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Protein profile of the HeLa cell line

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Abstract

HeLa cells are widely used for all kinds of in vitro studies in biochemistry, biology and medicine. Knowledge on protein expression is limited and no comprehensive study on the proteome of this cell type has been reported so far. We applied proteomics technologies to analyze the proteins of the HeLa cell line. The proteins were analyzed by two-dimensional (2D) gel electrophoresis and identified by matrix-assisted laser desorption ionization mass spectrometry (MS) on the basis of peptide mass fingerprinting, following in-gel digestion with trypsin. Approximately 3000 spots, excised from six two-dimensional gels, were analyzed. The analysis resulted in the identification of about 1200 proteins that were the products of 297 different genes. The HeLa cell database includes proteins with important functions and unknown functions, representing today one of the largest two-dimensional databases for eukaryotic proteomes and forming the basis for future expressional studies at the protein level.
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Keywords: HeLa cell line; Proteomics; Two-dimensional database; Proteins

1. Introduction

HeLa cells are well-documented and widely applied in biochemical, biological and medical experiments. Information on their proteome, however, is limited. The advent of proteomics technologies allowed generation of protein profiling and the construction of two-dimensional (2D) protein databases. Proteomics provides information in a high-throughput mode about the state of the gene products of a proteome as well as changes, resulting from disorders or the effect of external factors. Proteomics has as goal the discovery of novel drug targets and diagnostic markers. It usually comprises two steps, analysis of a protein mixture by 2D electrophoresis and identification of the proteins by mass spectrometry (MS) or other analytical methods [1,2]. 2D protein databases are useful tools in the quantification of differences in the protein levels because of various diseases by providing information on the protein identity and abundance.

We have applied proteomics technologies before in the study of neurological disorders, like Alzheimer's disease and Down syndrome [3–7] and constructed 2D databases for human and rat brain proteins, each including several hundreds of different gene products [8–12]. We further used proteomics to analyze the HeLa cell line and to investigate the role of the tuberous sclerosis (TSC) genes in the regulation of other proteins by studying differential protein expression in control and cell lines expressing the TSC proteins [13,14]. TSC is an autosomal dominantly inherited tumor syndrome characterized by mental retardation, epilepsy and the development of different growths, including cortical tubers [15]. Two genes have been shown to be responsible for the disease, TSC1, encoding hamartin, and TSC2, encoding tuberin [16,17]. The proteins exert regulatory functions in the cell cycle machinery [18,19] and regulate cell size control [20,21].

The currently available 2D databases for HeLa proteins include a low number of gene products. Shaw et al. [22] identified 21 proteins of the cell line and constructed a partial 2D polyacrylamide gel electrophoresis database. Decker et al. [23] added a series of 31 proteins to this information. Improved technologies, involving robot systems that

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enable high-throughput spot-picking, protein digestion and automated mass spectra acquisition and protein identification allowed us to create a more comprehensive HeLa protein map, representing an analytical tool and a database for further proteomic work. Here, we report the construction of a 2D protein database for the HeLa cell line, including 297 different gene products, one of the largest 2D protein databases for eukaryotic proteomes today.

2. Experimental

2.1. Materials

Immobilized pH-gradient (IPG) strips and IPG buffers were purchased from Amersham Pharmacia Biotechnology (Uppsala, Sweden). Acrylamide–piperazinediacrylamide (PDA) solution (37.5:1, w/v) was purchased from Biosolve (Valkenswaard, The Netherlands). CHAPS was obtained from Roche Diagnostics (Mannheim, Germany), urea from AppliChem (Darmstadt, Germany), thiourea from Fluka (Buchs, Switzerland), 1,4-dithioerythritol (DTE) from Merck (Darmstadt, Germany), tributylphosphine (TBP) from Pierce (Rockford, IL, USA) and the other reagents for the polyacrylamide gel preparation from Bio-Rad Labs. (Hercules, CA, USA). IPG strips were frozen at −20 °C and the other reagents were kept at 4 °C.

2.2. Sample preparation

HeLa cells (human cervical carcinoma cells) were obtained from the American Type Culture Collection (Manassas, VA, USA) and were grown in Dulbecco's modified Eagle's medium supplemented with 10% bovine serum and antibiotics (30 mg/l penicillin, 50 mg/l streptomycin sulphate) at 37 °C and 7% CO_2. As most studies in HeLa cells are addressing transfectional studies, transfection with the vector only was performed using the lipofectamine reagent from Invitrogen (Life Technologies, Lofer, Austria), following the transfection protocol provided by the supplier as previously described [24]. Forty-eight hours after transfection, the cells were harvested, washed three times in phosphate buffered saline and pellets were kept frozen at −70 °C until use.

To each tube, 0.8 ml of 20 mM Tris, containing 7 M urea, 2 M thiourea, 4% 3-[(cholamido propyl)dimethylamino]-1-propanesulfonate (CHAPS), 10 mM 1,4-dithioerythritol, 1 mM EDTA and protease inhibitors [1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 tablet Complete (Roche Diagnostics) per 50 ml of suspension buffer] and phosphatase inhibitors (0.2 mM Na_2VO_3 and 1 mM NaF) were added. Homogenization was performed with sonication twice, for 20 s each time. The suspension was centrifuged at 30 000 × *g* for 30 min and the supernatant was applied onto the IPG strips. The protein content was determined by the Coomassie blue method [25].

2.3. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was performed as reported [26,27]. Samples of 1.0 mg total protein were applied on immobilized pH 3–7 linear and 3–10 non-linear gradient strips in sample cups at their basic and acidic ends. Focusing started at 200 V and the voltage was gradually increased to 5000 V at 3 V/min using a computer-controlled power supply and kept constant for a further 4 h. The second-dimensional separation was performed on 12% sodium dodecyl sulfate (SDS)–polyacrylamide gels (180 mm × 200 mm × 1.5 mm) run at 40 mA per gel. After protein fixation for 12 h in 40% methanol, containing 5% phosphoric acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 24 h. Molecular masses were determined by running standard protein markers and p*I* values were used as given by the supplier of the IPG strips. Excess of dye was washed out from the gels with water and the gels were scanned in an Agfa Duoscan densitometer (resolution 200). Electronic images of the gels were recorded using Photoshop (Adobe) software. The images were stored as both tiff (about 5 MB/file) and jpeg (about 50 KB/file) formats.

2.4. Matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS)

MALDI-MS analysis was essentially performed as described [4,28]. The spots were excised and destained with 30% acetonitrile in 50 mM ammonium hydrogen carbonate and dried in a Speedvac evaporator. Each dried gel piece was rehydrated with 5 μl of 1 mM ammonium bicarbonate, containing 50 ng trypsin (Roche Diagnostics). After 16 h at room temperature, 20 μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid were added to each gel piece and incubated for 15 min with constant shaking. Sample application to the sample target was performed with a Cy-Well apparatus (Cybio, Jena, Germany). Peptide mixture (1.5 μl) was simultaneously applied with 1 μl of matrix solution, consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma) and the standard peptides des-Arg-bradykinin (Sigma, M_r 904.4681) and adrenocorticotropic hormone fragments 18–39 (Sigma, M_r 2465.1989) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Ultraflex, Bruker Daltonics, Bremen, Germany). Peptide matching and protein searches were performed automatically with the use of laboratory-developed software [29]. The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. The probability of a false positive match with a given MS spectrum was determined for each analysis. Unmatched peptides or miscleavage sites were not considered.

3. Results

3.1. Two-dimensional electrophoretic analysis and protein abundance

The proteins of the HeLa cell line were solubilized in the isoelectrofocusing-compatible reagents, urea, thiourea and CHAPS and analyzed by 2D gels. The 2D electrophoretic separation was performed on narrow and broad pH range IPG strips. Protein spots were visualized following stain with colloidal Coomassie blue. Figs. 1 and 2 show representative examples of HeLa proteins separated on a pH 3–7 and pH 3–10 IPG gel, respectively. On each gel, 1.0 mg of total protein was applied. The most abundant proteins were tubulin chains (P07437), heat shock protein hsp 90-α (hsp 86, P07900), heat shock cognate M_r 71 000 (P11142), nucleophosmin (P06748), glyceraldehyde 3-phosphate dehydrogenase (P04406) and α-enolase (P06733). The less abundant proteins were mainly hypothetical proteins, enzymes as well as structural proteins. Several high-abundance proteins, like serum albumin (P02769), protein α-2-hs-glycoprotein (fetuin, P12763) and α-1-antiproteinase (α-1-antitrypsin,

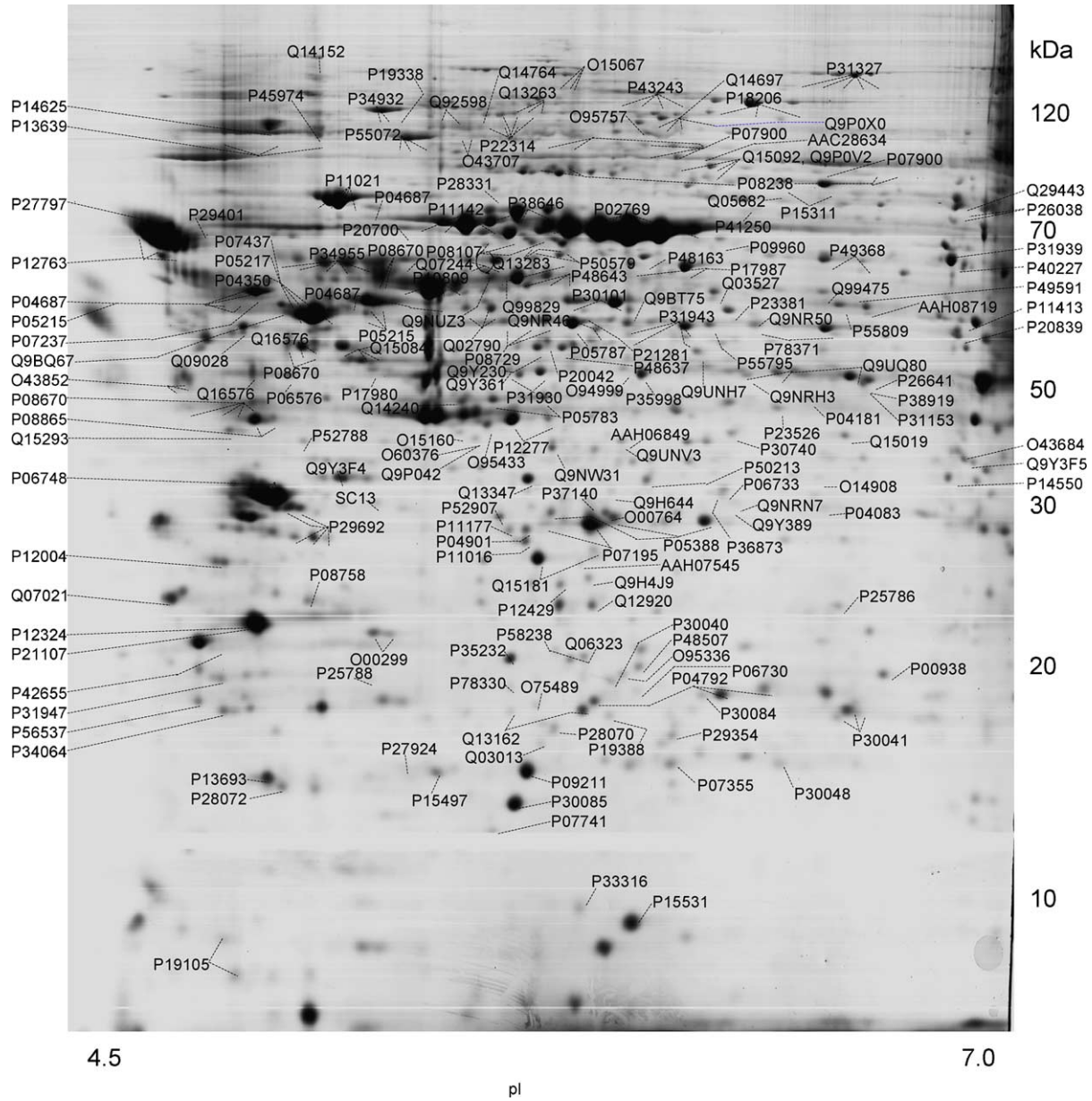

Fig. 1. Two-dimensional map of the HeLa cell line proteins. The proteins from control cultures were separated on a pH 3–7 linear IPG strip, followed by a 12% SDS–polyacrylamide gel, as stated in Section 2. The gel was stained with Coomassie blue. The spots were analyzed by MALDI-MS. The proteins identified are designated with their accession numbers. The names of the proteins are listed in Table 1. An electronic version of the map will be sent to the readers on request.

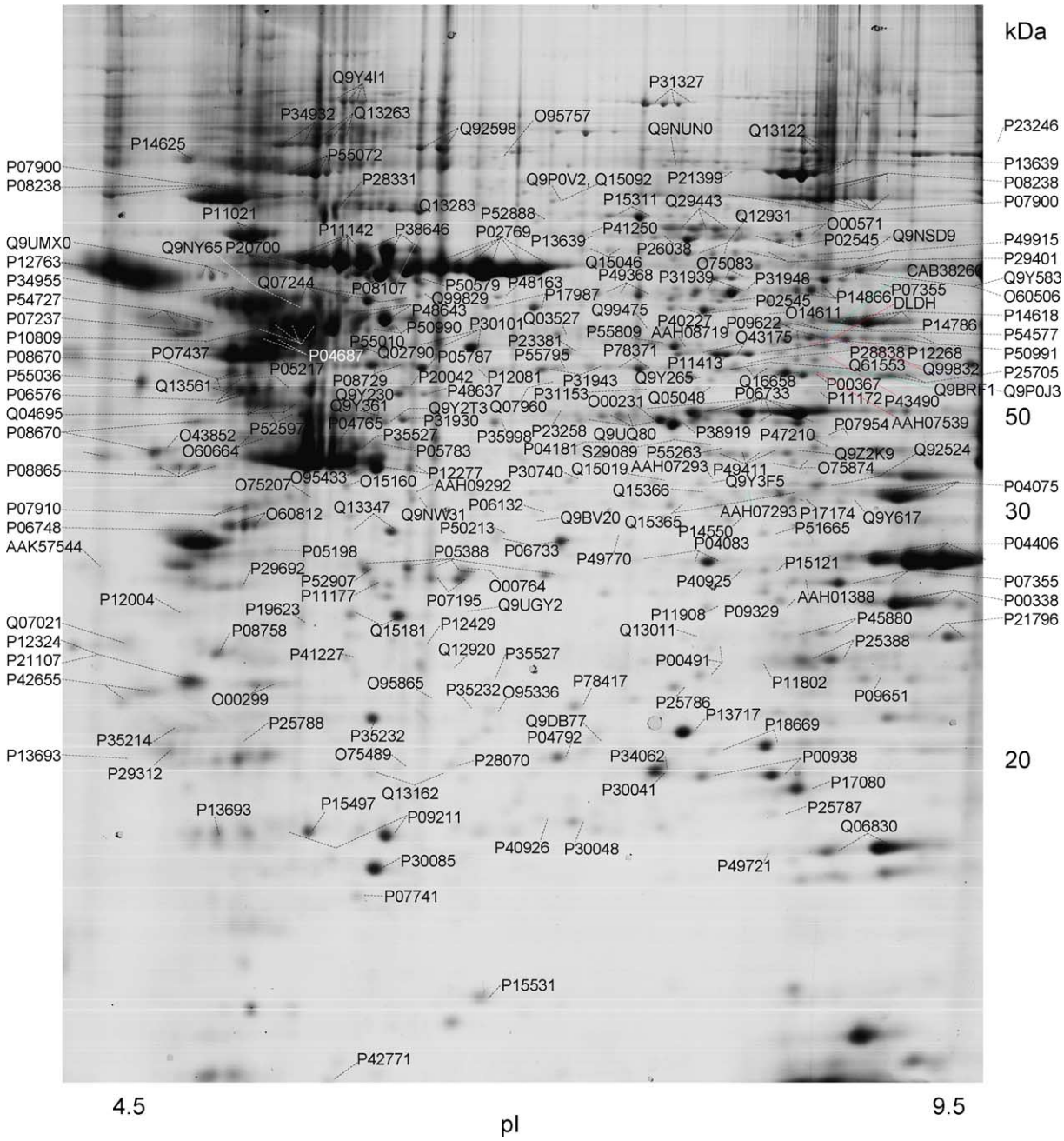

Fig. 2. Two-dimensional map of the HeLa cell line proteins. The proteins from control cultures were separated on a pH 3–10 non-linear IPG strip, followed by a 12% SDS–polyacrylamide gel, as stated in Section 2. The gel was stained with Coomassie blue. The spot analysis was performed as stated under legend to Fig. 1.

P34955), were of the bovine species and derived from the bovine serum of the culture medium.

3.2. Protein identification

Proteins were identified by MALDI-MS on the basis of peptide mass matching [30], following in-gel digestion with trypsin. About 500 spots were excised from each of six gels. Each excised spot was analyzed individually. The peptide masses were matched with the theoretical peptide masses of human proteins and if not successful, with all known proteins from all species. Totally about 3000 spots were analyzed and the MS analysis resulted in the identification of about 1200 polypeptides, which were the products of 297 different genes (Table 1). Identity could be assigned to about 40% of the analyzed spots. For about 50% of the unidentified spots, good MS data were collected, but no identity could be assigned. This may be due to insufficient precision in

Table 1
HeLa cell line proteins

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
AAC28634	PATCHX:AAC28634	Unknown, homo sapiens (human)	5.98	52897	6	3.35E-05	Fig. 1
AAH01388	SWTR_HUM:AAH01388	Annexin A2	7.82	38807	5	5.35E-05	Fig. 2
AAH06849	SWTR_HUM:AAH06849	Similar to RIKEN cDNA 2410044K02 gene	6.01	39431	6	3.74E-06	Fig. 1
AAH07293	SWTR_HUM:AAH07293	Unknown (protein for MGC:15668)	7.03	39703	9	1.00E-12	Fig. 2
AAH07539	SWTR_HUM:AAH07539	Unknown (protein for IMAGE:3029477) (fragment)	7.21	55728	7	1.00E-07	Fig. 2
AAH07545	SWTR_HUM:AAH07545	Unknown (protein for MGC:15444)	5.75	35290	6	3.48E-05	Fig. 1
AAH08719	SWTR_HUM:AAH08719	Nuclear matrix protein NMP200 related to splicing factor PRP19	6.60	55602	7	2.10E-09	Figs. 1 and 2
AAH09292	SWTR_HUM:AAH09292	COP9 (constitutive photomorphogenic), subunit 4 (arabidopsis)	5.71	46524	7	4.60E-08	Fig. 2
AAK57544	SWTR_HUM:AAK57544	NAC alpha (nascent-polypeptide-associated complex alpha polypeptide) (Q13765)	4.34	23369	4	1.00E-04	Fig. 2
CAB38260	SWTR_HUM:CAB38260	DJ149A16.6 (novel protein, human ortholog of worm F16A11.2 and bacterial and archea-bacterial predicted proteins)	7.20	55688	5	5.73E-05	Fig. 2
DLDH	HUMANGP:CHR7-DLDH	Dihydrolipoamide dehydrogenase, mitochondrial (EC 1.8.1.4) was used to identify this gene	7.50	53116	5	3.39E-05	Fig. 2
O00231	SWTR_HUM:O00231	Proteasome subunit P44.5	6.44	47719	5	1.00E-04	Fig. 2
O00299	SW:CLI1_HUMAN	Chloride intracellular channel protein 1 (nuclear chloride ion channel 27) (p64 clcp)	4.86	27248	7	1.24E-09	Figs. 1 and 2
O00571	SW:DDX3_HUMAN	Dead box protein 3 (helicase-like protein 2)	7.18	73597	9	1.92E-13	Fig. 2
O00764	SW:PDXK_HUMAN	Pyridoxine kinase (EC 2.7.1.35) (pyridoxal kinase)	6.07	35307	8	1.20E-11	Figs. 1 and 2
O14611	SWTR_HUM:O14611	HPAST (EH-domain containing protein 1)	6.99	60720	5	1.00E-04	Fig. 2
O14908	SW:GIPC_HUMAN	Gaip C-terminus interacting protein gipc (rgs-gaip interacting protein) (tax interaction protein 2) (tip-2)	6.22	36140	4	2.43E-05	Fig. 1
O15067	SW:PUR4_HUMAN	Phosphoribosylformylglycinamidine synthase (EC 6.3.5.3) (fgams) (formylglycinamide ribotide amidotransferase)	5.62	146226	11	1.00E-13	Fig. 1
O15160	SW:RPA5_HUMAN	DNA-directed rna polymerase i M_r 40 000 polypeptide (EC 2.7.7.6) (rpa40) (rpa39)	5.27	39453	6	1.45E-09	Figs. 1 and 2
O43175	SW:SERA_HUMAN	D-3-Phosphoglycerate dehydrogenase (EC 1.1.1.95) (pgdh)	6.69	57369	7	1.00E-07	Fig. 2
O43684	SW:BUB3_HUMAN	Mitotic checkpoint protein bub3	6.83	37587	5	6.56E-05	Fig. 1
O43707	SW:AAC4_HUMAN	Actinin 4 (non-muscle α-actinin 4) (f-actin cross linking protein)	5.22	105244	12	2.71E-15	Fig. 1
O43852	SW:CALU_HUMAN	Calumenin precursor	4.31	37197	5	1.50E-07	Figs. 1 and 2
O60376	SWTR_HUM:O60376	P1.11659_4 (stomatin (EPB72)-like 2)	6.82	38839	4	3.14E-05	Fig. 1
O60506	SWTR_HUM:O60506	GRY-RBP	9.18	69817	6	6.95E-05	Fig. 2
O60664	SW:TI47_HUMAN	Cargo selection protein tip47 (M_r 47 000 mannose 6-phosphate receptor-binding protein) (M_r 47 000 mpr-binding protein)	5.21	47174	5	1.00E-04	Fig. 2
O60812	HUMANGP:CHR14-O60812	DJ845O24.4 (heterogenous nuclear ribonucleoprotein hnrnp c1-like protein). Was used to identify this gene	4.78	32374	5	1.00E-05	Fig. 2
O75083	SW:WDR1_HUMAN	WD-repeat protein 1 (actin interacting protein 1) (nori-1)	6.64	66836	9	1.08E-12	Fig. 2
O75207	SWTR_HUM:O75207	Hypothetical M_r 33 600 protein (CUA001)	5.40	34131	5	1.00E-04	Fig. 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
O75489	SW:NUGM_HUMAN	NADH-ubiquinone oxidoreductase M_r 30 000 subunit (EC 1.6.5.3) (EC 1.6.99.3) (complex i-30 000) (ci-30 000)	7.57	30336	7	8.92E-10	Figs. 1 and 2
O75874	SW:IDHC_HUMAN	Isocitrate dehydrogenase [NADP] cytoplasmic (EC 1.1.1.42) (oxalosuccinate decarboxylase) (NADP+-specific icdh)	6.79	46943	6	6.43E-09	Fig. 2
O94999	SWTR_HUM:O94999	R30923_1 (fragment)	7.11	63399	5	1.50E-05	Fig. 1
O95336	SW:6PGL_HUMAN	6-Phosphogluconolactonase (EC 3.1.1.31) (6pgl)	5.98	27814	7	1.52E-10	Figs. 1 and 2
O95433	SW:C143_HUMAN	Protein c14orf3 (protein hspc322) (activator of M_r 90 000 heat shock protein ATPase homolog 1)	5.33	38421	7	1.00E-08	Figs. 1 and 2
O95757	SW:OS94_HUMAN	Osmotic stress protein 94 (heat shock 70-related protein apg-1)	5.73	95471	8	7.63E-07	Figs. 1 and 2
O95865	SW:DDH2_HUMAN	NG,-NG-dimethylarginine dimethylaminohydrolase 2 (EC 3.5.3.18) (dimethylarginine dimethylaminohydrolase 2)	5.93	29910	5	1.00E-04	Fig. 2
P00338	SW:LDHM_HUMAN	L–Lactate dehydrogenase m chain (EC 1.1.1.27) (ldh-a)	8.34	36819	7	1.00E-05	Fig. 2
P00367	SW:DHE3_HUMAN	Glutamate dehydrogenase 1 precursor (EC 1.4.1.3) (gdh)	7.83	61701	6	7.46E-08	Fig. 2
P00491	SW:PNPH_HUMAN	Purine nucleoside phosphorylase (EC 2.4.2.1) (inosine phosphorylase) (pnp)	6.94	32355	8	1.00E-11	Fig. 2
P00938	SW:TPIS_HUMAN	Triosephosphate isomerase (EC 5.3.1.1) (tim)	6.89	26806	7	1.17E-11	Figs. 1 and 2
P02545	SW:LAMA_HUMAN	Lamin a (M_r 70 000 lamin)	7.01	74379	8	1.00E-07	Fig. 2
P02769	SW:ALBU_BOVIN	Serum albumin precursor	6.11	71244	4	2.12E-04	Figs. 1 and 2
P04075	SW:ALFA_HUMAN	Fructose-bisphosphate aldolase (EC 4.1.2.13) a (muscle)	8.07	39720	7	1.74E-10	Fig. 2
P04083	SW:ANX1_HUMAN	Annexin I (lipocortin i) (calpactin ii) (chromobindin 9) (p35) (phospholipase A2 inhibitory protein)	7.02	38787	8	1.16E-08	Figs. 1 and 2
P04181	SW:OAT_HUMAN	Ornithine aminotransferase precursor (EC 2.6.1.13) (ornithine-oxo-acid aminotransferase)	7.04	48846	5	1.00E-07	Figs. 1 and 2
P04350	SW:TBB5_HUMAN	Tubulin β-5 chain	4.65	50055	4	5.68E-05	Fig. 1
P04406	SW:G3P2_HUMAN	Glyceraldehyde 3-phosphate dehydrogenase, liver (EC 1.2.1.12)	8.73	36070	6	6.80E-09	Fig. 2
P04687	SW:TBA1_HUMAN	Tubulin α-1 chain, brain-specific	4.90	50809	11	7.30E-20	Figs. 1 and 2
P04765	SW:IF41_HUMAN	Eukaryotic initiation factor 4a-i (eif-4a-i)	5.22	46352	6	1.50E-06	Fig. 2
P04792	SW:HS27_HUMAN	Heat shock M_r 27 000 protein (hsp 27) (stress-responsive protein 27) (srp27) (estrogen-regulated M_r 24 000 protein)	8.14	22427	6	2.21E-06	Figs. 1 and 2
P04901	SW:GBB1_HUMAN	Guanine nucleotide-binding protein g(i)/g(s)/g(t) beta s	5.87	38151	5	1.15E-04	Fig. 1
P05198	SW:IF2A_HUMAN	Eukaryotic translation initiation factor 2 α subunit (eif-2-α)	4.84	36243	5	1.00E-04	Fig. 2
P05215	SW:TBA4_HUMAN	Tubulin α-4 chain	4.80	50633	8	1.17E-11	Fig. 1
P05217	SW:TBB2_HUMAN	Tubulin β-2 chain	4.63	50255	6	2.87E-08	Figs. 1 and 2
P05388	SW:RLA0_HUMAN	60S acidic ribosomal protein p0 (l10e)	5.84	34422	7	8.75E-09	Figs. 1 and 2
P05783	SW:K1CR_HUMAN	Keratin, type i cytoskeletal 18 (cytokeratin 18) (k18) (ck 18)	5.23	47897	7	1.19E-07	Figs. 1 and 2
P05787	SW:K2C8_HUMAN	Keratin, type ii cytoskeletal 8 (cytokeratin 8) (k8) (ck 8)	5.83	53510	8	1.00E-09	Figs. 1 and 2
P06132	SW:DCUP_HUMAN	Uroporphyrinogen decarboxylase (EC 4.1.1.37) (uro-d)	6.08	41102	7	1.18E-06	Fig. 2
P06576	SW:ATPB_HUMAN	ATP synthase β chain, mitochondrial precursor (EC 3.6.1.34)	5.17	56524	9	4.23E-13	Figs. 1 and 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P06730	SW:IF4E_HUMAN	Eukaryotic translation initiation factor 4E (eif-4e) (eif4e) (mrna cap-binding protein) (eif-4f M_r 25 000 subunit)	6.07	25309	5	1.02E-04	Fig. 1
P06733	SW:ENOA_HUMAN	Alpha enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (non-neural enolase) (phosphopyruvate hydratase)	7.39	47350	8	8.41E-12	Figs. 1 and 2
P06748	SW:NPM_HUMAN	Nucleophosmin (npm) (nucleolar phosphoprotein b23) (numatrin) (nucleolar protein no38)	4.48	32725	5	1.00E-04	Figs. 1 and 2
P07195	SW:LDHH_HUMAN	L-Lactate dehydrogenase h chain (EC 1.1.1.27) (ldh-b)	5.96	36769	9	1.26E-12	Figs. 1 and 2
P07237	SW:PDI_HUMAN	Protein disulfide isomerase (pdi) (EC 5.3.4.1)/prolyl 4-hydroxylase beta subunit (EC 1.14.11.2)	4.59	57479	6	8.69E-08	Figs. 1 and 2
P07355	SW:ANX2_HUMAN	Annexin II (lipocortin ii) (calpactin i heavy chain) (chromobindin 8) (placental anticoagulant protein iv)	7.82	38676	6	7.46E-09	Figs. 1 and 2
P07437	SW:TBB1_HUMAN	Tubulin β-1 chain	4.59	50240	7	3.35E-08	Figs. 1 and 2
P07741	SW:APT_HUMAN	Adenine phosphoribosyltransferase (EC 2.4.2.7) (aprt)	5.88	19635	6	1.70E-09	Figs. 1 and 2
P07900	SW:HS9A_HUMAN	Heat shock protein hsp 90-alpha (hsp 86)	4.77	84888	9	1.34E-10	Figs. 1 and 2
P07910	SW:ROC_HUMAN	Heterogeneous nuclear ribonucleoproteins c1/c2 (hnrnp c1 and hnrnp c2)	4.95	33335	5	1.00E-06	Fig. 2
P07954	SW:FUMH_HUMAN	Fumarate hydratase, mitochondrial precursor (EC 4.2.1.2) (fumarase)	9.36	54773	6	1.00E-09	Fig. 2
P08107	SW:HS71_HUMAN	Heat shock M_r 70 000 protein 1 (hsp70.1) (hsp70-1/hsp70-2)	5.41	70294	7	9.25E-08	Figs. 1 and 2
P08238	SW:HS9B_HUMAN	Heat shock protein hsp 90-β (hsp 84) (hsp 90)	4.80	83453	9	1.56E-09	Figs. 1 and 2
P08670	SW:VIME_HUMAN	Vimentin	4.89	53579	10	1.65E-14	Figs. 1 and 2
P08729	SW:K2C7_HUMAN	Keratin, type ii cytoskeletal 7 (cytokeratin 7) (k7) (ck 7)	5.25	51286	5	4.92E-05	Figs. 1 and 2
P08758	SW:ANX5_HUMAN	Annexin v (lipocortin v) (endonexin ii) (calphobindin i) (cbp-i) (placental anticoagulant protein i) (pap-i) (pp4)	4.76	35840	9	5.82E-14	Fig. 2
P08865	SW:RSP4_HUMAN	40S ribosomal protein sa (p40) (M_r 34 000/67 000 laminin receptor) (colon carcinoma laminin-binding protein) (nem/1chd4)	4.62	32947	5	3.83E-06	Figs. 1 and 2
P09211	SW:GTP_HUMAN	Glutathione *S*-transferase p (EC 2.5.1.18) (class-pi) (gstp1-1)	5.38	23438	7	1.00E-10	Figs. 1 and 2
P09329	SW:KPR1_HUMAN	Ribose-phosphate pyrophosphokinase I (EC 2.7.6.1) (phosphoribosyl pyrophosphate synthetase i) (ppribp) (prs-i)	6.95	35194	6	1.50E-07	Fig. 2
P09622	SW:DLDH_HUMAN	Dihydrolipoamide dehydrogenase precursor (EC 1.8.1.4)	7.65	54686	7	1.00E-08	Fig. 2
P09651	SW:ROA1_HUMAN	Heterogeneous nuclear ribonucleoprotein a1 (helix-destabilizing protein) (single-strand binding protein)	10.06	38806	6	1.00E-05	Fig. 2
P09960	SW:LKHA_HUMAN	Leukotriene a-4 hydrolase (EC 3.3.2.6) (lta-4 hydrolase) (leukotriene a(4) hydrolase)	6.12	69737	5	5.87E-06	Fig. 1
P10809	SW:P60_HUMAN	Mitochondrial matrix protein P1 (p60 lymphocyte protein) (M_r 60 000 chaperonin) (heat shock protein 60) (hsp-60)	5.64	61187	6	1.88E-08	Figs. 1 and 2
P11016	SW:GBB2_HUMAN	Guanine nucleotide-binding protein g(I)/g(s)/g(t) β subunit 2 (transducin β chain 2)	5.87	38048	6	1.17E-05	Fig. 1

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P11021	SW:GR78_HUMAN	M_r 78 000 glucose-regulated protein precursor (grp 78) (immunoglobulin heavy chain binding protein) (bip)	4.87	72185	11	1.11E-16	Figs. 1 and 2
P11142	SW:HS7C_HUMAN	Heat shock cognate M_r 71 000 protein	5.26	71082	9	4.83E-12	Figs. 1 and 2
P11172	SW:PYR5_HUMAN	Uridine 5′-monophosphate synthase (orotate P-ribosyltransf (EC 2.4.2.10), orotidine 5′-P decarboxyl (EC 4.1.1.23))	7.22	52644	6	1.00E-08	Fig. 2
P11177	SW:ODPB_HUMAN	Pyruvate dehydrogenase e1 component, beta subunit precursor (EC 1.2.4.1) (pdhe1-b)	6.63	39536	6	7.85E-08	Figs. 1 and 2
P11413	SW:G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase (EC 1.1.1.49) (g6pd)	6.87	59553	5	9.11E-07	Figs. 1 and 2
P11802	SW:CDK4_HUMAN	Cell division protein kinase 4 (EC 2.7.1.-) (cyclin-dependent kinase 4) (psk-j3)	7.00	33936	4	2.73E-05	Fig. 2
P11908	SW:KPR2_HUMAN	Ribose-phosphate pyrophosphokinase II (EC 2.7.6.1) (phosphoribosyl pyrophosphate synthetase ii) (ppribp) (prs-ii)	6.60	35015	4	6.68E-05	Fig. 2
P12004	SW:PCNA_HUMAN	Proliferating cell nuclear antigen (pcna) (cyclin)	4.41	29092	8	1.00E-07	Figs. 1 and 2
P12081	SW:SYH_HUMAN	Histidyl-trna synthetase (EC 6.1.1.21) (histidine--trna ligase) (hisrs)	5.66	57944	7	1.00E-07	Fig. 2
P12268	SW:IMD2_HUMAN	Inosine-5′-monophosphate dehydrogenase 2 (EC 1.1.1.205) (imp dehydrogenase 2) (impdh-ii) (impd 2)	6.89	56225	7	1.50E-05	Fig. 2
P12277	SW:KCRB_HUMAN	Creatine kinase, b chain (EC 2.7.3.2) (b-ck)	5.37	42902	7	1.15E-06	Figs. 1 and 2
P12324	SW:TPMN_HUMAN	Tropomyosin, cytoskeletal type (tm30-nm)	4.57	29242	6	1.50E-07	Figs. 1 and 2
P12429	SW:ANX3_HUMAN	Annexin III (lipocortin iii) (placental anticoagulant protein iii) (pap-iii) (35-α calcimedin)	5.76	36523	7	2.38E-08	Figs. 1 and 2
P12763	SW:A2HS_BOVIN	α-2-hs-Glycoprotein precursor (fetuin)	5.26	39192	6	1.50E-07	Figs. 1 and 2
P13639	SW:EF2_HUMAN	Elongation factor 2 (ef-2)	6.81	96246	10	7.71E-14	Figs. 1 and 2
P13693	SW:TCTP_HUMAN	Translationally controlled tumor protein (tctp) (p23)	4.68	19696	5	1.16E-05	Figs. 1 and 2
P13717	SW:NUCA_SERMA	Nuclease precursor (EC 3.1.30.2) (endonuclease)	7.43	29154	8	8.61E-11	Fig. 2
P14550	SW:ALDX_HUMAN	Alcohol dehydrogenase (nadp(+)) (EC 1.1.1.2) (aldehyde reductase)	6.78	36760	7	1.87E-06	Figs. 1 and 2
P14618	SW:KPY1_HUMAN	Pyruvate kinase, M1 (muscle) isozyme (EC 2.7.1.40) (cytosolic thyroid hormone-binding protein) (cthbp) (thbp1)	7.63	58280	7	1.28E-07	Fig. 2
P14625	SW:ENPL_HUMAN	Endoplasmin 6 (M_r 94 000 glucose-regulated protein) (grp94) (gp96 homolog) (tumor rejection antigen 1)	4.59	92696	8	1.99E-09	Figs. 1 and 2
P14786	SW:KPY2_HUMAN	Pyruvate kinase, M2 isozyme (EC 2.7.1.40)	7.80	58316	6	1.00E-04	Fig. 2
P14866	SW:ROL_HUMAN	Heterogeneous nuclear ribonucleoprotein l (hnrnp l)	7.11	60719	8	1.00E-09	Fig. 2
P15121	SW:ALDR_HUMAN	Aldose reductase (EC 1.1.1.21) (ar) (aldehyde reductase)	6.97	36098	6	1.00E-07	Fig. 2
P15311	SW:EZRI_HUMAN	Ezrin (p81) (cytovillin) (villin-2)	6.21	69338	6	1.66E-06	Figs. 1 and 2
P15497	SW:APA1_BOVIN	Apolipoprotein A-I precursor (apo-ai)	5.84	30257	7	8.24E-09	Figs. 1 and 2
P15531	SW:NDKA_HUMAN	Nucleoside diphosphate kinase A (EC 2.7.4.6) (ndk a) (tumor metastatic process-associated protein)	6.13	17308	6	7.05E-08	Figs. 1 and 2
P17080	SW:RAN_HUMAN	GTP-binding nuclear protein ran (tc4)	7.11	24509	5	9.66E-05	Fig. 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P17174	SW:AATC_HUMAN	Aspartate aminotransferase, cytoplasmic (EC 2.6.1.1) (transaminase a) (glutamate oxaloacetate transaminase-1)	7.21	46334	7	4.77E-09	Fig. 2
P17980	SW:PRSA_HUMAN	26S protease regulatory subunit s6a (tat-binding protein 1) (tbp-1)	4.92	49372	5	7.91E-05	Figs. 1 and 2
P17987	SW:TCPA_HUMAN	T-complex protein 1, alpha subunit (tcp-1-alpha) (cct-alpha)	6.00	60818	7	2.28E-07	Figs. 1 and 2
P18206	SW:VINC_HUMAN	Vinculin	5.94	117088	6	8.73E-08	Fig. 1
P18669	SW:PMGB_HUMAN	Phosphoglycerate mutase, brain form (EC 5.4.2.1) (pgam-b) (EC 5.4.2.4) (EC 3.1.3.13) (bpg-dependent pgam)	7.21	28768	7	1.97E-10	Fig. 2
P19105	SW:MLRM_HUMAN	Myosin regulatory light chain 2, non-sarcomeric (myosin rlc)	4.49	19707	4	1.50E-05	Fig. 1
P19338	SW:NUCL_HUMAN	Nucleolin (protein c23)	4.41	76224	5	1.50E-05	Fig. 1
P19388	SW:RPB5_HUMAN	DNA-directed RNA polymerase II M_r 23 000 polypeptide (EC 2.7.7.6) (rpb25) (xap4) (rpb5)	5.53	24710	5	1.00E-05	Fig. 1
P19623	SW:SPEE_HUMAN	Spermidine synthase (EC 2.5.1.16) (putrescine aminopropyltransferase) (spdsy)	5.27	34372	6	1.50E-08	Fig. 2
P20042	SW:IF2B_HUMAN	Eukaryotic translation initiation factor 2 β subunit (eif-2-beta)	5.56	38718	5	1.26E-05	Figs. 1 and 2
P20700	SW:LAM1_HUMAN	Lamin b1	4.94	66521	10	1.00E-12	Figs. 1 and 2
P20839	SW:IMD1_HUMAN	Inosine-5′-monophosphate dehydrogenase 1 (EC 1.1.1.205) (imp dehydrogenase 1) (impdh-i) (impd 1)	6.59	55813	8	2.28E-10	Fig. 1
P21107	SW:TPMI_MOUSE	Tropomyosin 5, cytoskeletal type	4.57	29230	5	1.00E-05	Figs. 1 and 2
P21281	SW:VAT2_HUMAN	Vacuolar ATP synthase subunit b, brain (EC 3.6.1.34) (endomembrane proton pump M_r 58 000 subunit) (v-ATPase b)	5.63	56823	6	5.67E-09	Fig. 1
P21399	SW:IRE1_HUMAN	Iron-responsive element binding protein 1 (ire-bp 1) (iron regulatory protein 1) (ferritin repressor protein)	6.67	98849	5	3.07E-07	Fig. 2
P21796	SW:POR1_HUMAN	Voltage-dependent anion-selective channel protein 1 (VDAC1) (outer mitochondrial membrane protein porin)	9.04	30736	7	1.00E-09	Fig. 2
P22314	SW:UBA1_HUMAN	Ubiquitin-activating enzyme E1 (a1s9 protein)	5.71	118798	10	1.00E-11	Fig. 1
P23246	SW:PSF_HUMAN	PTB-associated splicing factor (psf)	10.25	76215	6	1.00E-06	Fig. 2
P23258	SW:TBG_HUMAN	Tubulin γ chain	6.08	51507	5	1.00E-05	Fig. 2
P23381	SW:SYW_HUMAN	Tryptophanyl-tRNA synthetase (EC 6.1.1.2) (tryptophan—tRNA ligase) (trprs) (ifp53)	6.17	53473	7	1.50E-08	Figs. 1 and 2
P23526	SW:SAHH_HUMAN	Adenosylhomocysteinase (EC 3.3.1.1) (*S*-adenosyl-l-homocysteine hydrolase) (adohcyase)	6.44	48254	6	4.54E-07	Fig. 1
P25388	SW:GBLP_HUMAN	Guanine nucleotide-binding protein β subu	7.64	35510	9	1.10E-13	Fig. 2
P25705	SW:ATPA_HUMAN	ATP synthase α chain, mitochondrial precursor (EC 3.6.1.34)	9.93	59827	6	1.50E-08	Fig. 2
P25786	SW:PRC2_HUMAN	Proteasome component C2 (EC 3.4.99.46) (macropain subunit c2) (multicatalytic endopeptidase complex C2)	6.60	29821	6	1.50E-07	Fig. 1
P25787	SW:PRC3_HUMAN	Proteasome component C3 (ec 3.4.99.46) (macropain subunit c3) (multicatalytic endopeptidase complex C3)	7.51	25865	4	1.50E-07	Fig. 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P25788	SW:PRC8_HUMAN	Proteasome component C8 (EC 3.4.99.46) (macropain subunit c8) (multicatalytic endopeptidase complex C8)	5.07	28512	6	1.00E-08	Fig. 2
P26038	SW:MOES_HUMAN	Moesin (membrane-organizing extension spike protein)	6.35	67760	7	8.00E-07	Figs. 1 and 2
P26641	SW:EF1G_HUMAN	Elongation factor 1-γ (ef-1-γ)	6.64	50429	5	5.44E-05	Fig. 1
P27797	SW:CRTC_HUMAN	Calreticulin precursor (crp55) (calregulin) (hacbp) (erp60) (M_r 52 000 ribonucleoprotein autoantigen ro/ss-a)	4.13	48282	7	1.00E-09	Fig. 1
P27924	SW:UBC1_HUMAN	Ubiquitin-conjugating enzyme e2-25 000 (EC 6.3.2.19) (ubiquitin-protein ligase) (ubiquitin carrier protein)	5.21	22506	4	1.00E-04	Fig. 1
P28070	SW:PRCB_HUMAN	Proteasome β chain (EC 3.4.99.46) (macropain β chain) (multicatalytic endopeptidase complex β chain)	5.83	29230	4	1.16E-05	Figs. 1 and 2
P28072	SW:PRCD_HUMAN	Proteasome δ chain (EC 3.4.99.46) (macropain δ chain) (multicatalytic endopeptidase complex δ chain)	4.64	25527	5	1.31E-06	Fig. 1
P28331	SW:NUAM_HUMAN	NADH-ubiquinone oxidoreductase M_r 75 000 subunit (EC 1.6.5.3) (EC 1.6.99.3) (complex i-75 000)	5.98	80548	6	1.00E-07	Fig. 2
P28838	SW:AMPL_HUMAN	Cytosol aminopeptidase (EC 3.4.11.1) (leucine aminopeptidase) (lap) (EC 3.4.11.5) (prolyl aminopeptidase)	6.72	53005	6	1.00E-07	Fig. 2
P29312	SW:143Z_HUMAN	14-3-3 protein ζ/δ (protein kinase c inhibitor protein-1) (kcip-1) (factor-activating exoenzyme s) (fas)	4.55	27898	7	1.00E-09	Fig. 2
P29354	SW:GRB2_HUMAN	Growth factor receptor-bound protein 2 (grb2 adaptor protein) (ash protein)	6.27	25304	7	1.79E-10	Fig. 1
P29401	SW:TKT_HUMAN	Transketolase (EC 2.2.1.1) (tk)	7.63	68518	7	1.50E-08	Figs. 1 and 2
P29692	SW:EF1D_HUMAN	Elongation factor 1-δ (ef-1-δ)	4.80	31315	6	2.02E-05	Figs. 1 and 2
P30040	SW:ER29_HUMAN	Endoplasmic reticulum protein erp29 precursor (erp31) (erp28)	8.36	29054	4	6.56E-05	Fig. 1
P30041	SW:AOP2_HUMAN	Antioxidant protein 2 (EC 1.11.1.7) (M_r 24 000 protein) (liver 2d page spot 40/red blood cells page spot 12)	6.31	25002	6	1.12E-10	Figs. 1 and 2
P30048	SW:TDXM_HUMAN	Mitochondrial thioredoxin-dependent peroxide reductase precursor (antioxidant protein 1) (aop-1)	7.76	28017	6	6.35E-07	Figs. 1 and 2
P30084	SW:ECHM_HUMAN	Enoyl-CoA hydratase, mitochondrial (EC 4.2.1.17) (short chain enoyl-CoA hydratase) (sceh) (enoyl-CoA hydratase 1)	8.03	31807	5	1.70E-05	Fig. 1
P30085	SW:KCY_HUMAN	UMP-CMP kinase (EC 2.7.4.14) (cytidylate kinase) (deoxycytidylate kinase)	5.31	22436	4	1.00E-04	Figs. 1 and 2
P30101	SW:ER60_HUMAN	Probable protein disulfide isomerase er-60 (EC 5.3.4.1) (erp60) (M_r 58 000 microsomal protein) (p58) (grp58)	6.30	57145	6	5.14E-08	Figs. 1 and 2
P30740	SW:ILEU_HUMAN	Leukocyte elastase inhibitor (lei) (monocyte/neutrophil elastase inhibitor) (ei)	6.22	42828	4	1.24E-04	Figs. 1 and 2
P31153	SW:METK_HUMAN	*S*-Adenosylmethionine synthetase gamma form (EC 2.5.1.6) (methionine adenosyltransferase) (mat-ii)	6.46	43975	6	4.26E-07	Figs. 1 and 2
P31327	SW:CPSM_HUMAN	Carbamoyl-phosphate synthase [ammonia] mitochondrial (EC 6.3.4.16) (carbamoyl-phosphate synthetase i)	6.71	165975	13	2.55E-16	Figs. 1 and 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P31930	SW:UCR1_HUMAN	Ubiquinol-cytochrome-*c* reductase complex core protein i precursor (EC 1.10.2.2)	6.33	53269	6	5.44E-07	Figs. 1 and 2
P31939	SW:PUR9_HUMAN	Phosphoribosylaminoimidazole-carboxamide formyltransferase (EC 2.1.2.3)/imp cyclohydrolase (EC 3.5.4.10)	6.83	64938	7	2.09E-08	Figs. 1 and 2
P31943	SW:ROH1_HUMAN	Heterogeneous nuclear ribonucleoprotein h (hnrnp h)	6.26	49483	10	2.63E-14	Figs. 1 and 2
P31947	SW:143S_HUMAN	14-3-3 Protein σ (stratifin) (epithelial cell marker protein 1)	4.51	27870	8	1.50E-08	Fig. 1
P31948	SW:IEFS_HUMAN	Transformation-sensitive protein ief ssp 3521 (stress-induced-phosphoprotein 1, Hsp70/Hsp90-organizing protein)	6.78	63226	6	1.00E-07	Fig. 2
P33316	SW:DUT_HUMAN	Deoxyuridine 5'-triphosphate nucleotidohydrolase (EC 3.6.1.23) (dutpase) (dutp pyrophosphatase)	10.40	26974	7	4.03E-09	Fig. 1
P34062	SW:PRCI_HUMAN	Proteasome iota chain (EC 3.4.99.46) (multicatalytic endopeptidase complex iota) (M_r 27 000 prosomal prot.)	6.72	27837	5	1.00E-05	Fig. 2
P34064	SW:PRCZ_RAT	Proteasome ζ chain (EC 3.4.99.46) (macropain ζ chain) (multicatalytic endopeptidase complex ζ chain)	4.63	26545	5	1.00E-04	Fig. 1
P34932	SW:HS74_HUMAN	Heat shock M_r 70 000 protein 4 (hsp70ry) (fragment)	4.97	85330	10	7.27E-13	Figs. 1 and 2
P34955	SW:A1AT_BOVIN	α-1-Antiproteinase precursor (α-1-antitrypsin) (α-1-proteinase inhibitor)	6.51	46416	8	1.06E-10	Figs. 1 and 2
P35214	SW:143G_RAT	14-3-3 Protein γ (protein kinase c inhibitor protein-1) (kcip-1)	4.63	28324	6	1.00E-06	Fig. 2
P35232	SW:PHB_HUMAN	Prohibitin	5.55	29842	7	2.62E-10	Figs. 1 and 2
P35527	SW:K1CI_HUMAN	Keratin, type i cytoskeletal 9 (cytokeratin 9) (k9) (ck 9)	5.00	62177	5	8.15E-05	Fig. 2
P35998	SW:PRS7_HUMAN	26S protease regulatory subunit 7 (mss1 protein)	5.73	49002	10	9.86E-15	Figs. 1 and 2
P36873	SW:PP1G_HUMAN	Serine/threonine protein phosphatase pp1-γ catalytic subunit (EC 3.1.3.16) (pp-1g)	6.50	37701	4	1.00E-05	Fig. 1
P37140	SW:PP1B_HUMAN	Serine/threonine protein phosphatase pp1-β catalytic subunit (EC 3.1.3.16) (pp-1b)	6.08	37960	7	1.00E-09	Fig. 1
P38646	SW:GR75_HUMAN	Mitochondrial stress-70 protein (M_r 75 000 glucose-regulated protein) (grp 75) (peptide-binding protein 74) (pbp74)	6.22	74018	9	5.14E-12	Figs. 1 and 2
P38919	SW:IF4N_HUMAN	Eukaryotic initiation factor 4a-like nuk-34 (ha0659)	6.41	47088	5	3.79E-05	Figs. 1 and 2
P40227	SW:TCPZ_HUMAN	T-complex protein 1, ζ subunit (tcp-1-ζ) (cct-ζ) (tcp20) (htr3)	6.66	58443	8	1.00E-09	Figs. 1 and 2
P40925	SW:MDHC_HUMAN	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37)	7.38	36500	5	1.00E-06	Fig. 2
P40926	SW:MDHM_HUMAN	Malate dehydrogenase, mitochondrial precursor (EC 1.1.1.37)	8.81	35964	5	1.00E-05	Fig. 2
P41227	SW:ARDH_HUMAN	N-terminal acetyltransferase complex ard1 subunit homolog	5.47	26612	6	1.00E-07	Fig. 2
P41250	SW:SYG_HUMAN	Glycyl-tRNA synthetase (EC 6.1.1.14) (glycine--trna ligase) (glyrs)	6.18	78165	8	7.07E-09	Figs. 1 and 2
P42655	SW:143E_HUMAN	14-3-3 Protein epsilon (mitochondrial import stimulation factor l subunit) (protein kinase c inhibitor protein-1) (kcip-1)	4.46	29326	6	1.00E-07	Figs. 1 and 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P42771	SW:CDN2_HUMAN	Cyclin-dependent kinase 4 inhibitor a (cdk4i) (p16-ink4) (p16-ink4a) (multiple tumor suppressor 1) (mts1)	5.66	16579	4	1.00E-06	Fig. 2
P43243	SW:MAT3_HUMAN	Matrin 3 (fragment)	5.15	47348	6	1.85E-06	Fig. 1
P43490	SW:PBEF_HUMAN	Pre-B cell enhancing factor precursor	7.17	55771	5	1.89E-07	Fig. 2
P45880	SW:POR2_HUMAN	Voltage-dependent anion-selective channel protein 2 (VDAC2) (outer mitochondrial membrane protein porin)	6.74	38638	4	4.81E-05	Fig. 2
P45974	SW:UBP1_HUMAN	Ubiquitin carboxyl-terminal hydrolase t (EC 3.1.2.15) (ubiquitin-specific processing protease t)	4.76	96637	5	1.00E-07	Fig. 1
P47210	SW:PRS8_HUMAN	26S protease regulatory subunit 8 (proteasome subunit p45) (thyroid hormone receptor interacting protein 1)	8.36	45795	6	1.50E-08	Fig. 2
P48163	SW:MAOX_HUMAN	Malate oxidoreductase (EC 1.1.1.40) (malic enzyme) (me)	6.05	64679	9	1.00E-11	Fig. 1
P48507	SW:GSH0_HUMAN	Glutamate-cysteine ligase regulatory subunit (EC 6.3.2.2) (gamma-glutamylcysteine synthetase) (g-ecs)	5.95	31049	5	7.12E-06	Fig. 1
P48637	SW:GSHB_HUMAN	Glutathione synthetase (EC 6.3.2.3) (glutathione synthase) (gsh synthetase) (gsh-s)	5.80	52523	9	1.85E-13	Figs. 1 and 2
P48643	SW:TCPE_HUMAN	T-complex protein 1, ε subunit (tcp-1-epsilon) (cct-ε) (kiaa0098)	5.43	60088	6	2.34E-07	Figs. 1 and 2
P49368	SW:TCPG_HUMAN	T-complex protein 1, γ subunit (tcp-1-gamma) (cct-γ)	6.61	60862	8	1.39E-09	Figs. 1 and 2
P49591	SW:SYS_HUMAN	Seryl-tRNA synthetase (EC 6.1.1.11) (serine--trna ligase) (serrs)	6.39	59226	4	1.30E-05	Fig. 1
P49411	SW:EFTU_HUMAN	Elongation factor tu, mitochondrial precursor (p43)	7.61	49852	5	2.04E-06	Fig. 2
P49721	SW:PRCG_HUMAN	Proteasome component c7-i (EC 3.4.99.46) (multicatalytic endopeptidase complex subunit c7-I)	7.03	22992	4	6.20E-06	Fig. 2
P49770	SW:E2BB_HUMAN	Translation initiation factor eif-2b β subunit (eif-2b gdp-gtp exchange factor) (s20i15) (s20iii15)	6.14	39192	5	1.50E-05	Fig. 2
P49915	SW:GUAA_HUMAN	GMP synthase (glutamine-hydrolysing) (EC 6.3.5.2) (glutamine amidotransferase) (GMP synthetase)	6.85	77408	6	8.52E-09	Fig. 2
P50213	SW:IDHA_HUMAN	Isocitrate dehydrogenase (NAD), mitochondrial subunit alpha (EC 1.1.1.41) (isocitric dehydrogenase)	6.91	40022	6	5.58E-08	Figs. 1 and 2
P50579	SW:AMP2_HUMAN	Methionine aminopeptidase 2 (EC 3.4.11.18) (metap 2) (initiation factor 2 associated M_r 67 000 glycoprotein) (p67)	5.64	53713	7	4.49E-09	Figs. 1 and 2
P50990	SW:TCPQ_HUMAN	T-complex protein 1, θ subunit (tcp-1-θ) (cct-θ) (kiaa0002)	5.50	60166	14	3.20E-22	Fig. 2
P50991	SW:TCPD_HUMAN	T-complex protein 1, δ subunit (tcp-1-δ) (cct-δ) (stimulator of tar rna binding)	7.57	58315	7	1.00E-06	Fig. 2
P51665	SW:PRSC_HUMAN	26S proteasome regulatory subunit S12 (proteasome subunit p40) (mov34 protein)	6.54	37094	5	1.00E-05	Fig. 2
P52597	SW:ROF_HUMAN	Heterogeneous nuclear ribonucleoprotein f (hnrnp f)	5.40	45984	6	1.50E-05	Fig. 2
P52788	SW:SPSY_HUMAN	Spermine synthase (EC 2.5.1.22) (spermidine aminopropyltransferase)	4.72	41852	5	1.00E-05	Fig. 1

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
P52888	SW:MEPD_HUMAN	Thimet oligopeptidase (EC 3.4.24.15) (endopeptidase 24.15) (mp78)	5.97	79570	6	4.63E-07	Fig. 2
P52907	SW:CAZ1_HUMAN	F-actin capping protein α-1 subunit (capz)	5.50	33073	7	1.00E-11	Figs. 1 and 2
P54577	SW:SYY_HUMAN	Tyrosyl-tRNA synthetase (EC 6.1.1.1) (tyrosyl--tRNA ligase) (tyrrs)	9.25	44028	9	1.00E-10	Fig. 2
P54727	SW:R23B_HUMAN	UV excision repair protein rad23 homolog b (hhr23b) (xp-c repair complementing complex M_r 58 000 protein)	4.60	43201	6	1.00E-06	Fig. 2
P55010	SW:IF5_HUMAN	Eukaryotic translation initiation factor 5 (eif-5)	5.37	49548	4	2.05E-06	Fig. 2
P55036	SW:PSD4_HUMAN	26S proteasome regulatory subunit s5a (multiubiquitin chain binding protein) (antisecretory factor-1) (af) (asf)	4.52	40939	6	1.50E-07	Fig. 2
P55072	SW:TERA_HUMAN	Transitional endoplasmic reticulum ATPase (15s mg(2+)-ATPase p97 subunit) (valosin containing protein)	4.99	89949	9	1.18E-09	Figs. 1 and 2
P55263	SW:ADK_HUMAN	Adenosine kinase (EC 2.7.1.20) (ak) (adenosine 5'-phosphotransferase)	6.73	37866	5	1.00E-06	Fig. 2
P55795	SW:ROH2_HUMAN	Heterogeneous nuclear ribonucleoprotein h' (hnrnp h') (ftp-3)	6.26	49517	9	5.27E-12	Figs. 1 and 2
P55809	SW:SCOT_HUMAN	Succinyl-CoA:3-ketoacid-coenzyme A transferase (EC 2.8.3.5) (succinyl CoA:3-oxoacid CoA-transferase) (oxct)	7.44	56578	5	4.27E-05	Figs. 1 and 2
P56537	SW:IF6_HUMAN	Eukaryotic translation initiation factor 6 (eif-6) (b4 integrin interactor) (cab) (p27(bbp))	4.40	27095	5	1.30E-07	Fig. 1
P58238	SW:PSE1_MACFA	Proteasome activator complex subunit 1 (proteasome activator 28-alpha subunit) (pa28alpha) (pa28a)	6.22	28788	6	3.78E-06	Fig. 1
P78330	SW:SERB_HUMAN	L-3-phosphoserine phosphatase (EC 3.1.3.3) (psp) (o-phosphoserine phosphohydrolase) (pspase)	5.51	25176	5	5.75E-05	Fig. 1
P78371	SW:TCPB_HUMAN	T-complex protein 1, β subunit (tcp-1-beta) (fragment)	6.27	22924	7	7.54E-08	Figs. 1 and 2
P78417	SW:GTXH_HUMAN	Glutathione-*S*-transferase homolog	6.55	27833	4	1.57E-04	Fig. 2
Q02790	SW:FKB4_HUMAN	p59 protein (hsp binding immunophilin) (hbi) (possible peptidyl-prolyl *cis–trans* isomerase) (EC 5.2.1.8)	5.24	52057	4	1.21E-04	Figs. 1 and 2
Q03013	SW:GTM4_HUMAN	Glutathione *S*-transferase mu 4 (EC 2.5.1.18) (gstm4-4) (gts-mu2) (gst class-mu)	5.74	25772	5	1.52E-06	Fig. 1
Q03527	SW:PRS4_HUMAN	26S protease regulatory subunit 4 (p26s4)	5.81	49325	9	2.43E-11	Figs. 1 and 2
Q04695	SW:K1CQ_HUMAN	Keratin, type i cytoskeletal 17 (cytokeratin 17) (k17) (ck 17) (39.1) (version 1)	4.80	48230	7	1.00E-07	Fig. 2
Q05048	SW:CST1_HUMAN	Cleavage stimulation factor, M_r 50 000 subunit (cstf 50 000 subunit) (cf-1 50 000 subunit)	6.56	49125	9	5.82E-14	Fig. 2
Q05682	SW:CALD_HUMAN	Caldesmon (cdm)	5.48	93251	5	3.09E-06	Fig. 1
Q06323	SW:IGUP_HUMAN	Interferon γ up-regulated i-5111 protein precursor (igup i-5111) (proteasome activator complex subunit 1)	5.90	28876	6	3.78E-06	Fig. 1
Q06830	SW:TDX2_HUMAN	Thioredoxin peroxidase 2 (thioredoxin-dependent peroxide reductase 2) (proliferation-associated protein pag)	8.19	22324	5	4.98E-05	Fig. 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
Q07021	SW:MA32_HUMAN	Complement component 1, q subcomponent binding protein (glycoprotein gc1qbp) (hyaluronan-binding protein 1)	4.58	31741	4	1.00E-05	Figs. 1 and 2
Q07244	SW:ROK_HUMAN	Heterogeneous nuclear ribonucleoprotein k (hnrnp k) (dc-stretch binding protein) (csbp) (transform	5.28	51229	6	4.60E-12	Figs. 1 and 2
Q07960	SW:RHG5_HUMAN	GTPase-activating protein rhogap (rho-related small gtpase protein activator) (cdc42 gtpase-activating protein)	6.25	50461	7	3.25E-07	Fig. 2
Q09028	SW:RB48_HUMAN	Chromatin assembly factor 1 p48 subunit (caf-1 p48 subunit) (retinoblastoma binding protein p48)	4.60	47911	7	1.00E-07	Fig. 1
Q12920	SW:PSE3_HUMAN	Proteasome activator complex subunit 3 (proteasome activator 28-gamma subunit) (pa28gamma) (pa28g)	5.82	29601	5	1.40E-04	Figs. 1 and 2
Q12931	SW:TRA1_HUMAN	Tumor necrosis factor type 1 receptor-associated protein (trap-1) (fragment)	8.34	75694	13	1.00E-20	Fig. 2
Q13011	SW:ECH1_HUMAN	Probable peroxisomal enoyl-CoA hydratase (EC 4.2.1.17)	7.06	36313	5	1.60E-06	Fig. 2
Q13122	SWTR_HUM:Q13122	M_r 100 000 coactivator	7.04	100312	9	1.00E-09	Fig. 2
Q13162	SW:TDXN_HUMAN	Antioxidant enzyme aoe372 (aoe37-2)	6.25	30748	6	2.24E-10	Figs. 1 and 2
Q13263	SW:TF1B_HUMAN	Transcription intermediary factor 1-β (nuclear corepressor kap-1) (krab-associated protein 1)	5.62	90261	6	1.50E-09	Figs. 1 and 2
Q13283	SW:G3BP_HUMAN	Ras-gtpase-activating protein binding protein 1 (gap sh3-domain binding protein 1) (g3bp-1)	5.32	52189	6	1.49E-07	Figs. 1 and 2
Q13347	SW:IF34_HUMAN	Eukaryotic translation initiation factor 3 δ subunit (eif-3 δ) (eif3 p36) (tgf-β receptor interacting protein 1)	5.45	36877	6	4.52E-08	Figs. 1 and 2
Q13561	SW:DYNC_HUMAN	Dynactin, M_r 50 000 isoform (M_r 50 000 dynein-associated polypeptide) (dynamitin)	4.92	44906	5	1.00E-05	Fig. 2
Q14152	SW:IF3A_HUMAN	Eukaryotic translation initiation factor 3 subunit 10 (eif-3 theta) (eif3 p167) (eif3 p180) (eif3 p185) (kiaa0139)	6.74	166867	6	6.71E-09	Fig. 1
Q14240	SW:IF42_HUMAN	Eukaryotic initiation factor 4a-ii (eif-4a-ii)	5.23	46592	5	1.03E-04	Fig. 1
Q14697	SWTR_HUM:Q14697	Glucosidase II (KIAA0088 protein)	6.03	107288	13	9.34E-17	Fig. 2
Q14764	SW:MVP_HUMAN	Major vault protein (mvp) (lung resistance-related protein)	5.26	100135	6	1.00E-09	Fig. 1
Q15019	SW:NED5_HUMAN	Nedd5 protein homolog (kiaa0158)	6.59	41689	7	6.97E-07	Figs. 1 and 2
Q15046	SW:SYK_HUMAN	Lysyl-tRNA synthetase (EC 6.1.1.6) (lysine--trna ligase) (lysrs) (kiaa0070)	6.32	68446	5	1.60E-05	Fig. 2
Q15084	SW:ERP5_HUMAN	Probable protein disulfide isomerase p5 precursor (EC 5.3.4.1)	4.80	48490	5	1.16E-05	Fig. 1
Q15092	SWTR_HUM:Q15092	Transmembrane protein	6.45	84015	9	9.96E-10	Figs. 1 and 2
Q15181	SW:IPYR_HUMAN	Inorganic pyrophosphatase (EC 3.6.1.1) (pyrophosphate phosphohydrolase) (ppase)	5.69	33095	8	1.12E-12	Figs. 1 and 2
Q15293	SW:RCN1_HUMAN	Reticulocalbin 1 precursor	4.71	38866	5	1.00E-05	Fig. 1
Q15365	SW:PCB1_HUMAN	Poly(rc)-binding protein 1 (hnrnp-e1) (nucleic acid binding protein sub2.3) (alpha-cp1)	7.07	38015	4	1.80E-04	Fig. 2
Q15366	SW:PCB2_HUMAN	Poly(rc)-binding protein 2 (hnrnp-e2)	6.76	38954	5	1.00E-05	Fig. 2
Q16576	SW:RB46_HUMAN	Histone acetyltransferase type b subunit 2 (retinoblastoma binding protein p46)	4.77	48132	7	5.31E-09	Fig. 1

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
Q16658	SW:FASC_HUMAN	Fascin (actin bundling protein)	7.21	55123	12	4.09E-16	Fig. 2
Q29443	SW:TRFE_BOVIN	Serotransferrin precursor (siderophilin) (beta-1-metal binding globulin)	7.01	79869	8	1.15E-06	Figs. 1 and 2
Q61553	SW:FASC_MOUSE	Fascin	6.64	55112	6	1.50E-06	Fig. 2
Q92524	SW:PRSX_HUMAN	26S protease regulatory subunit s10b (p42)	7.49	44418	8	1.50E-10	Fig. 2
Q92598	SW:H110_HUMAN	Heat shock protein M_r 110 000 (kiaa0201)	5.15	97716	9	2.53E-07	Figs. 1 and 2
Q99475	SWTR_HUM:Q99475	KM-102-derived reductase-like factor (thioredoxin reductase)	6.66	60972	6	1.97E-05	Figs. 1 and 2
Q99829	SW:CNE1_HUMAN	Copine I	5.67	59648	6	5.39E-07	Figs. 1 and 2
Q99832	SW:TCPH_HUMAN	T-complex protein 1, eta subunit (tcp-1-eta) (cct-eta) (hiv-1 nef interacting protein)	7.62	59842	6	1.06E-08	Fig. 2
Q9BQ67	SW:GRWD_HUMAN	Glutamate-rich wd repeat protein	4.67	49787	6	1.30E-07	Fig. 1
Q9BRF1	SWTR_HUM:Q9BRF1	Singed (drosophila)-like (sea urchin fascin homolog like)	7.21	55151	5	8.60E-07	Fig. 2
Q9BT75	SWTR_HUM:Q9BT75	Similar to peptidase D	5.93	55311	8	5.52E-10	Fig. 1
Q9BV20	HUMANGP:CHR19-Q9BV20	Similar to CG11334 gene product was used to identify this gene	6.26	39467	5	1.00E-04	Fig. 2
Q9DB77	SW:UCR2_MOUSE	Ubiquinol-cytochrome *c* reductase complex core protein 2, mitochondrial (EC 1.10.2.2) (complex iii subunit ii)	10.08	48262	8	1.00E-09	Fig. 2
Q9H4J9	HUMANGP:CHR10-Q9H4J9	DJ1099D15.1 (A putative DNAJ protein) was used to identify this gene	7.36	28411	6	3.54E-05	Fig. 1
Q9H644	SWTR_HUM:Q9H644	CDNA: FLJ22618 FIS, CLONE HSI05382 (unknown) (protein for MGC:5585)	6.79	25787	5	1.18E-04	Fig. 1
Q9NR46	SWTR_HUM:Q9NR46	SH3-containing protein SH3GLB2	5.85	44174	7	1.50E-08	Fig. 1
Q9NR50	SW:E2BG_HUMAN	Translation initiation factor eif-2b gamma subunit (eif-2b gdp-gtp exchange factor)	6.41	50949	5	1.50E-05	Fig. 1
Q9NRH3	SW:TBG2_HUMAN	Tubulin γ-2 chain (γ-2 tubulin)	5.65	51401	7	1.00E-07	Fig. 1
Q9NRN7	SWTR_HUM:Q9NRN7	HAH-P	7.80	38700	5	1.65E-04	Fig. 1
Q9NSD9	SW:SYFB_HUMAN	Phenylalanyl-tRNA synthetase β chain (EC 6.1.1.20) (phenylalanine—tRNA ligase β chain) (phers)	6.82	66714	6	1.00E-07	Fig. 2
Q9NUN0	SWTR_HUM:Q9NUN0	CDNA FLJ11260 FIS, Clone PLACE1009060, waekly similar to BRO1 protein	6.26	68477	6	1.50E-06	Fig. 2
Q9NUZ3	SWTR_HUM:Q9NUZ3	CDNA FLJ11039 FIS, Clone PLACE1004376	5.11	48999	5	1.07E-04	Fig. 1
Q9NW31	SWTR_HUM:Q9NW31	CDNA FLJ10348 FIS, Clone NT2RM2001065	5.71	45243	6	5.79E-07	Figs. 1 and 2
Q9NY65	SW:TBA8_HUMAN	Tubulin α-8 chain (alpha-tubulin 8)	4.80	50745	6	1.20E-07	Fig. 2
Q9P042	SWTR_HUM:Q9P042	HSPC108 (stomatin-like protein 2)	5.95	37293	4	3.14E-05	Fig. 1
Q9P0J3	SWTR_HUM:Q9P0J3	Putative M_r 55 000 protein	7.36	55481	5	5.58E-05	Fig. 2
Q9P0V2	SWTR_HUM:Q9P0V2	Mitofilin (fragment)	5.60	68316	9	6.63E-11	Figs. 1 and 2
Q9P0X0	SWTR_HUM:Q9P0X0	Glucosidase ii α subunit	6.20	109825	10	1.81E-10	Fig. 1
Q9UGY2	HUMANGP:CHR16-Q9UGY2	DJ37E16.5 (novel protein similar to nitrophenylphosphatases from various organisms) (hypothetical M_r 31 700 protein)	7.51	33495	5	1.00E-06	Fig. 2
Q9UMX0	SWTR_HUM:Q9UMX0	Ubiquilin	5.03	63143	5	1.00E-06	Fig. 2
Q9UNH7	SW:SNX6_HUMAN	Sorting nexin 6	6.08	46904	5	4.57E-06	Fig. 1
Q9UNV3	PSDD_HUMAN	26S proteasome non-ATPase regulatory subunit 13 (26S proteasome regulatory subunit S11)	5.67	43188	6	1.00E-06	Fig. 1
Q9UQ80	SW:P2G4_HUMAN	Proliferation-associated protein 2g4 (cell cycle protein p38-2g4 homolog) (hg4-1)	6.53	44101	9	5.63E-12	Figs. 1 and 2

Table 1 (*Continued*)

Number	Protein	Full name	p*I*	M_r	Matches	Probability	Figure
Q9Y230	SWTR_HUM:Q9Y230	Erythrocyte cytosolic protein of M_r 51 000, ECP-51 (REPTIN52)	5.43	51295	10	1.06E-09	Figs. 1 and 2
Q9Y265	SWTR_HUM:Q9Y265	Erythrocyte cytosolic protein of M_r 54 000, ECP-54	6.39	50538	9	1.00E-12	Fig. 2
Q9Y2T3	SW:GUAD_HUMAN	Guanine deaminase (EC 3.5.4.3) (guanase) (guanine aminase) (guanine aminohydrolase) (gah) (p51-nedasin)	5.53	51483	6	3.67E-07	Fig. 2
Q9Y361	SWTR_HUM:Q9Y361	CGI-46 protein (RuvB-like 2)	6.65	48489	8	5.43E-06	Figs. 1 and 2
Q9Y389	SWTR_HUM:Q9Y389	CGI-80 protein	7.71	36225	5	1.43E-04	Fig. 1
Q9Y3f4	SW:UNRI_HUMAN	Unr-interacting protein (wd-40 repeat protein pt-wd)	4.85	38756	6	1.00E-08	Fig. 1
Q9Y3F5	SWTR_HUM:Q9Y3F5	Agrelated protein (protein tyrosine kinase 9-like PTK9L), (A6-related protein, A6RP)	6.85	39751	5	6.31E-05	Figs. 1 and 2
Q9y4L1	SW:OXRP_HUMAN	M_r 150 000 oxygen-regulated protein precursor (orp150)	5.00	111494	11	1.00E-13	Fig. 2
Q9Y583	SWTR_HUM:Q9Y583	NSAP1 protein	7.34	62845	6	6.55E-07	Fig. 2
Q9Y617	SW:SERC_HUMAN	Phosphoserine aminotransferase (EC 2.6.1.52) (psat)	6.66	35508	6	1.19E-05	Fig. 2
S29089	PIR2:S29089	α-Centractin-human (P42024)	6.63	42700	6	2.06E-06	Fig. 2

Proteins from the HeLa cell line were extracted and separated by 2D electrophoresis as described in section Experimental. The proteins were identified by MALDI-MS, following in-gel digestion with trypsin. The search in protein databases was performed with in house developed software. At least 4 matching peptides were required for an identity assignment. The number of matching peptides is listed in Table 1 (matches). The spots representing the identified proteins are shown in Figs. 1 and 2 and are designated with their accession numbers of SWISS-PROT or the other databases. The theoretical M_r and p*I* values, as well as the probability of assignment of a random protein identity are given. The probability was determined as described by Berndt et al. [29]. In the column "Protein", the abbreviated name of the protein and the database used for protein search are indicated. The data are sorted according to acceding accession numbers.

mass determination, or to spot overlapping, which did not allow unambiguous identity assignment. For about 40% of the unidentified spots, MS data were insufficient for protein identification (usually a low number of peptides were found mainly from spots of low intensity) and for the remaining 10% of the spots no MS data could be acquired. The major reasons for the latter were no signal acquisition for one of the standard peptides, very weak spots, which did not deliver a sufficient amount of peptides or peptide losses. In Table 1, the proteins identified are listed together with their theoretical M_r and isoelectric point (p*I*) values and the data from the mass spectrometry analysis, i.e. the numbers of matching peptides and the probability that the protein identity assigned could be random.

The measured peptide masses were corrected with the use of internal peptide standards. The correction allowed the use of narrow windows of mass tolerance (0.0025%) and increased thus the confidence of identification. In most cases, identification was based on five or more (up to 14) matching peptides and the probability of a random identity assignment was usually lower than 10^{-5}. In some cases, mainly for proteins of low molecular mass, which deliver few peptides [31], identification was based on four matching peptides. The number of matching peptides is related to the molecular mass of the protein analyzed and usually increases with protein size, as larger proteins carry a higher number of lysine and arginine residues, i.e. more trypsin cleavage sites, than their shorter counterparts. This gives rise to a larger number of proteolytic products and consequently the identification relies on a higher number of matching peptides. The average molecular mass of proteins identified with, e.g. four matches was 31 000 and those identified with five matches 44 000.

Approximately 70% of the identified proteins have masses between 20 and 60 kDa. Eleven proteins have molecular masses higher than 100 000, up to 166 000. No protein smaller than M_r 15 000 was identified. In general, low- and high-molecular-mass proteins are underrepresented in Table 1, probably on account of limitations of the technology. Small proteins can be hardly identified by MALDI-MS as mentioned above and large proteins enter the IPG strips with low efficiency and a high percentage of them are not detected in gels. About 70% of the identified, etc. proteins have theoretical p*I* values between 5 and 8 and 15% between 4 and 5. Acidic proteins with p*I* values lower than 4 were not detected probably due to detection limitations (the lower p*I* limit was about 3.5). Four polypeptides have theoretical p*I* values higher than 10 (Table 1).

Most of the proteins identified in the HeLa cell line had been detected in other samples as well. The most frequently detected proteins are heat shock proteins, like (P11142, heat shock M_r 70 000 protein (P08107) and M_r 78 000 glucose-regulated protein (P11021), which have been found in more than 300 samples analyzed by mass spectrometry in our laboratory. Other frequently detected proteins are tubulin chains, and high-abundance enzymes, such as P06733, ATP synthase β-chain (P06576) and protein disulfide isomerase (P30101).

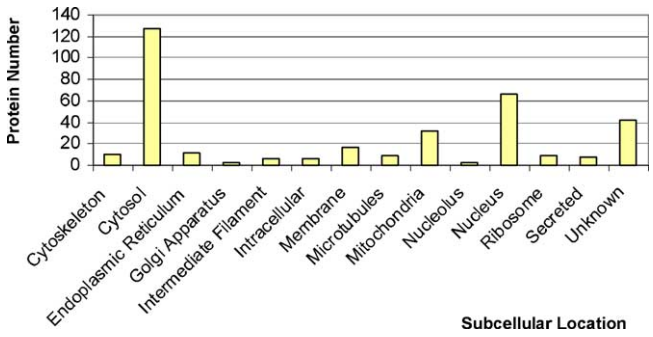

Fig. 3. Subcellular location of the HeLa cell line proteins. The proteins identified in this study were classified according to their subcellular location as annotated in the SWISS-PROT and other public domains. For about 15% of the proteins, no annotation existed.

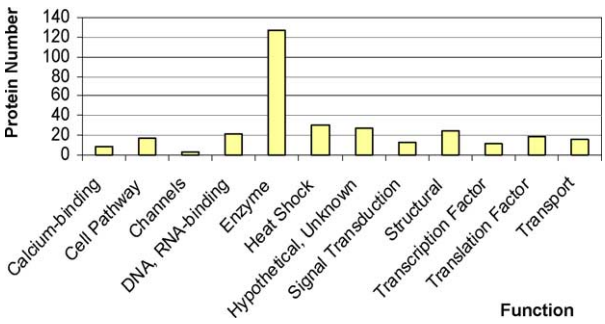

Fig. 4. Function of the HeLa cell line proteins. The proteins were classified into major functional groups. About 10% of the proteins are annotated as hypothetical or unknown.

A large percentage of the proteins showed a heterogeneity and were represented by more than one spot. These were mainly high-abundance enzyme subunits and structural proteins. We estimate that in average about three to five spots correspond to one gene product. The multiple spots may be partly the consequence of different splicing, processing and post-transational modification, which result in alteration of the p*I* of the polypeptides and consequently of the focusing position. Heterogeneity may also result from artifacts of the technology, such as carbamylation of the proteins upon prolonged contact of the sample with urea. For most of the observed heterogeneities, we do not know the origin or biological significance.

3.3. Subcellular location and protein function

Approximately 42% of the identified proteins are annotated to be localized in the cytosol, about 22% in the nucleus, 10% in mitochondria, including mitochondrial membranes, and 6% in membranes. For about 15% of the proteins, no annotation about their subcellular location was found. Fig. 3 shows the distribution of the HeLa cells proteins according to their subcellular location.

Forty-five percent of the proteins of Table 1 are enzymes or enzyme subunits (about 127) with various catalytic activities. Also, structural proteins such as tubulin chains, are largely represented in the gels (about 25 strucutral proteins). Other major classes of identified proteins include about 30 heat shock proteins (glucose-regulated proteins, T-complex protein chains, etc.), DNA- and RNA-binding proteins (about 22), proteins involved in cell pathways (about 18), in signal transduction (about 13), transport (about 16), channels, transcription, translation factors (and proteins with other, non-catalytic functions. About 27 hypothetical or unknown gene products, like unknown proteins AAH07539, AAH07545, AAH07293, Q9NW31 and many others, were detected for which there was so far no indication about their existence at the protein level. In Fig. 4, the proteins are distributed according to their function.

4. Discussion

A major goal of this study was to generate a comprehensive HeLa cell 2D protein database that can be used for future proteomic investigations, forming the background for work on protein expression. Several protein classes with several members of the individual pathways and cascades, signaling, cytoskeleton, proteasome, antioxidant, chaperone, nucleic acid binding and metabolism-related were defined. Moreover, a series of hypothetical proteins were unambiguously identified and their different expression forms are shown (Figs. 1 and 2). Many of them have been only described at the nucleic acid level so far and others have never been detected before by a protein chemical method or have been simply detected by immunochemical methods, which depend on antibody specificity and availability. This is of importance as the experiment now reveals the real existence of these gene products in the human system. Further analysis of the predicted or hypothetical proteins using bioinformatics and genomics tools will extend our knowledge on already existing systems and may indicate or even represent HeLa- or tumor-specific proteins. Using similar technologies, we have identified a series of hypothetical proteins in the brain and neurological cell lines [3,6,11,12,32–35].

Several of the observed proteins have been annotated to be tumor-associated, tumor-linked, protooncogens or proliferation-linked structures, although also found in non-tumor tissue. For example, cyclin-dependent kinase 4 inhibitor A (P42771) is involved in tumor formation in a wide range of tissues [36,37]. Similarly, erythrocyte cytosolic protein M_r 51 000 (Q9Y230) is an essential cofactor in the c-Myc oncogenic transformation [38]. Proteins of Table 1 which have been associated to various carcinomas also include the eukaryotic translation initiation factors 4E and 6 (P06730 and P56537, respectively), prohibitin (P35232) and cell division protein kinase 4 (P11802).

Our list includes several keratin proteins of types I and II. Keratins and cytokeratins are considered to reflect contamination during sample analysis and the search software can exclude this group of proteins from successful identifications. However, it is highly unlikely the keratins repre-

sent contamination products in our case as several cytokeratins were observed in the 2D gels following non-sensitive Coomassie staining and thus can be considered as abundant proteins. Instrumentation used in this study was able to reliably analyze some isoforms (Table 1) despite of inherent problems with identification of keratins and cytokeratins due to high sulfur content, insolubility, poor trypsin cleavage and high sequence homology [39].

Heat shock M_r 70 000 protein 4, matrin 3, mitofilin, protein R30923_1, T-complex protein 1 β subunit, tumor necrosis factor type 1 receptor-associated protein and hypothetical protein IMAGE:3029477 were detected as fragments only and can be therefore not reliably and definitively assigned even if matches were appropriate. Truncation as a post-translational modification or limited proteolysis may have been responsible for fragmentation and as the whole sequence is not covered, these proteins can only be assigned to the category of "related" or highly homologous proteins or protein families.

Membrane proteins are underrepresented in our list. Detection of this class of proteins represents one of the major challenges of proteomics. In general, hydrophobic proteins cannot be detected in two-dimensional gels [4,12]. Lack of detection of hydrophobic proteins in two-dimensional gels may have two reasons: (i) the protein is not soluble in solubilizing agents which are compatible with isoelectric focusing, the first-dimensional separation, or (ii) the protein includes strong hydrophobic stretches which hinder it from entering the immobilized pH gradient strips [40]. The hydrophobicity of proteins is characterized by the grand average hydrophobicity (GRAVY) scores [41] which provide a picture of the hydrophobicity of the whole protein molecule and usually vary in a range of ±2. Positive scores indicate hydrophobic, and negative scores hydrophilic, proteins. Proteins from membrane fractions visualized in 2D gels are rather hydrophilic when judged with their GRAVY values, or their transmembrane domains may not be strongly hydrophobic, or they may be contaminants from other fractions. However, GRAVY values do not appear to represent a reliable criterion whether a protein will enter the IPG strip. It seems that the amino acid sequence of the hydrophobic stretches is decisive whether a protein will enter the IPG strip and not the hydrophobicity of the entire protein. For example, cytochrome P450 2D6, a hydrophilic protein with one transmembrane region, was not detected in 2D gels, but at the sample application position [42]. The other major limitation of proteomics is the detection of low-abundance proteins [40]. For their detection, protein enriching steps are required, like chromatography and electrophoretic techniques [43–45].

In summary, we constructed a 2D database for the HeLa cell line transfected with an empty vector. The database comprises 297 different gene products, resulting from MALDI-MS analysis of approximately 3000 spots, which were taken from six 2D gels. The database represents today one of the largest 2D databases for eukaryotic proteomes. About 45% of the proteins were reflecting enzyme subunits. It further includes proteins of various classes with important functions and also hypothetical proteins and may be a useful analytical tool and form the basis for studies at the protein level, independent of antibody availability and specificity by a fair protein chemical method.

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