, 2.43)
3	P73409	hypothetical protein slr1841	67559	4.47	integral to membrane	Outer Membrane	-0.147	0	SpI	7161 (11/157, 28%, 0.77)	458 (14/16, 23%, 0.14)
4	Q01952	allophycocyanin b chain	17205	5.43	membrane, phycobilisome, thylakoid	Unknown	0.111	0	NS	2362 (9/70, 78%, 6.20)	290 (9/12, 70%, 2.51)
5	P73103	hypothetical protein slr1908	64471	5.07	integral to membrane	Outer Membrane	-0.281	0	SpI	1475 (18/40, 46%, 1.57)	259 (8/10, 15%, 0.28)
6	P74227	elongation factor Tu	43705	5.16	cytoplasm	Cytoplasmic	-0.240	0	NS	1053 (20/28, 66%, 2.71)	216 (7/8, 27%, 0.55)

7	P73204	phycocyanin associated linker protein	30779	9.39	cellular thylakoid membrane; peripheral membrane protein; cytoplasmic side	Unknown	-0.502	0	NS	635 (14/18, 52%, 1.52)	283 (8/10, 37%, 0.85)
8	P29255	P700 apo protein subunit lb (PSA-B)	81384	6.43	cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.202	11	NS	618 (14/27, 21%, 0.49)	131 (4/4, 6%, 0.08)
	P29255	P700 apo protein subunit lb	81354	6.43	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.205	11	NS		
9	Q55544	phycobilisome LCM core-membrane linker polypeptide	100234	9.25	membrane, phycobilisome, thylakoid	Unknown	-0.395	0	NS	697 (21/24, 31%, 0.78)	255 (8/8, 11%, 0.05)
10	P29254	P700 apo protein subunit la (PSA-A)	82896	7.33	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.234	9	NS	463 (14/26, 19%, 0.47)	195 (6/7, 9%, 0.12)
	P29254	Photosystem IP700 chlorophyll a apo protein A1 (PsaA)	82896	7.33	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.234	9	NS		
	P29254	P700 apo protein subunit la	82882	7.33	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.234	9	NS		
11	P26527	ATP synthase subunit B	51701	4.89	Cellular thylakoid membrane; Peripheral membrane protein	Cytoplasmic	-0.063	0	NS	653 (14/14, 48%, 1.23)	216 (7/7, 23%, 0.45)
12	Q55199	phosphate-binding Periplasmic protein precursor	39998	4.57	inorganic phosphate transmembrane transporter activity	Periplasmic	-0.058	1	SpII	420 (8/14, 35%, 1.05)	271 (5/7, 29%, 0.49)
13	Q01951	allophycocyanin a chain	17344	4.86	membrane, phycobilisome, thylakoid	Unknown	-0.053	0	NS	480 (6/15, 45%, 2.46)	189 (6/6, 48%, 1.03)
14	P73203	phycocyanin associated linker protein	32501	9.35	membrane, phycobilisome, thylakoid	Outer membrane	-0.379	0	NS	414 (12/15, 54%, 1.64)	152 (5/5, 25%, 0.48)
15	Q01903	sulfate binding protein; SbpA	38104	4.83	Periplasm	Periplasmic	-0.230	0	SpII	493 (11/15, 42%, 1.50)	188 (6/6, 27%, 0.52)
	Q01903	sulfate binding protein; SbpA	38019	4.83	Periplasm	Periplasmic	-0.230	0	SpII		
16	P09193	Photosystem II 44 kDa reaction center protein (P6 protein) (CP43)	51728	6.11	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.255	7	NS	353 (9/14, 28%, 0.54)	62 (2/2, 5%, 0.29)
	S06469	Photosystem II 44 kDa reaction center protein (P6 protein) (CP43)	50470	3.47	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.255	7	NS		
	P09193	Photosystem II 44 kDa reaction center protein (P6 protein) (CP43)	32688	6.11	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.534	6	NS		

	P09193	Photosystem II 44 kDa reaction center protein (P6 protein) (CP43)	51757	6.11	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.268	7	NS		
17	P73785	periplasmicPhosphate binding protein	35268	9.92	transmembrane transporter	Periplasmic	-0.096	1	Spl	377 (13/14, 47%, 1.24)	264 (8/9, 32%, 0.31)
18	P29256	photosystem I subunit III	18351	6.82	plasma membrane-derived photosystem I	Unknown	0.075	2	Spl	357 (6/12, 38%, 2.29)	162 (5/5, 36%, 0.97)
19	P74625	phycobilisome rod-core linker polypeptide; CpcG	28505	9.14	phycobilisome attached to thylakoid	Unknown	-0.240	1	NS	366 (13/14, 63%, 2.76)	91 (3/3, 17%, 0.28)
20	P27179	ATP synthase subunit A	54046	4.96	membrane, thylakoid	Unknown	-0.025	0	NS	317 (10/12, 25%, 0.70)	182 (6/6, 16%, 0.27)
21	P74625	phycobilisome rod-core linker polypeptide; CpcG	27375	9.14	phycobilisome attached to thylakoid	Unknown	-0.240	1	NS	329 (11/13, 42%, 1.50)	84 (2/2, 10%, 0.26)
22	P80505	glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating)	36476	6.00	Cytoplasm	Cytoplasmic	-0.114	0		319 (8/8, 33%, 0.84)	83 (2/2, 8%, 0.09)
	P80505	glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating)	36470	6.00	Cytoplasm	Cytoplasmic	-0.114	0			
23	P05429	photosystem II CP47 protein/Photosystem IIP680 chlorophyll A apo protein	55924	5.52	Cellular thylakoid membrane; Multi-pass membrane protein.	Cytoplasmic membrane	0.117	6	NS	311 (7/8, 22%, 0.49)	73 (2/3, 6%, 0.19)
24	P54206	ribulose bisphosphate carboxylase small subunit	13231	5.36	chloroplast ribulose	Cytoplasmic	-0.577	0	NS	281 (4/6, 38%, 1.51)	179 (1/2, 15%, 0.30)
25	Q05972	GroEL	57661	5.01	Cytoplasm	Cytoplasmic	-0.173	0	NS	306 (13/15, 29%, 0.56)	250 (8/9, 17%, 0.13)
	Q05972	chaperonin GroEL	57617	5.01	Cytoplasm	Cytoplasmic	-0.163	0	NS		
26	P77961	glutamate--ammonia ligase	52966	4.98	Cytoplasm	Cytoplasmic	-0.394	0		147 (6/6, 19%, 0.27)	98 (3/3, 13%, 0.46)
27	P73282	transketolase	71680	5.35	NA	Unknown	-0.072	0		245 (7/7, 16%, 0.37)	62 (2/2, 5%, 0.05)
28	Q55013	Chain A,Psii Associated Cytochrome C549	15110	4.58	Cellular thylakoid membrane; peripheral membrane	Unknown	-0.286	0	NS	235 (4/4, 41%, 1.00)	98 (3/3, 9%, 0.48)
	Q55013	lowPotential cytochrome C	15086	4.58	Cellular thylakoid membrane; peripheral membrane	Unknown	-0.599	0	NS		
	Q55013	cytochrome c550	17873	4.58	Cellular thylakoid membrane; peripheral membrane	Unknown	-0.286	1	Spl		

29	P74534	hypothetical protein sll1338	20170	4.52	NA	Unknown	-0.058	0	Spl	253 (3/6, 17%, 0.59)	107 (2/2, 10%, 0.42)
30	Q59991	hypothetical protein slr0042	61812	4.53	integral to membrane	Unknown	-0.001	0	Spl	270 (7/8, 18%, 0.44)	122 (4/4, 12%, 0.04)
31	P54205	methionine sulfoxide reductase A	52457	5.78	chloroplast ribulose	Unknown	-0.289	0	NS	339 (11/12, 31%, 0.63)	128 (4/4, 10%, 0.20)
32	P72700	hypothetical protein slr0244	31185	5.12	NA	Cytoplasmic	-0.013	0	NS	222 (2/3, 11%, 0.22)	89 (2/2, 11%, 0.11)
33	B1XQ09	carbon dioxide concentrating mechanism protein; CcmK	11128	5.33	NA	Cytoplasmic	0.117	0	NS	217 (5/8, 45%, 1.95)	95 (3/4, 36%, 0.72)
34	P72761	phosphoglycerate kinase	38786	5.33	NA	Cytoplasmic	0.125	0	NS	217 (6/7, 21%, 0.51)	199 (6/6, 21%, 0.39)
35	P96017	fructose-1,6-bisphosphatase	41480	5.19	NA	Unknown	0.012	0	NS	183 (6/9, 18%, 0.26)	134 (4/4, 13%, 0.08)
	P73922	GlpX protein	37051	5.10	NA	Cytoplasmic	-0.019	0	NS		
36	P19569	photosystem I subunit II	15691	8.94	plasma membrane-derived Photosystem I	Unknown	-0.660	0	NS	206 (9/10, 55%, 2.25)	155 (5/6, 36%, 1.67)
37	P74135	hypothetical protein sll1873	12898	10.00	NA	Unknown	-0.502	0	NS	197 (4/5, 49%, 1.03)	109 (2/2, 27%, 0.60)
38	P26287	apocytochrome fPrecursor	35209	4.88	Cellular thylakoid membrane; Single-pass membrane protein	Unknown	0.056	2	NS	195 (6/7, 29%, 0.57)	
39	P37277	photosystem I reaction center protein subunit XI	16613	4.67	membrane, photosystem I, thylakoid	Cytoplasmic membrane	0.398	2	NS	235 (4/8, 45%, 1.52)	137 (4/4, 29%, 0.24)
40	P74182	hypothetical protein slr1270	57700	4.82	outer membrane-bounded periplasmic space	Outer Membrane	-0.368	0	Spl	183 (5/7, 13%, 0.25)	69 (2/2, 6%, 0.06)
41	P16033	photosystem II D1 protein	39924	5.49	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.304	5	NS	181 (4/4, 13%, 0.27)	131 (4/4, 14%, 0.05)
	P16033	photosystem II D1 protein	39696	5.49	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.304	5	NS		
42	P72758	carbon dioxide concentrating mechanism protein; CcmM	73076	8.83	chloroplast ribulose	Cytoplasmic	-0.276	0	NS	156 (5/5, 10% 0.14)	
43	P52231	thioredoxin	11741	4.79	NA	Cytoplasmic	-0.053	0	NS	170 (4/4, 51%, 1.79)	

44	Q55707	chloroplast membrane-associated 30 kD protein	28888	4.95	NA	Cytoplasmic	-0.558	0	NS	169 (3/3, 13%, 0.24)	94 (3/3, 15%, 0.12)
45	Q8VV05	phycocyanin associated linker protein	9317	8.16	phycobilisome attached to thylakoid	Unknown	-0.627	0	NS	157 (5/5, 71%, 2.56)	66 (1/1, 16%, 0.37)
46	P74655	hypothetical protein sll1550	58595	4.5	integral to membrane	Outer Membrane	-0.096	0	Spl	155 (5/6, 12%, 0.18)	122 (4/4, 12%, 0.15)
47	P12975	photosystem I reaction center subunit IV	8140	8.27	Cellular thylakoid membrane; peripheral membrane protein	Unknown	-0.299	0	NS	152 (2/3, 37%, 1.06)	81 (2/2, 31%, 0.54)
	P12975	Chain E, Psae Sub-Unit Of ThePhotosystem I	8049	4.71	Cellular thylakoid membrane; peripheral membrane protein	Unknown	-0.353	0	NS		
48	P09192	photosystem II D2 protein	39581	5.35	membrane, photosystem II, thylakoid	Cytoplasmic membrane	0.361	6	NS	285 (8/8, 26%, 0.49)	134 (4/5, 13%, 0.06)
	P09192	photosystem II D2 protein	39467	4.92	membrane, photosystem II, thylakoid	Cytoplasmic membrane	0.361	6	NS		
	P09192	unnamed proteinProduct	14294	5.55	membrane, photosystem II, thylakoid	Unknown	-0.094	1	NS		
49	P74720	hypothetical protein sll1106	18019	4.71	NA	Cytoplasmic	0.229	1	NS	160 (2/2, 20%, 0.41)	104 (2/2, 16%, 0.19)
50	P72760	carbon dioxide concentrating mechanism protein; CcmK	12094	6.10	NA	Cytoplasmic	0.194	0	NS	162 (3/7, 25%, 0.79)	68 (2/3, 32%, 0.28)
51	P22034	60kD chaperonin 2	57739	4.89	cytoplasm	Cytoplasmic	0.040	0	NS	332 (9/28, 22%, 0.25)	164 (5/5, 13%, 0.13)
52	P73704	general secretionPathway protein G	17563	4.68	type II protein secretion system complex	Unknown	-0.061	1	NS	95 (2/3, 13%, 0.70)	36 (1/1, 5%, 0.19)
53	P73452	nitrate transport 45kD protein	49163	4.88	Cell inner membrane; Peripheral membrane protein	Cytoplasmic membrane	-0.306	0	NS	94 (3/3, 9%, 0.07)	
54	P23349	50S ribosomal protein L7/L12	13251	4.74	Ribosomal	Unknown	0.047	0	NS	228 (5/6, 47%, 1.51)	190 (6/6, 73%, 0.58)
55	Q55195	phosphate transport ATP-binding protein;PstB	29226	6.13	Cell inner membrane; Peripheral membrane protein	Unknown	-0.093	0	NS	120 (4/4, 23%, 0.24)	
56	P73297	DNA-directed RNAPolymerase alpha subunit	34982	4.72	intracellular	Cytoplasmic	-0.236	0	NS	117 (3/3, 19%, 0.20)	
57	P72655	erthyrocyte band 7 integral membrane protein, protein 7.2B, stomatin	35705	5.55	membrane	Unknown	-0.142	0	NS	125 (3/4, 14%, 0.30)	

58	P27180	ATP synthase subunit D	20081	6.33	CF(1)	Cytoplasmic	0.098	0	NS	96 (3/3, 35%, 0.36)	90 (3/5, 22%, 0.17)
59	P26522	NADH dehydrogenase subunit H	40579	4.95	membrane, thylakoid	Cytoplasmic membrane	0.833	8	NS	114 (2/2, 10%, 0.17)	
60	P73722	SOS function regulatory protein	22730	5.84	NA	Cytoplasmic	-0.330	0	NS	139 (4/5, 31%, 0.32)	63 (2/2, 14%, 0.15)
61	P74102	Orange carotenoid-binding protein	34637	4.94	Cellular thylakoid membrane	Unknown	0.005	0	NS	109 (3/3, 16%, 0.32)	61 (2/2, 9%, 0.10)
62	Q55332	12 kDa extrinsic protein of Photosystem II	14222	4.31	Cellular thylakoid membrane	Unknown	0.027	1	Spl	145 (4/4, 38%, 1.36)	61 (1/1, 7%, 0.24)
	Q55332	12 kDa extrinsic protein of Photosystem II	14236	4.31	Cellular thylakoid membrane	Unknown	0.027	1	Spl		
63	P27178	ATP synthase subunit A	30679	5.96	CF(0), membrane	Cytoplasmic membrane	0.566	6	NS	105 (3/4, 16%, 0.23)	
64	Q55200	SphX protein precursor	36756	4.62	NA	Unknown	-0.176	0	SpII	104 (3/3, 11%, 0.19)	
65	P73728	Thioredoxin reductase	21153	4.94	NA	Unknown	-0.267	0	NS	102 (3/3, 18%, 0.55)	
66	P73348	rehydrin	23545	5.08	NA	Cytoplasmic	-0.291	0	NS	98 (3/3, 16%, 0.49)	
67	P72939	alkaline Phosphatase	149299	3.93	NA	Unknown	-0.066	0	NS	174 (4/4, 6%, 0.09)	211 (7/7, 7%, 0.03)
68	Q05971	10 kDa chaperonin (protein Cpn10) (groES protein)	10853	5.06	cytoplasm	Cytoplasmic	-0.137	0	NS	133 (4/4, 56%, 0.74)	63 (2/2, 26%, 0.32)
	Q05971	10 kDa chaperonin (protein Cpn10) (groES protein)	11172	5.06	cytoplasm	Cytoplasmic	-0.137	0	NS		
69	P28371	elongation factor G	76721	4.90	cytoplasm	Cytoplasmic	-0.347	0	NS	239 (7/11, 11%, 0.09)	
	P28371	elongation factor EF-2	76702	4.90	cytoplasm	Cytoplasmic	-0.347	0	NS		
70	Q55835	periplasmic iron-binding protein	38132	5.16	Cellular thylakoid membrane; peripheral membrane protein; Lumenal side	Periplasmic	-0.376	0	Spl	102 (2/2, 8%, 0.09)	91 (3/4, 14%, 0.09)
71	P26290	plastoquinol--plastocyanin reductase	18984	4.87	Cellular thylakoid membrane; Single-pass membrane protein	Cytoplasmic membrane	-0.086	1	NS	119 (3/4, 24%, 0.18)	

	P26290	plastoquinol--plastocyanin reductase	20529	4.87	Cellular thylakoid membrane; Single-pass membrane protein	Cytoplasmic membrane	-0.021	0	NS		
	P26290	Cytochrome b6-f complex iron-sulfur subunit 2 (Rieske iron-sulfur protein 2)	18984	4.87	Cellular thylakoid membrane; Single-pass membrane protein	Cytoplasmic membrane	-0.086	1	NS		
72	Q57038	cytochrome b6	25038	9.10	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.559	5	NS	183 (4/6, 27%, 0.15)	
73	P72870	allophycocyanin-B	17912	5.44	phycobilisome attached to thylakoid	Unknown	-0.253	0	NS	135 (5/5, 49%, 0.68)	131 (4/4, 25%, 0.19)
74	P74426	hypothetical protein sll0359	17208	9.18	NA	Unknown	-0.605	0	NS	147(4/3, 21%, 0.43)	159 (5/5, 37%, 0.20)
75	Q02925	phycobilisome LC linker polypeptide	7800	10.94	Cellular thylakoid membrane; Peripheral membrane protein; Cytoplasmic side	Unknown	-0.506	0	NS	96 (3/3, 49%, 2.07)	
76	P10549	Photosystem II manganese-stabilizing polypeptide precursor (MSP)	29893	4.69	plasma membrane-derived thylakoid photosystem II	Unknown	-0.206	1	SpI	127 (4/4, 20%, 0.37)	38 (1/1, 3%, 0.11)
77	P17253	ATP synthase subunit C	34584	6.50	plasma membrane proton-transporting ATP synthase complex	Unknown	-0.114	0	NS	80 (2/2, 7%, 0.10)	98 (3/3, 16%, 0.05)
78	P72797	transaldolase	43078	4.93	cytoplasm	Cytoplasmic	-0.154	0	NS	103 (3/4, 14%, 0.08)	69 (2/2, 7%, 0.08)
79	P80507	Inorganic pyrophosphatase (EC 3.6.1.1)	26245	4.69	cytoplasm	Cytoplasmic	-0.241	0	NS	97 (3/3, 36%, 0.10)	139 (4/4, 29%, 0.13)
	P80507	Inorganic pyrophosphatase (EC 3.6.1.1)	19076	4.69	cytoplasm	Cytoplasmic	-0.241	0	NS		
80	P14835	photosystem II reaction center proteinPsbH	7112	6.26	membrane, photosystem II, thylakoid	Unknown	0.708	1	NS	70 (1/1, 22%, 0.50)	66 (2/2, 39%, 0.28)
81	P74466	ATP-dependent Clp protease proteolytic subunit	24866	4.86	NA	Cytoplasmic	-0.076	0	NS	70 (2/2, 14%, 0.13)	
82	P27183	ATP synthase subunit B	16236	5.16	Membrane	Cytoplasmic	-0.429	1	NS	164 (3/3, 34%, 0.77)	96 (3/3, 28%, 0.46)
83	P73048	hypothetical protein sll1638	16524	6.72	plasma membrane-derived thylakoid Photosystem II	Unknown	-0.117	0	SpII	98 (3/3, 28%, 0.21)	67 (2/2, 11%, 0.11)
84	Q55432	hypothetical protein sll0822	13953	8.63	NA	Unknown	-0.518	0	NS	97 (3/3, 33%, 0.24)	

85	P73821	phosphoglycerate dehydrogenase	59185	5.48	NA		Cytoplasmic	0.170	0	NS	137 (4/4, 15%, 0.06)	161 (5/8, 15%, 0.11)
86	P21697	plastocyaninPrecursor	13138	5.53	NA		Periplasmic	0.286	1	Spl	75 (1/1, 14%, 0.26)	
87	Q59987	protochlorophyllide oxidoreductase	36039	8.60	chloroplast		Unknown	-0.222	0	NS	97 (3/3, 14%, 0.19)	95 (3/3, 14%, 0.09)
88	P37101	phosphoribulokinase	37890	5.28	NA		Cytoplasmic	-0.373	0	NS	63 (2/2, 8%, 0.09)	97 (3/3, 17%, 0.09)
89	Q55318	ferredoxin-NADP oxidoreductase	46330	5.72	Cellular thylakoid membrane; Peripheral membrane protein; Cytoplasmic side		Cytoplasmic	-0.570	0	NS	105 (3/3, 12%, 0.07)	
90	P73319	50S ribosomal protein L4	23342	10.21	ribosome		Unknown	-0.472	0	NS	68 (2/2, 16%, 0.14)	
91	Q55664	fructose-bisphosphate aldolase	38948	5.47	NA		Cytoplasmic	-0.231	0	NS	266 (7/7, 29%, 0.63)	281 (8/8, 32%, 0.28)
92	P09190	cytochrome b559 subunit alpha	9443	4.49	Cellular thylakoid membrane; Single-pass membrane protein		Unknown	-0.212	1	NS	73 (2/2, 26%, 0.37)	65 (2/2, 26%, 0.37)
93	P74344	molybdopterin biosynthesis protein MoeB	42732	4.93	NA		Unknown	-0.049	1	NS	66 (2/2, 7%, 0.08)	
94	P09191	cytochrome b559 subunit beta	4930	10.84	membrane, thylakoid, photosystem II		Unknown	0.598	1	NS	102 (3/7, 68%, 0.43)	
95	P73946	hypothetical protein slr1506	68804	5.16	NA		Unknown	-0.118	0	NS	71 (2/2, 6%, 0.05)	
96	P73472	chloroplast import-associated channel IAP75	92251	4.29	chloroplast outer membrane		Outer Membrane	-0.224	0	Spl	75 (2/2, 3%, 0.04)	
97	Q59977	citrate synthase	44802	5.70	cytoplasm		Cytoplasmic	-0.137	0	NS	115 (3/3, 10%, 0.07)	
98	Q55247	Nitrogen regulatory proteinP-II (PII signal transducing protein)	12390	6.33	NA		Unknown	-0.094	0	NS	103 (3/7, 26%, 0.28)	65 (2/2, 26%, 0.22)
	Q55247	PII protein	12390	6.33	NA		unknown	-0.094	0	NS		
99	P74233	hypothetical protein slr1160	22248	5.45	Cellular component: outer membrane-bounded Periplasmic space		Unknown	-0.099	0	Spl	67 (1/1, 8%, 0.15)	

100	Q55329	photosystem I reaction center subunit IX	4529	5.36	membrane, photosystem I, thylakoid	Unknown	0.830	1	NS	66 (1/2, 23%, 0.81)	40 (1/1, 23%, 0.81)
101	P27589	cytochrome b6-f complex subunit 4	17432	5.72	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.571	3	NS	93 (3/3, 25%, 0.11)	
102	Q55146	hypothetical protein sll0064	30159	4.37	Cellular component: outer membrane-bounded Periplasmic space	Periplasmic	0.095	0	Spl	69 (2/2, 14%, 0.11)	91 (3/3, 16%, 0.11)
103	Q55113	hypothetical protein slr0431	26995	7.93	NA	Periplasmic	-0.111	0	Spl	98 (3/3, 20%, 0.23)	
104	Q55356	photosystem II 13 kD protein	12582	4.96	Cellular thylakoid membrane; Peripheral membrane protein; Cytoplasmic side.	Cytoplasmic	-0.375	0	NS	117 (3/3, 36%, 0.27)	
105	P73603	hypothetical protein slr1852	21821	5.05	NA	Cytoplasmic	-0.513	0	NS	68 (2/2, 15%, 0.15)	
106	P52415	glucose-1-phosphate adenylyltransferase	49335	6.06	NA	Unknown	-0.221	0	NS	75 (2/2, 7%, 0.07)	
107	P19125	NADH dehydrogenase subunit J	20580	4.44	NA	Cytoplasmic	-0.302	0	NS	60 (1/1, 8%, 0.18)	45 (1/1, 8%, 0.21)
	P19125	NADH dehydrogenase subunit J	18275	4.44	NA	Cytoplasmic	-0.292	0	NS		
108	P73418	DNA binding protein HU	14884	8.69	NA	Unknown	-0.452	0	NS	152 (4/4, 33%, 0.23)	132 (4/4, 52%, 0.23)
109	P27181	ATP synthase subunit B	19793	5.08	Membrane; Single-pass membrane protein	Cytoplasmic	-0.379	0	NS	136 (4/4, 25%, 0.37)	
	P27181	ATP synthase subunit B	19776	5.08	Membrane; Single-pass membrane protein	Cytoplasmic	-0.379	0	NS		
110	P73392	secreted protein MPB70	14096	4.87	NA	Unknown	0.489	1	NS	35 (1/1, 14%, 0.24)	
111	P32422	photosystem I subunit VII	9335	5.65	membrane, photosystem I, thylakoid	Unknown	0.086	0	NS	66 (2/3, 22%, 0.40)	
112	P74791	hypothetical protein ssr0692	5847	10.83	NA	Unknown	-0.700	0	NS	71 (2/2, 61%, 0.61)	
113	P72659	polyribonucleotideNucleotidyltransferase	77783	5.13	cytoplasm	Unknown	-0.129	0	NS	172 (4/4, 9%, 0.04)	194 (6/6, 15%, 0.04)

114	Q55171	hypothetical protein slr0476	14385	6.92	NA	Unknown	-0.606	0	NS	61 (1/1, 13%, 0.13)	
115	P73676	Photosystem II protein Y	4199	5.88	Cellular thylakoid membrane; Single-pass membrane protein	Unknown	0.892	1	NS	140 (3/4, 69%, 0.51)	68 (1/1, 28%, 0.79)
116	P72805	hypothetical protein sll1665	63569	3.44	NA	Cytoplasmic	-0.541	1	Spl	138 (2/2, 5%, 0.11)	
117	P73953	hypothetical protein slr1512	39622	5.33	NA	Unknown	0.632	8	NS	109 (3/3, 10%, 0.09)	98 (3/5, 12%, 0.08)
118	P48939	30S ribosomal protein S4	23168	10.48	small ribosomal subunit	Unknown	-0.594	0	NS	103 (1/2, 8%, 0.14)	
119	P73393	hypothetical protein sll1734	50006	5.55	NA	Unknown	-0.228	0	NS	107 (3/3, 9%, 0.14)	
120	P29107	ketol-acid reductoisomerase	39926	4.91	NA	Unknown	-0.026	0	NS	168 (4/4, 13%, 0.08)	
121	Q55162	hypothetical protein sll0051	17787	4.41	NA	Cytoplasmic	-0.328	0	NS	62 (2/2, 18%, 0.19)	
122	P74071	30S ribosomal protein S2	30131	5.14	small ribosomal subunit	Cytoplasmic	-0.455	0	NS	77 (2/2, %, 0.11)	
123	P24602	bacterioferritin	18319	4.71	NA	Cytoplasmic	-0.505	0	NS	57 (1/1, 11%, 0.18)	60 (2/12, 23%, 0.18)
124	P77968	superoxide dismutase	21663	4.90	NA	Unknown	-0.247	0	NS	96 (3/3, 23%, 0.33)	71 (1/1, 8%, 0.16)
125	P72986	photosystem IPsaM subunit	3378	5.82	Cellular thylakoid membrane	Unknown	1.187	1	NS	74 (2/3, 100%, 1.15)	
126	P52983	glucose-6-phosphate isomerase	58320	5.48	cytoplasm	Cytoplasmic	-0.226	0	NS	96 (3/3, 7%, 0.06)	
127	P74061	ribulose-phosphate 3-epimerase	24955	5.24	NA	Cytoplasmic	0.093	0	NS	57 (1/1, 7%, 0.13)	
128	P73068	rubredoxin	12562	6.55	NA	Cytoplasmic	-0.095	1	NS	52 (1/1, 15%, 0.27)	
129	P73287	bacterioferritin	20213	4.59	NA	Unknown	-0.516	0	NS	62 (2/3, 13%, 0.17)	
130	P74361	ClpB protein	98062	5.16	cytoplasm	Cytoplasmic	-0.445	0	NS	191 (6/6, 9%, 0.03)	

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131	Q55662	ATP-dependent ClpProtease regulatory subunit	91118	5.40	NA	Cytoplasmic	-0.287	0	NS	151 (5/5, 9%, 0.04)	173 (5/5, 7%, 0.04)
132	P74459	ClpB protein	101329	5.23	cytoplasm	Cytoplasmic	-0.445	0	NS	61 (2/2, 4%, 0.03)	184 (5/5, 8%, 0.03)
133	P73057	hypothetical protein slr1847	12127	4.62	NA	Cytoplasmic	-0.332	0	NS	61 (2/2, 18%, 0.28)	
134	P74551	phycobilisome core component	18880	4.96	phycobilisome attached to thylakoid	Cytoplasmic	-0.170	0	NS	60 (2/2, 14%, 0.18)	
135	Q55457	hypothetical protein slr0038	15231	4.83	NA	Unknown	-0.304	0	NS	68 (2/2, 19%, 0.22)	
136	P74215	aspartyl/glutamyl-tRNA amidotransferase subunit B	57570	5.40	NA	Cytoplasmic	-0.331	0	NS	93 (3/3, 8%, 0.03)	
137	P72932	high light inducible protein	6468	4.71	NA	Unknown	-0.060	1	NS	41 (1/1, 28%, 0.56)	
138	P26525	NADH dehydrogenase subunit I	22136	6.31	Cellular thylakoid membrane; Peripheral membrane protein	Unknown	-0.344	0	NS	37 (1/1, 5%, 0.15)	
139	Q55274	iron-stress chlorophyll-binding protein	37197	6.17	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.461	7	NS	36 (1/1, 2%, 0.09)	
140	P77972	phosphopyruvate hydratase	46500	5.02	Cytoplasm. Secreted. Cell surface.	Cytoplasmic	-0.206	0	NS	65 (2/2, 6%, 0.07)	
141	Q59993	ATP-dependent ClpProteaseProteolytic subunit	24808	4.69	cytoplasm	Cytoplasmic	-0.094	0	NS	75 (2/2, 17%, 0.14)	
142	P73824	glutathione Peroxidase	16636	4.63	NA	Periplasmic	-0.183	0	NS	31 (1/1, 8%, 0.20)	
143	P74463	twitching motility protein	40590	6.57	intracellular	Cytoplasmic	-0.103	0	NS	92 (3/3, 20%, 0.08)	
144	Q55196	phosphate transport ATP-binding protein;PstB	30191	5.75	plasma membrane	Cytoplasmic membrane	-0.350	0	NS	87 (2/2, 10%, 0.11)	
145	P73654	hypothetical protein ssl3364	8290	4.18	NA	Cytoplasmic	-0.899	0	NS	95 (3/3, 70%, 0.42)	
146	P22358	molecular chaperone DnaK	67573	4.72	NA	Cytoplasmic	-0.275	0	NS	176 (5/5, 9%, 0.10)	102 (3/3, 8%, 0.05)
147	P52208	6-phosphogluconate dehydrogenase	52840	5.12	NA	Unknown	-0.158	0	NS	65 (2/2, 10%, 0.06)	

148	P74189	general secretion pathway protein D	84590	4.64	cell outer membrane	Outer Membrane	-0.106	0	Spl	151 (5/5, 7%, 0.04)	
149	P27724	NADH dehydrogenase delta subunit	45505	5.74	thylakoid membrane	Cytoplasmic	-0.276	0	NS	98 (3/3, 11%, 0.15)	
150	P73954	hypothetical protein slr1513	11999	6.82	Membrane associated protein	Cytoplasmic	-0.152	0	NS	48 (1/1, 8%, 0.29)	
151	P74494	nucleoside diphosphate kinase	16681	5.43	cytoplasm	Cytoplasmic	-0.159	0	NS	66 (2/2, 15%, 0.20)	
152	P73853	inositol-5-monophosphate dehydrogenase	40209	5.22	NA	Cytoplasmic	0.251	0	NS	70 (2/2, 9%, 0.04)	
153	Q55765	RNA-binding protein	16606	7.75	NA	Unknown	-0.874	0	NS	67 (2/2, 13%, 0.20)	
154	P27182	ATP synthase subunit C	7963	4.87	Cell membrane; Multi-pass membrane protein	Unknown	0.960	2	NS	35 (1/1, 11%, 0.44)	75 (2/2, 32%, 0.55)
155	Q55354	photosystem II reaction center L	4470	8.34	Cellular thylakoid membrane; Single-pass membrane protein.	Unknown	0.195	1	NS	34 (1/1, 18%, 0.84)	
156	P48944	30S ribosomal protein S14	11846	10.75	ribosome	Cytoplasmic	-1.100	0	NS	31 (1/1, 8%, 0.29)	
157	P74690	putativePhosphoketolase	92389	5.78	NA	Unknown	-0.364	0	NS	230 (7/7, 15%, 0.04)	
158	P07826	photosystem II D1 protein	39625	5.71	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.414	6	NS	78 (2/2, 7%, 0.05)	81 (1/1, 3%, 0.08)
	P07826	photosystem II D1 protein	39580	5.71	Cellular thylakoid membrane; Multi-pass membrane protein	Cytoplasmic membrane	0.414	6	NS		
159	P74627	transposase	14737	10.28	NA	Cytoplasmic	-0.598	0	NS		65 (2/3, 18%, 0.26)
160	P74195	transposase	13924	10.07	NA	Cytoplasmic	-0.800	0	NS		65 (2/3, 20%, 0.28)
161	P72938	extracellular nuclease	196415	3.77	integral to membrane	Unknown	-0.048	0	NS		334 (11/13, 10%, 0.03)
162	P54735	eukaryotic protein kinase	55180	6.55	NA	Cytoplasmic membrane	-0.333	1	NS		61 (2/5, 3%, 0.03)
163	P72929	hypothetical protein sll1021	74378	5.08	Membrane; Single-pass membrane protein	Unknown	-0.360	1	NS		98 (3/3, 6%, 0.09)

164	P72991	cell division protein; FtsH	67209	5.11	Cell membrane; Multi-pass membrane protein	Cytoplasmic membrane	-0.169	2	Spl	91 (3/3, 9%, 0.05)
165	P73530	30S ribosomal protein S1	36547	4.57	ribosome	Cytoplasmic	-0.305	0	NS	134 (4/5, 14%, 0.09)
166	P73609	hypothetical protein slr1859	11982	4.69	NA	Unknown	0.117	0	NS	62 (2/2, 25%, 0.29)
167	P74367	photosystem II 11 kD protein	14908	8.80	plasma membrane-derived thylakoid	Unknown	-0.326	0	SplI	63 (2/2, 17%, 0.23)
168	Q55954	hypothetical protein sll0780	10916	5.58	NA	Unknown	0.001	0	NS	62 (2/8, 10%, 0.32)
169	Q55923	hypothetical protein slr0316	28734	8.76	NA	Cytoplasmic membrane	0.984	6	NS	165 (5/6, 25%, 0.08)
170	Q6YRQ9	hypothetical protein slr6095	53499	5.77	NA	Cytoplasmic	-0.334	0	NS	98 (3/3, 8%, 0.04)
171	Q55517	hypothetical protein sll0529	78267	5.36	NA	Unknown	-0.066	0	NS	67 (2/2, 5%, 0.04)
172	Q55770	hypothetical protein sll0185	46964	4.99	NA	Unknown	-0.424	0	NS	60 (2/2, 8%, 0.07)
173	P74145	hypothetical protein sll1390	27377	4.61	plasma membrane-derived thylakoid	Unknown	0.095	2	Spl	40 (1/1, 7%, 0.12)
174	P74339	hypothetical protein slr1624	50977	4.25	NA	Unknown	-0.424	1	Spl	64 (2/2, 6%, 0.06)
175	P72596	hypothetical protein sll1204	45987	9.10	NA	Cytoplasmic membrane	0.917	12	NS	30 (1/1, 2%, 0.07)
176	Q55176	hypothetical protein slr0483	16874	6.59	NA	Unknown	-0.007	2	NS	30 (1/1, 9%, 0.20)
177	P74643	phosphoglucomutase	61087	5.71	NA	Unknown	-0.195	0	NS	184 (6/6, 13%, 0.03)