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Structural Genomics of human proteins – target selection and generation of a public catalogue of expression clones

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Abstract

Background

The availability of suitable recombinant protein is still a major bottleneck in protein structure analysis. The Protein Structure Factory, part of the international structural genomics initiative, targets human proteins for structure determination. It has implemented high throughput procedures for all steps from cloning to structure calculation. This article describes the selection of human target proteins for structure analysis, our high throughput cloning strategy, and the expression of human proteins in *Escherichia coli* host cells.

Results and Conclusion

Protein expression and sequence data of 1414 *E. coli* expression clones representing 537 different proteins are presented. 139 human proteins (18%) could be expressed and purified in soluble form and with the expected size. All *E. coli* expression clones are publicly available to facilitate further functional characterisation of this set of human proteins.

Background

The Protein Structure Factory

The Protein Structure Factory (PSF) is a joint endeavour of universities, research institutes and companies from the Berlin area [1, 2]. It takes part in the international structural genomics initiative [3, 4] and aims at the determination of human protein structures by X-ray diffraction methods and NMR spectroscopy using standardised high-throughput procedures. A complete pipeline has been established for this purpose that comprises cloning, protein expression in small and large scale, biophysical protein characterisation, crystallisation, X-ray diffraction and structure calculation.

It is known that eukaryotic proteins are often difficult to express in *Escherichia coli* [5]. Only a certain fraction of these proteins can be overproduced in *E. coli* in sufficient yield without formation of inclusion body aggregates or proteolytic degradation. Alternative expression systems include cell cultures of various eukaryotic organisms and cell-free, *in vitro* protein expression. These systems have been greatly improved since 1999, when the PSF project was initiated. In the meantime, *E. coli* [5-7] and wheat germ [8] *in vitro* protein synthesis is routinely used by structural genomics projects. At the PSF, yeast expression hosts, *Saccharomyces cerevisiae* and *Pichia pastoris*, were successfully established as alternative systems to *E. coli*, as described in detail previously [9-11]. We will focus here on the results obtained with the *E. coli* expression system.

E. coli strains and vectors

The T7 RNA polymerase-dependent *E. coli* expression vector system (pET-vectors) is a universal system to generate recombinant protein for structural analysis [12]. pET vectors are usually combined with the *E. coli* B strain BL21 and derivatives that are engineered to carry the T7 RNA polymerase gene. These strains, however, have limitations in cloning and stable propagation of the expression constructs. Expression vectors which are regulated by the *lac* operator are independent of the host strain. Recombination-deficient *E. coli* K-12 strains are suitable for cloning because of their high transformation rates and because they allow for stable propagation of recombinant constructs. The strain SCS1 (Stratagene; *hsd*R17(r_K^- m_K^+) *recA1 endA1 gyrA96 thi-1 relA1 supE44*) was found to perform well at the PSF in cloning experiments. It grows relatively fast and allows for robust protein expression.

Affinity tags allow for standardised protein purification procedures. The first vector that was used routinely in the PSF, pQStrep2 (GenBank AY028642, Figure 1), is based on pQE-30 (Qiagen) and adds an N-terminal His-tag [13] for metal chelate affinity chromatography (IMAC) and a C-terminal Strep-tag II [14, 15] to the expression product. pQStrep2 allows for an efficient two-step affinity purification of the encoded protein, as demonstrated in a study of an SH3 domain [16]. The eluate of the initial IMAC is directly loaded onto a Streptactin column. Thereby, only full-length expression products are purified and degradation products are removed. However, the two tags, which are flexible unfolded peptides, remain on the

protein and may interfere with protein crystallisation, although we could show that crystal growth may be possible in their presence even for small proteins [16]. To exclude any negative influence by the affinity tags, another vector, pQTEV (GenBank AY243506, Figure 1), was constructed. pQTEV allows for expression of N-terminal His-tag fusion proteins that contain a recognition site of the tobacco etch virus (TEV) protease for proteolytic removal of the tag.

Codon usage has a major influence on protein expression levels in *E. coli* [17], and eukaryotic sequences often contain codons that are rare in *E. coli*. Especially the arginine codons AGA and AGG lead to low protein yield [18]. This can be alleviated by introducing genes for overexpression of the corresponding tRNAs into the *E. coli* host cells. We have used the plasmid pSE111 (Figure 1) carrying the *argU* gene for this purpose. pSE111 is compatible with pQTEV and other common expression vectors. It carries the $lacI^Q$ gene for overexpression of the Lac repressor, which is required when using promoters regulated by *lac* operators. pSE111 was used at the PSF in combination with the expression vectors pQStrep2 and pGEX-6P-1. Strains for overexpression of rare tRNAs are available from Invitrogen (BL21 Codon Plus) and Novagen (Rosetta). The Rosetta strain contains the chloramphenicol-resistant pRARE plasmid that supplies tRNAs for the codons AUA, AGG, AGA, CUA, CCC, GGA [19]. This plasmid is used at the PSF in combination with pQTEV and pGEX-6P-2.

The supplementary file psfClones.xml lists the vector and helper plasmid for overexpression of rare tRNAs that was used for each individual clone.

Selection of target proteins

We selected target proteins with higher-than-average chances of successful expression in *E. coli* and crystallisation [1]. Proteins were excluded for which sequence analysis predicted that structure determination would be difficult. Starting from the complete set of known human proteins, potentially difficult target proteins and proteins of known structure were excluded according to the following criteria:

- Membrane proteins are known to be complicated targets for structure determination and were excluded. Membrane proteins were identified with the program TMHMM [20, 21].
- Since very large proteins are often difficult to express, the maximal length of target proteins was set to 500 amino acids.
- Protein regions that are unstructured or only partially structured [22] may lead to difficulties during protein expression and purification. Unstructured regions are susceptible to proteolyic attack, and represent an obstacle to protein crystallisation. A large proportion of intrinsically unstructured protein sequences are characterised by sequence stretches of low complexity and tandem repeats [23]. Proteins with low

complexity regions of more than 20 amino acids length, detected by the SEG program, or with more than one region were excluded [24, 25].

- Coiled-coil proteins were excluded from our target list, since this fold is not novel, and structural analysis of coiled coils requires special attention. Many coiled coil proteins form hetero-complexes with other coiled-coil proteins and cannot be studied without their binding partner. Coiled-coils domains are long, extended structures which can usually only be crystallised as domains, i.e. expression constructs lacking other domains have to be prepared. To identify coiled-coil proteins, the program COILS was used [26-28].
- The cellular localisation of target proteins was assigned with the Meta_A(nnotator) [29, 30]. Target proteins annotated to be localised in the extracellular space, endoplasmic reticulum, Golgi stack, peroxisome or mitochondria were excluded from expression in *E. coli*. Many of these proteins require formation of disulphide bonds for correct folding, but these are generally not formed in the reducing environment of the *E. coli* cytoplasm. Therefore, these proteins were allocated only for extracellular expression by yeast host cells. Proteins with predicted intracellular localisation or which were not assigned with a localisation by Meta_A(nnotator) were expressed in the cytosol of *E. coli*.
- Potential target proteins were matched to the sequences of proteins with known structure at the Protein Data Bank (PDB) [31]. PSI-BLAST [32] was used to detect even very distinct homologies to PDB entries to rule out proteins with known folds. This filter was later replaced by a less stringent one, which considers the sequence identity and 'coverage' of matches to PDB sequences. The coverage is the length of the sequence match divided by the protein length. According to the less stringent filter, proteins with 60% or more sequence identity over 90% or more of the sequence length were excluded. Thereby target proteins could be included of which only a part, e.g. a single domain, has a known structure.

Results

Target lists

The first target list was generated in 1999, when the PSF project started, from the set of all human proteins known at that time. This set was filtered as described above. PSI-BLAST was used to match potential target proteins to the PDB and to include only proteins of presumably novel folds. To enable high-throughput cloning, we selected target proteins for which full-length cDNA clones were available. In 1999, cDNA clones of the IMAGE consortium represented the main public source of sequenced cDNA clones [33]. However, only partial sequence information existed for these clones – the EST sequences of the dbEST database [34] – and only a small proportion contained complete open reading frames (full-ORF clones).

490 proteins were selected which met the selection criteria, had no match to PDB detected by PSI-BLAST and for which full-ORF IMAGE cDNA clones were available.

In 2003, a second target list was compiled from novel full-length cDNAs discovered by the German cDNA consortium [35, 36]. The same filter criteria as for the first target list were applied, except that cellular localisation was not taken into account. Proteins with a PDB match of 60% or more sequence identity and 90% sequence coverage were excluded, resulting in a target list of 259 proteins.

A third set of target proteins was selected from a human cDNA expression library (hEx1), which was cloned in a bacterial expression vector. This library was screened for expression clones on high density arrays and by high-throughput protein expression and purification experiments [37-40]. This identified 2,700 clones expressing soluble His-tag fusion proteins that could be purified by affinity chromatography [39]. The cDNA inserts of these clones were sequenced and assigned to sequences of the Ensembl database [41]. 141 proteins represented by clones of the expression library where selected as targets for structural analysis [39]. These clones express soluble, full-length proteins, of which the three-dimensional structure was unknown.

The numbers of targets and success rates grouped by the type of target cDNA clone are summarised in Table 1.

Generation and characterisation of expression clones

We established a common cloning strategy that allows for easy shuttling of cDNA fragments between different *E. coli* and yeast vectors. We adopted a cloning system that adds only a minimal number of extra amino acids to the protein of interest and therefore decided to clone with restriction enzymes instead of using alternative systems, such as Invitrogen's Gateway system or ligation independent cloning [42].

The PSF has been working with more than a thousand target proteins to date. Suitable cDNA clones were selected and subcloned into the *E. coli* expression vectors pQTEV [GenBank: AY243506] and pQStrep2 [GenBank:AY028642] [16]. These vectors provide for an N-terminal His-tag; pQStrep2 also encodes an C-terminal Strep-tag-II. Some proteins have also been expressed as GST fusion proteins using the vectors pGEX-4T2 or pGEX-6P1 (Amersham Biosciences).

Expression of protein coding genes from multiple transformants per target was tested under multiple conditions. Standardisation and automation was introduced to achieve this throughput. Expression clones were characterised by small scale protein synthesis at different temperatures, 37°C, 30°C and 25°C, in 1 ml volumes in deep-96-well microplates. Proteins were purified in parallel by a pipetting robot, as described previously [43]. 10% of the purified protein eluate from a 1 ml culture was analysed by SDS-PAGE. For each protein expression experiment, the size of the expression product was recorded and the amount of protein was classified into four categories: none, weak, moderate and strong expression. This classifica-

tion is arbitrary to a certain degree, however, we found it sufficient to select suitable clones for protein production scale-up.

1414 clones for 537 target proteins were successfully cloned in *E. coli* expression vectors, with 473, 191 and 94 target proteins corresponding to target lists one (IMAGE clones), two (DKFZ clones) and three (hEx1 clones), respectively. Clones for 139 different target proteins were found to be expressed in soluble form by *E. coli*. Figure 2 and Table 2 show the result of small scale expression and purification of these proteins. The yield varied significantly among different target proteins. The supplementary file psfClones.xml contains further details on the expression clones, such as vector, strain and helper plasmid for overexpression of rare tRNAs.

Biophysical properties of proteins which could be expressed in soluble form in *E. coli* were compared against all tested proteins. We found no significant correlation between expression success and either protein length or mean net charge (data not shown). However, when analysing the mean hydrophobicity, we found that hydrophobic proteins are less likely to be expressed in soluble form. Only one of 139 well expressed proteins has a mean hydrophobicity of more than 0.2, while 8% of the other proteins are above this value. This group of proteins does not contain transmembrane helices according to TMHMM, and therefore may represent peripheral membrane proteins with hydrophobic surface regions.

The *E. coli* expression clones of the PSF are publicly available from the RZPD German Resource Center [44]. The supplementary file 1 is an XML list of these clones. It can be viewed in a web browser (Figure 3). The supplementary file contains, for each clone:

- Gene ID and name,
- Accession number,
- Cloning details,
- Strain and vector,
- Expected sequence,
- Protein expression results,
- Sequence verification.

Solved structures

As a result of the target selection and cloning described in this paper, ten novel X-ray structures of human proteins were determined (

Table 3). The structure of one protein, TRAPPC3/BET-3, was determined after protein expression in *S. cerevisae*, while the other proteins were produced in *E. coli*.

Discussion

We describe here the strategies and experiments of our structural genomics project on human proteins. In addition to the expression of full length proteins, the Protein Structure Factory has also studied protein domains by NMR spectroscopy, which has been described elsewhere [45-47]. Our selection of full length target proteins was mainly determined by the availability of full length cDNA clones. In addition, biophysical and bioinformatical criteria were applied, leading to a biased selection of target proteins from the human proteome. Therefore, we expect that the percentage of proteins that we could express and purify in soluble form, 18%, is higher than it would be in a randomly selected set. The low proportion of successfully expressed proteins indicates that *E. coli* is not the appropriate expression host for many full length human proteins. High throughput protein expression in alternative system such as yeast [9-11] or insect cells/baculovirus [48] has been established and will lead to better success rates in future projects.

Generally, clones that did express a soluble protein were verified by DNA sequencing, while clones that did not express or expressed an insoluble product were usually not sequence verified. It cannot be ruled out that some of the unsuccessful clones contain sequence errors introduced during cloning. Since template cDNA clones of the IMAGE consortium with only partial sequence information were used for most cloning experiments, expression clones that were not sequence verified might represent splice variants or isoforms of the original target. The distribution of mean net charge and length was similar among successfully expressed and all proteins, while very hydrophobic proteins were generally not expressed well in our *E. coli* expression system.

Future efforts in structural genomics of mammalian proteins will benefit from a much better supply of full length cDNA clones. Clones prepared for protein expression by resource centres and commercial suppliers are becoming available now. With such resources, alternative target selection strategies will become feasible that will not be restricted by the availability of cDNA clones. Instead, all potential target proteins, including splice variants, could be clustered by similarity and the most suitable members of each cluster could be selected by appropriate criteria as outlined in the Background section.

In our approach, we have excluded certain types of proteins such as membrane proteins and very large proteins. A structural genomics approach that includes membrane proteins would require standard protocols to optimise expression conditions and detergents [49]. The best strategy to study large proteins is to divide them into domains and smaller regions. However, such smaller constructs usually have to be designed manually.

All clones listed in the supplementary file and Table 2 are available to the research community. Thereby we hope to facilitate further functional characterisation of this set of human proteins.

Materials and Methods

Cloning with restriction enzymes

cDNA inserts were amplified by PCR primers carrying tails with *Bam*HI and *Not*I sites and cloned into the respective sites of one of the expression vectors pQTEV, pQStrep2 or pGEX-6p1. This had the drawback that the restriction sites chosen for cloning might occur in the insert. In such cases, compatible overhangs were produced by alternative enzymes or by the hetero-stagger cloning method [50]. Alternative enzymes are *Bgl*II for *Bam*HI and the type IIs enzymes *Bpi*I, *Eco*31I, *Esp*3I, which can replace both *Bam*HI and *Not*I. Type IIs enzymes cut outside their recognition sequence and can produce arbitrary overhangs.

PCR Primer design

PCR primers with tails carrying restriction enzyme cleavage sites were designed automatically by a Perl program. The primer design program adjusts the length of the primers to achieve a melting temperature close to a common default. Then, restriction enzymes that do not cut within the respective cDNA sequence are selected by the program and restriction enzyme sites are attached to the primer sequences. Finally, since restriction enzymes do not cut well at the very end of a DNA molecule, an additional short nucleotide tail is automatically attached to the primers. The sequence of this tail is optimised to minimise formation of secondary structure, hairpins or dimerisation. A Java version of the primer design software, 'ORFprimer', is publicly available [51].

Automated high-throughput cloning, protein expression and purification

PCR primers and cDNA clones were delivered in 96-well microplate format. Upon delivery, plates with PCR primers and template clones were reformatted to obtain corresponding plate positions by a Zinsser Speedy pipetting robot. The PCR master mix (Roche Expand) and cDNA primers (10 µM stocks) were pipetted into a PCR microplate with a multichannel pipet. Template clone bacteria were added with a 96-pin steel replicating device from overnight cultures in microtitre plates. PCR product size and yield was determined by agarose gel electrophoresis and the software Phoretix 1D Quantifier (Nonlinear dynamics). PCR products were purified with magnetic beads on the pipetting robot with a system that has been developed at the Max Planck Institute of Molecular Genetics in collaboration with Bruker Daltonics (Bruker genopure kit). The correct restriction enzyme master mixes were automatically added and the digested fragments were purified again, analysed by agarose gel electrophoresis and quantified. The robot then adjusted DNA concentrations to a common default by dilution. Ligations were set up manually with a multichannel pipet, and SCS1 *E. coli* cells carrying pRARE

were transformed in a PCR microplate by chemical transformation on a PCR machine [52]. Transformed cells were manually plated on individual agar plates. Four clones were picked per transformation and were checked by PCR using vector primers. *E. coli* expression clones were ready for protein expression at this stage.

Primer sequences and template clones for cloning of the target cDNAs are listed in the supplementary XML file.

The characterisation of expression clones by parallel expression and protein purification is described in reference [43].

Sequence analysis

Sequence analysis software was run with default settings unless indicated otherwise. The mean charge of a protein was calculated as the difference of the number of positive and negatively charged amino acids (Lys, Arg and Glu, Asp, respectively) divided by the protein length. The mean hydrophobicity was calculated with the Kyte and Doolittle hydropathy index [53], obtained from the EMBOSS package [54]. The index values were added up for a given protein and divided by the protein length.

Authors' contributions

- Study design and coordination: KB, HL, UHe
- Bioinformatics: UHa, BS, JS, PB, VS, KB
- *E. coli* cloning: VS, KB
- *E. coli* protein work: CS, KB
- Manuscript, figures, XML file preparation: KB

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Figures and Tables

Figure 1, Vector maps.

Vector maps of pQStrep2, pQTEV and pSE111

Figure 2, SDS-PAGE of purified human proteins.

15% SDS-PAGE (Coomassie-stained) of proteins expressed in small scale in *E. coli* and purified by automated immobilised metal chelate affinity chromatography as described in [43]. The identities of the purified proteins are indicated in Table 2. Protein expression was induced at the temperature that is optimal for the individual clone. These temperatures are listed in the supplementary file psfClones.xml. M: Molecular weight marker.

Figure 3, The supplementary XML file displayed in a web browser.

Supplementary Files

psfClones.xml

XML list of PSF clones available at the RZPD. 1414 Clones for 537 target proteins are described in this file. It lists the gene, cloning details, expected sequence, protein expression results and the degree of sequence verification for each clone. The file can be viewed in a current web browser like Mozilla or Internet Explorer with the supplied style sheet.

psfToHtml.xsl

XSL style sheet for PsfExpressionClones.xml. To display the XML file in a web browser, place it in the same folder with psfToHtml.xsl.

Tables

Table 1, Origin of template cDNA clones

Numbers of targets grouped by type of template cDNA clone

Target list	Number of targets	Number of successfully cloned cDNA	Number of proteins with soluble expression	Number of structures
1 – IMAGE clones	490	264	54	3
2 – cDNA consortium	259	185	34	2
3 – hEx1 library	141	88	51	3
all	890	537	139	8

Table 2, PSF *E. coli* expression clones with soluble expression products.

The table corresponds to the proteins shown in Figure 2. More detailed information is available in the supplementary XML file.

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
39	ACAT2, acetyl-Coenzyme A acetyltransferase 2	[GenBank:AAM00223]	PSFEp250C082	partly, none		44,177	D, 24
203	AK1, adenylate kinase 1	[GenBank:BAA78534]	PSFEp250B112	yes, none	[GenBank:DQ000549]	24,558	C, 21
689	BTF3, basic transcription factor 3	[GenBank:CAA37376]	PSFEp758D0224	no		20,622	A, 27
830	CAPZA2, capping protein (actin filament) muscle Z-line, alpha 2	[GenBank:AAC60382]	PSFEp758H1226	no		59,649	A, 4
1036	CDO1, cysteine dioxygenase, type I	[GenBank:BAA12872]	PSFEp758D0124	yes, none	[GenBank:DQ000531]	25,895	E, 16
1428	CRYM, crystallin, mu	[GenBank:AAC16914]	PSFEp758B0810	yes, none	[GenBank:DQ000499]	36,275	D, 4
1460	CSNK2B, casein kinase 2, beta polypeptide	[GenBank:CAA34379]	PSFEp758H0422	no		26,209	B, 12
1606	DGKA, diacylglycerol kinase, alpha 80kDa	[GenBank:AAC34806]	PSFEp758F0324	yes, none	[GenBank:DQ000529]	16,408	A, 23
1627	DBN1, drebrin 1	[GenBank:AAH07567]	PSFEp250C052	partly, none		74,354	C, 31
1635	DCTD, dCMP deaminase	[GenBank:AAC37579]	PSFEp250B121	yes, none	[GenBank:DQ000535]	22,938	E, 25
1937	EEF1G, eukaryotic translation elongation	[GenBank:AAH15	PSFEp250H061	partly, none		53,045	C, 11

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
	factor 1 gamma	813]					
1974	EIF4A2, eukaryotic translation initiation factor 4A, isoform 2	[GenBank:AAH48105]	PSFEp250H052	partly, none		49,301	D, 20
2963	GTF2F2, general transcription factor IIF, polypeptide 2, 30kDa	[GenBank:CAA42419]	PSFEp250B012	yes, none	[GenBank:DQ000547]	31,304	C, 17
2992	GYG, glycogenin	[GenBank:AAB00114]	PSFEp758H1122	yes, none	[GenBank:DQ000517]	40,403	B, 4
3151	HMGN2, high-mobility group nucleosomal binding domain 2	[GenBank:AAA52678]	PSFEp250E102	yes, none	[GenBank:DQ000559]	12,314	D, 27
3312	HSPA8, heat shock 70kDa protein 8	[GenBank:BAB18615]	PSFEp250E022	partly, none		56,444	D, 31
3735	KARS, lysyl-tRNA synthetase	[GenBank:AAH04132]	PSFEp250D032	partly, A116G		70,976	D, 16
3925	STMN1, stathmin 1/oncoprotein 18	[GenBank:CAC16020]	PSFEp250F072	yes, none	[GenBank:DQ000556]	20,225	D, 19
4043	LRPAP1, low density lipoprotein receptor-related protein associated protein 1	[GenBank:AAC67373]	PSFEp250G031	yes, none	[GenBank:DQ000540]	44,391	C, 22
4695	NDUFA2, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2	[GenBank:AAD27762]	PSFEp250D091	no		13,843	C, 13
4698	NDUFA5, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5	[GenBank:AAD21526]	PSFEp250A0510	no		16,381	A, 13
5184	PEPD, peptidase D	[GenBank:AAH28295]	PSFEp250H122	yes, none	[GenBank:DQ000560]		D, 29
5202	PFDN2, prefoldin 2	[GenBank:AAF17218]	PSFEp250D112	yes, none	[GenBank:DQ000555]	19,570	D, 17
5412	UBL3, ubiquitin-like 3	[GenBank:AAD02323]	PSFEp758A0510	yes, none	[GenBank:DQ000501]	15,657	
5502	PPP1R1A, protein phosphatase 1, regulatory (inhibitor) subunit 1A	[GenBank:AAB02402]	PSFEp758G0124	yes, none	[GenBank:DQ000526]	21,862	A, 15
5716	PSMD10, proteasome (prosome, macropain) 26S subunit, non-ATPase, 10 (gankyrin)	[GenBank:AAH11960]	PSFEp250A062	yes, none	[GenBank:DQ000544]	27,351	C, 9
5717	PSMD11, proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	[GenBank:AAH04430]	PSFEp250B122	yes, none	[GenBank:DQ000548]	50,390	C, 19

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
5877	RABIF, RAB interacting factor	[GenBank:AAB18264]	PSFEp250C021	no		16,761	E, 23
6133	RPL9, ribosomal protein L9	[GenBank:BAA03401]	PSFEp758E0124	yes, none	[GenBank:DQ000524]	24,786	A, 28
6156	RPL30, ribosomal protein L30	[GenBank:CAA55820]	PSFEp758A1113	yes, none	[GenBank:DQ000503]	15,284	D, 6
6191	RPS4X, ribosomal protein S4, X-linked	[GenBank:BC007308]	PSFEp758A0923	no		28,670	
6342	SCP2, sterol carrier protein 2	[GenBank:AAA03559]	PSFEp758C0723	yes, none	[GenBank:DQ000515]	16,668	B, 1
6451	SH3BGRL, SH3 domain binding glutamic acid-rich protein like	[GenBank:AAC27445]	PSFEp758C0713	no		15,274	D, 2
6728	SRP19, signal recognition particle 19kDa	[GenBank:CAA31280]	PSFEp758E0317	no		41,156	
6888	TALDO1, transaldolase 1	[GenBank:AAB53943]	PSFEp758B0711	partly, none		40,040	D, 1
6990	TCTE1L, t-complex-associated-testis-expressed 1-like	[GenBank:AAA57444]	PSFEp758D0814	no		38,062	A, 3
6993	TCTEL1, t-complex-associated-testis-expressed 1-like 1	[GenBank:BAA09317]	PSFEp758F1123	yes, none	[GenBank:DQ000521]	13,719	A, 14
7001	PRDX2, peroxiredoxin 2	[GenBank:AAH03022]	PSFEp250A042	yes, none	[GenBank:DQ000546]	24,815	C, 30
7178	TPT1, tumor protein, translationally-controlled 1	[GenBank:CAA34200]	PSFEp250G011	yes, none	[GenBank:DQ000539]	22,518	C, 14
7247	TSN, translin	[GenBank:CAA55341]	PSFEp250B111	yes, none	[GenBank:DQ000538]	29,106	C, 12
7353	UFD1L, ubiquitin fusion degradation 1-like	N[GenBank:P_005650]	PSFEp758G0123	yes, none	[GenBank:DQ000519]	41,777	D, 9
7390	UROS, uroporphyrinogen III synthase	[GenBank:AAA60273]	PSFEp758G0323	no		29,894	D, 11
7518	XRCC4, X-ray repair complementing defective repair in Chinese hamster cells 4	[GenBank:AAH16314]	PSFEp250A033	partly, none		41,211	D, 26
7531	YWHAE, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	[GenBank:AAH01440]	PSFEp250H041	yes, A146G	[GenBank:DQ000541]	32,097	C, 24
8125	ANP32A, acidic (leucine-rich) nuclear	[GenBank:AAB91	PSFEp250D092	yes, none	[GenBank:DQ000	31,509	D, 22

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
	phosphoprotein 32 family, member A	548]			553]		
8214	DGCR6, DiGeorge syndrome critical region gene 6	[GenBank:CAA65339]	PSFEp758G0324	yes, none	[GenBank:DQ000533]	12,111	E, 19
8407	TAGLN2, transgelin 2	[GenBank:BAA04802]	PSFEp250F062	yes, none	[GenBank:DQ000550]	25,314	C, 27
8544	PIR, pirin	[GenBank:CAA69195]	PSFEp758A0213	yes, none	[GenBank:DQ000498]	34,613	C, 1
8575	PRKRA, protein kinase, interferon-inducible double stranded RNA dependent activator	[GenBank:CAB66550]	PSFEp250D014	yes, none	[GenBank:DQ000578]	37,328	E, 9
8677	STX10, syntaxin 10	[GenBank:AAC05087]	PSFEp250D102	yes, none	[GenBank:DQ000552]	25,369	D, 18
8724	SNX3, sorting nexin 3	[GenBank:AAC16040]	PSFEp758A0323	yes, none	[GenBank:DQ000514]	20,029	B, 15
8896	G10, maternal G10 transcript	[GenBank:AAH22821]	PSFEp250F011	yes, A394G	[GenBank:DQ000536]	19,766	C, 16
8926	SNURF, SNRPN upstream reading frame	[GenBank:AAD31391]	PSFEp250A0610	no		11,334	C, 25
9049	AIP, aryl hydrocarbon receptor interacting protein	[GenBank:AAB59004]	PSFEp758F1024	yes, none	[GenBank:DQ000528]	40,589	A, 21
9158	FIBP, fibroblast growth factor (acidic) intracellular binding protein	[GenBank:CAG33030]	PSFEp250B022	partly, T602C		44,803	C, 20
9168	TMSB10, thymosin, beta 10	[GenBank:AAB25225]	PSFEp758A0124	yes, none	[GenBank:DQ000522]	6,292	A, 18
9337	CNOT8, CCR4-NOT transcription complex, subunit 8	[GenBank:AAH17366]	PSFEp758E0524	yes, none	[GenBank:DQ000523]	36,354	A, 24
9453	GGPS1, geranylgeranyl diphosphate synthase 1	[GenBank:AAH05252]	PSFEp250E052	partly, none		37,795	D, 23
9465	AKAP7, A kinase (PRKA) anchor protein 7	[GenBank:AAC39715]	PSFEp758E0415	no		34,000	A, 1
9796	PHYHIP, phytanoyl-CoA hydroxylase interacting protein	[GenBank:BAA13402]	PSFEp758C1124	yes, T806C	[GenBank:DQ000530]	40,497	A, 29
10000	AKT3, v-akt murine thymoma viral oncogene homolog 3	[GenBank:CAB55977]	PSFEp250B063	yes, none		56,516	E, 20
10063	COX17, COX17 homolog, cytochrome c oxidase assembly protein (yeast)	[GenBank:AAA98114]	PSFEp758D0723	yes, none	[GenBank:DQ000516]	8,182	B, 3

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
10094	ARPC3, actin related protein 2/3 complex, subunit 3	[GenBank:AAB64191]	PSFEp758G0811	yes, none	[GenBank:DQ000507]	23,047	D, 7
10169	SERF2, small EDRK-rich factor 2	[GenBank:AAC63516]	PSFEp250A057	no		9,821	C, 23
10228	STX6, syntaxin 6	[GenBank:AAH09944]	PSFEp758G0526	yes, none	[GenBank:DQ000509]	32,100	B, 7
10247	HRSP12, heat-responsive protein 12 (14.5 kDa translational inhibitor protein, p14.5)	[GenBank:CAA64670]	PSFEp758H0822	yes, none	[GenBank:DQ000518]	15,760	D, 12
10290	APEG1, aortic preferentially expressed protein 1	[GenBank:AAH06346]	PSFEp250B082	yes, none	[GenBank:DQ000543]	15,614	E, 18
10539	TXNL2, thioredoxin-like 2	[GenBank:AAH05289]	PSFEp250A022	partly, none		40,357	C, 26
10588	MTHFS, 5,10-methenyltetrahydrofolate synthetase	[GenBank:AAC41945]	PSFEp758B0824	no		24,522	D, 15
10589	DRAP1, DR1-associated protein 1 (negative cofactor 2 alpha)	[GenBank:AAH10025]	PSFEp250C042	yes, none	[GenBank:DQ000542]	25,273	E, 3
10598	AHSA1, AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	[GenBank:AAD09623]	PSFEp250B091	no		41,199	C, 15
10606	PAICS, phosphoribosylaminoimidazole carboxylase	[GenBank:AAH19255]	PSFEp758G0226	partly, none		50,005	A, 5
10842	C7orf16, chromosome 7 open reading frame 16	[GenBank:AAF03537]	PSFEp758F0923	no		19,132	B, 2
10856	RUVBL2, RuvB-like 2 (E. coli)	[GenBank:AAH04531]	PSFEp250C072	partly, none		54,083	D, 25
10912	GADD45G, growth arrest and DNA-damage-inducible, gamma	[GenBank:AAC83329]	PSFEp758F0111	yes, none	[GenBank:DQ000504]	19,621	C, 4
10933	MORF4L1, mortality factor 4 like 1	[GenBank:AAH22845]	PSFEp250G051	partly, none		40,155	C, 10
10963	STIP1, stress-induced-phosphoprotein 1	[GenBank:AAA58682]	PSFEp250A012	partly, A86C		65,566	C, 28
11140	CDC37, CDC37 cell division cycle 37 homolog (S. cerevisiae)	[GenBank:AAH08793]	PSFEp250E042	partly, none		47,394	D, 28
11316	COPE, coatomer protein complex, subunit epsilon	[GenBank:AAH07250]	PSFEp758G1126	yes, none	[GenBank:DQ000510]	37,406	B, 9
11333	PDAP1, PDGFA associated protein 1	[GenBank:AAH07	PSFEp250A073	yes, none	[GenBank:DQ000	23,553	D, 30

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
		873]			554]		
11337	GABARAP, GABA(A) receptor-associated protein	[GenBank:AAD02337]	PSFEp758H0922	no		15,185	D, 10
11344	PTK9L, PTK9L protein tyrosine kinase 9-like (A6-related protein)	N[GenBank:P_009215]	PSFEp758A0127	yes, none	[GenBank:DQ000525]	42,473	E, 13
22818	COPZ1, coatomer protein complex, subunit zeta 1	[GenBank:AAD34115]	PSFEp758A0812	yes, none	[GenBank:DQ000497]	22,698	D, 3
22919	MAPRE1, microtubule-associated protein, RP/EB family, member 1	[GenBank:CAB53072]	PSFEp250C112	yes, none	[GenBank:DQ000557]	32,923	D, 21
22931	RAB18, RAB18, member RAS oncogene family	[GenBank:CAB66668]	PSFEp250C118	no		25,900	B, 27
23589	CARHSP1, calcium regulated heat stable protein 1	[GenBank:AAD25021]	PSFEp778D072	no		40,934	
25842	ASF1A, ASF1 anti-silencing function 1 homolog A (S. cerevisiae)	[GenBank:AAD34093]	PSFEp758E1224	yes, none	[GenBank:DQ000532]	25,891	E, 17
25843	PREI3, preimplantation protein 3	[GenBank:AAD34090]	PSFEp758B0812	yes, none	[GenBank:DQ000500]	28,532	C, 2
25996	DKFZP566E144, small fragment nuclease	[GenBank:AAD34109]	PSFEp758G0423	yes, none	[GenBank:DQ000520]	25,021	D, 13
26289	AK5, adenylate kinase 5	[GenBank:AAH12467]	PSFEp250G042	yes, 3 bp insertion at 3' end	[GenBank:DQ000558]	24,897	E, 14
26353	HSPB8, heat shock 22kDa protein 8	[GenBank:CAB66870]	PSFEp250G125	yes, none	[GenBank:DQ000565]	24,527	B, 16
27095	TRAPPC3, trafficking protein particle complex 3	[GenBank:AAB96936]	PSFEp758A0513	yes, none	[GenBank:DQ000502]	22,774	C, 3
27249	C2orf25, chromosome 2 open reading frame 25	[GenBank:AAD20048]	PSFEp758D1124	no		35,864	A, 22
27335	eIF3k, eukaryotic translation initiation factor 3 subunit 12	[GenBank:BAA76626]	PSFEp758D0923	no		26,326	D, 8
27430	MAT2B, methionine adenosyltransferase II, beta	[GenBank:CAB66599]	PSFEp250A114	yes, none	[GenBank:DQ000575]	40,476	B, 21
28970	PTD012, PTD012	[GenBank:CAB66540]	PSFEp250B014	yes, none	[GenBank:DQ000586]	38,042	E, 24
29789	PTD004, GTP-binding protein PTD004	[GenBank:CAB66481]	PSFEp250H073	yes, none	[GenBank:DQ000564]	47,609	B, 18
30819	KCNIP2, Kv channel interacting protein 2	[GenBank:CAB66656]	PSFEp250G064	yes, none	[GenBank:DQ000562]	28,964	E, 5

NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
51001	CGI-12, CGI-12	[GenBank:AAH12995]	PSFEp250B092	yes, none	[GenBank:DQ000545]	40,832	C, 8
51076	CUTC, cutC copper transporter homolog (E.coli), CGI-32	[GenBank:AAH21105]	PSFEp758E1112	yes, none	[GenBank:DQ000505]	31,811	C, 5
51078	THAP4, THAP domain containing 4	[GenBank:AAD27745]	PSFEp250H116	no		21,550	C, 7
51155	HN1, hematological and neurological expressed 1	[GenBank:AAH01420]	PSFEp758G0126	yes, 3 bp deletion at 5' end, G457A	[GenBank:DQ000508]	18,937	C, 6
51160	VPS28, vacuolar protein sorting 28 (yeast)	[GenBank:AAF00499]	PSFEp758C0323	yes, none	[GenBank:DQ000513]	26,692	B, 14
51335	NEUGRIN, mesenchymal stem cell protein DSC92	[GenBank:CAD39160]	PSFEp250B084	yes, none	[GenBank:DQ000566]	27,342	B, 28
51397	COMMD10, COMM domain containing 10	[GenBank:AAD44489]	PSFEp758H0811	yes, none	[GenBank:DQ000506]	25,466	D, 5
51433	ANAPC5, anaphase promoting complex subunit 5	[GenBank:BAA76629]	PSFEp758E0214	no		47,174	B, 8
51451	LCMT1, leucine carboxyl methyltransferase 1	[GenBank:AAH01214]	PSFEp758H0126	partly, none		41,303	B, 11
51534	C6orf55, chromosome 6 open reading frame 55	[GenBank:AAF76210]	PSFEp758H0426	partly, none		41,303	B, 13
51629	CGI-69, CGI-69	[GenBank:AAD34064]	PSFEp758D0316	no		63,564	B, 6
51678	MPP6, membrane protein, palmitoylated 6	[GenBank:CAB66770]	PSFEp250E023	yes, none	[GenBank:DQ000583]	64,044	E, 27
54816	SUHW4, suppressor of hairy wing homolog 4 (Drosophila)	[GenBank:CAB66569]	PSFEp250G074	yes, none	[GenBank:DQ000587]	27,032	E, 22
55255	WDR41, WD repeat domain 41	[GenBank:CAD38853]	PSFEp250E105	yes, none	[GenBank:DQ000563]	26,313	
55276	PGM2, phosphoglucomutase 2	[GenBank:CAB66640]	PSFEp250B113	partly, none		71,239	B, 26
56681	SARA1, SAR1a gene homolog 1 (S. cerevisiae)	[GenBank:CAB66658]	PSFEp250G115	yes, none	[GenBank:DQ000570]	25,290	B, 17
56911	C21orf7, chromosome 21 open reading frame 7	[GenBank:CAD28500]	PSFEp250B086	yes, none	[GenBank:DQ000571]	19,032	E, 6
57019	LOC57019, cytokine induced apoptosis inhibitor 1	[GenBank:AAC24311]	PSFEp758E0424	yes, none	[GenBank:DQ000534]	36,506	E, 21
58485	TRAPPC1, trafficking	[GenBan	PSFEp250D0	yes, T350C	[GenBan	19,75	E, 1

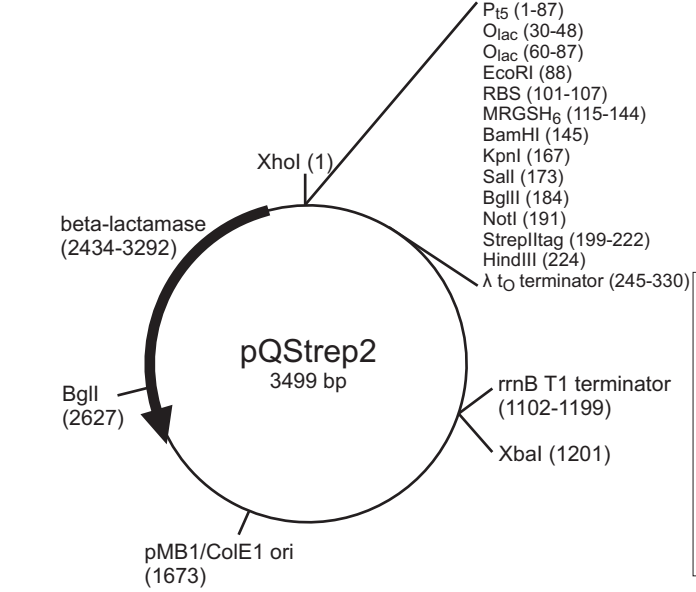
NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
	protein particle complex 1	k:AAD44697]	71		k:DQ000537]	4	
64284	RAB17, RAB17, member RAS oncogene family	[GenBank:CAB66580]	PSFEp250E095	yes, none	[GenBank:DQ000572]	26,398	A, 6
79632	C6orf60, chromosome 6 open reading frame 60	[GenBank:CAB66701]	PSFEp250B044	yes, none	[GenBank:DQ000568]	34,217	B, 20
79666	PLEKHF2, pleckstrin homology domain containing, family F member 2	[GenBank:CAD39132]	PSFEp250D054	yes, A271G	[GenBank:DQ000581]	30,721	A, 9
79791	FBXO31, F-box protein 31	[GenBank:CAB66696]	PSFEp250F085	yes, none	[GenBank:DQ000585]	18,588	E, 12
80347	COASY, Coenzyme A synthase	[GenBank:AAF87955]	PSFEp250A053	yes, none	[GenBank:DQ000551]	33,147	C, 29
80895	ILKAP, integrin-linked kinase-associated serine/threonine phosphatase 2C	[GenBank:CAB66784]	PSFEp250D083	yes, none	[GenBank:DQ000569]	45,832	B, 19
81876	RAB1B, RAB1B, member RAS oncogene family	[GenBank:CAB66570]	PSFEp250C128	no		25,094	A, 12
81889	DKFZP566J2046, fumarylacetoacetate hydrolase domain containing 1	[GenBank:CAB66654]	PSFEp250B074	yes, none	[GenBank:DQ000567]	27,766	B, 24
83538	DKFZP434H0115, hypothetical protein DKFZp434H0115	[GenBank:CAB66694]	PSFEp250G093	partly, none		79,584	E, 11
83543	C9orf58, chromosome 9 open reading frame 58	[GenBank:CAB66501]	PSFEp250B085	yes, none	[GenBank:DQ000573]	19,990	B, 25
83667	SESN2, sestrin 2	[GenBank:CAB66486]	PSFEp250F043	yes, none	[GenBank:DQ000576]	57,420	E, 7
84072	NOHMA, HORMA domain containing protein	[GenBank:CAB66689]	PSFEp250F083	yes, none	[GenBank:DQ000561]	47,343	E, 4
84324	CIP29, cytokine induced protein 29 kDa	[GenBank:CAC37950]	PSFEp758H1026	yes, none	[GenBank:DQ000512]	26,594	A, 2
84457	PHYHIPL, phytanoyl-CoA hydroxylase interacting protein-like	[GenBank:CAD39006]	PSFEp250A084	yes, none	[GenBank:DQ000579]	45,425	E, 10
84557	MAP1LC3A, microtubule-associated protein 1 light chain 3 alpha	[GenBank:CAD38714]	PSFEp250E106	yes, none	[GenBank:DQ000584]	17,194	E, 26
91603	MGC20398, hypothetical protein MGC20398	[GenBank:BAB70992]	PSFEp758H0526	yes, none	[GenBank:DQ000511]	44,915	B, 10
94240	EPSTI1, epithelial stromal interaction 1	[GenBank:CAD38	PSFEp250C015	yes, none	[GenBank:DQ000	25,527	A, 11

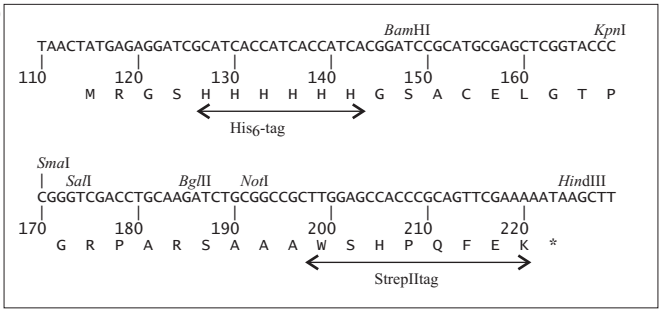
NCBI Entrez gene ID	Gene symbol, name	Protein accession	RZPD clone ID	Sequence verified, non-silent mutations	Clone accession	Protein size [Da]	Protein gel, lane
	(breast)	599]			580]		
112611	RWDD2, RWD domain containing 2	[GenBank:CAB52345]	PSFEp758A1024	partly, T709C		35,159	B, 5
118812	C10orf83, chromosome 10 open reading frame 83	[GenBank:CAD38849]	PSFEp250H085	yes, none	[GenBank:DQ000574]	19,158	B, 23
122060	FLJ30046, hypothetical protein FLJ30046	[GenBank:CAD38891]	PSFEp250E015	yes, none	[GenBank:DQ000577]	23,468	E, 8
136319	MTPN, myotrophin	[GenBank:CAD38909]	PSFEp250D016	yes, none	[GenBank:DQ000582]	15,817	A, 7
140856	C20orf79, chromosome 20 open reading frame 79	[GenBank:CAB56175]	PSFEp758G0224	yes, G258T	[GenBank:DQ000527]	20,585	A, 19

Table 3, Novel human protein structures

The structures of full length proteins solved by the Protein Structure Factory.

NCBI Entrez gene ID	Name	GenBank protein accession	PDB ID	Reference
5716	Gankyrin	[GenBank:AAH11960]	[PDB:1QYM]	[55]
10290	APEG1, aortic preferentially expressed protein 1	[GenBank:AAH06346]	[PDB:1U2H]	
81889	Fumarylacetoacetate hydrolase family member FLJ36880	[GenBank:CAB66654]	[PDB:1SAW]	[56]
28970	PTD012	[GenBank:CAB66540]	[PDB:1XCR]	
10247	14.5 kDa translational inhibitor protein, p14.5	[GenBank:CAA64670]	[PDB:1ONI]	[57]
27095	BET3, trafficking protein particle subunit	[GenBank:AAB96936]	[PDB:1SZ7]	[58]
5184	Peptidase D	[GenBank:AAH28295]		
51076	CutC copper transporter homolog, CGI-32	[GenBank:AAH21105]		
122553	TPC6	[GenBank:CAI46185]	[PDB:2BJN]	[59]
6449	Nicotinamide mononucleotide adenylyltransferase	[GenBank:NP_003012]	[PDB:1GZU]	[60]


Pt5 (1-87)
Olac (30-48)
Olac (60-87)
EcoRI (88)
RBS (101-107)
MRGSH6 (115-144)
BamHI (145)
KpnI (167)
SalI (173)
BglII (184)
NotI (191)
StrepIItag (199-222)
HindIII (224)
λ tO terminator (245-330)
XhoI (1)
beta-lactamase
(2434-3292)
pQStrep2
3499 bp
BglI
(2627)
rrnB T1 terminator
(1102-1199)
XbaI (1201)
pMB1/ColE1 ori
(1673)


BamHI
KpnI
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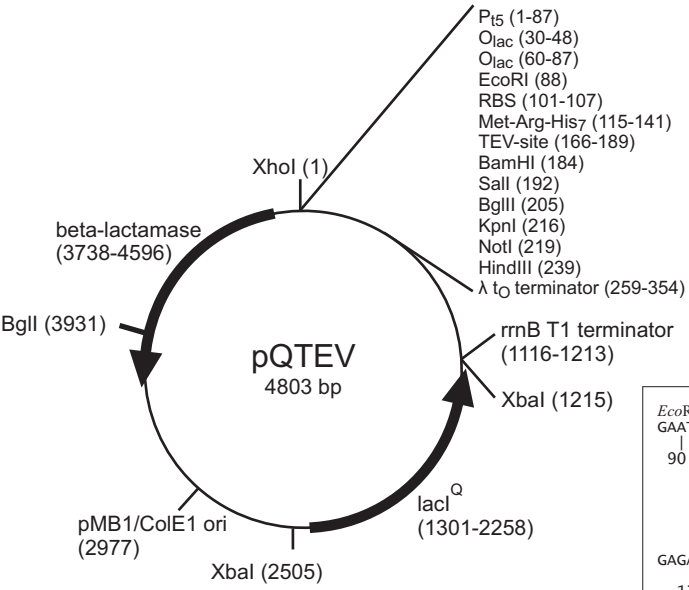
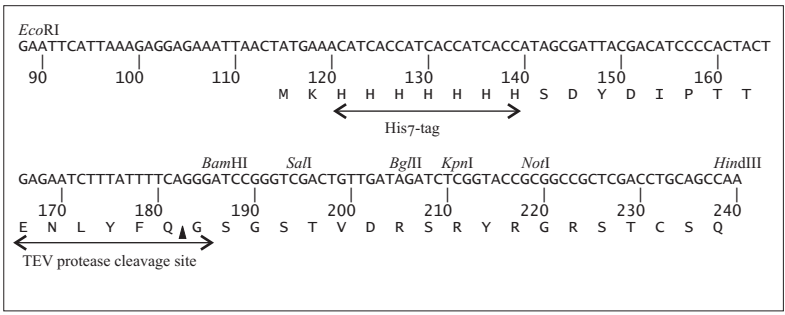
110 120 130 140 150 160
M R G S H H H H H H G S A C E L G T P
His6-tag
SmaI
SalI
BglII
NotI
HindIII
CGGGTCGACCTGCAAGATCTGCGGCCGCTTGGAGCCACCCGCAGTTCGAAAAATAAGCTT
170 180 190 200 210 220
G R P A R S A A A W S H P Q F E K *
StrepIItag


Pt5 (1-87)
Olac (30-48)
Olac (60-87)
EcoRI (88)
RBS (101-107)
Met-Arg-His7 (115-141)
TEV-site (166-189)
BamHI (184)
SalI (192)
BglII (205)
KpnI (216)
NotI (219)
HindIII (239)
λ tO terminator (259-354)
XhoI (1)
beta-lactamase
(3738-4596)
BglI (3931)
pQTEV
4803 bp
rrnB T1 terminator
(1116-1213)
XbaI (1215)
lacIQ
(1301-2258)
pMB1/ColE1 ori
(2977)
XbaI (2505)


EcoRI
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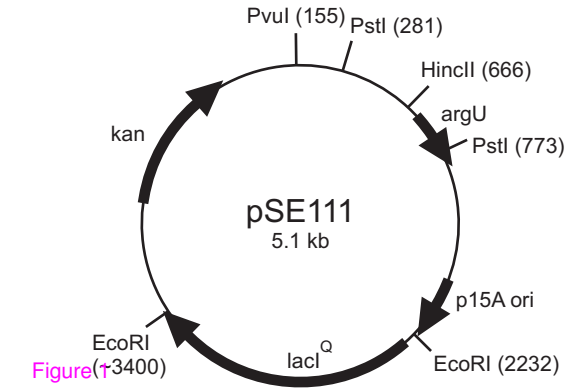
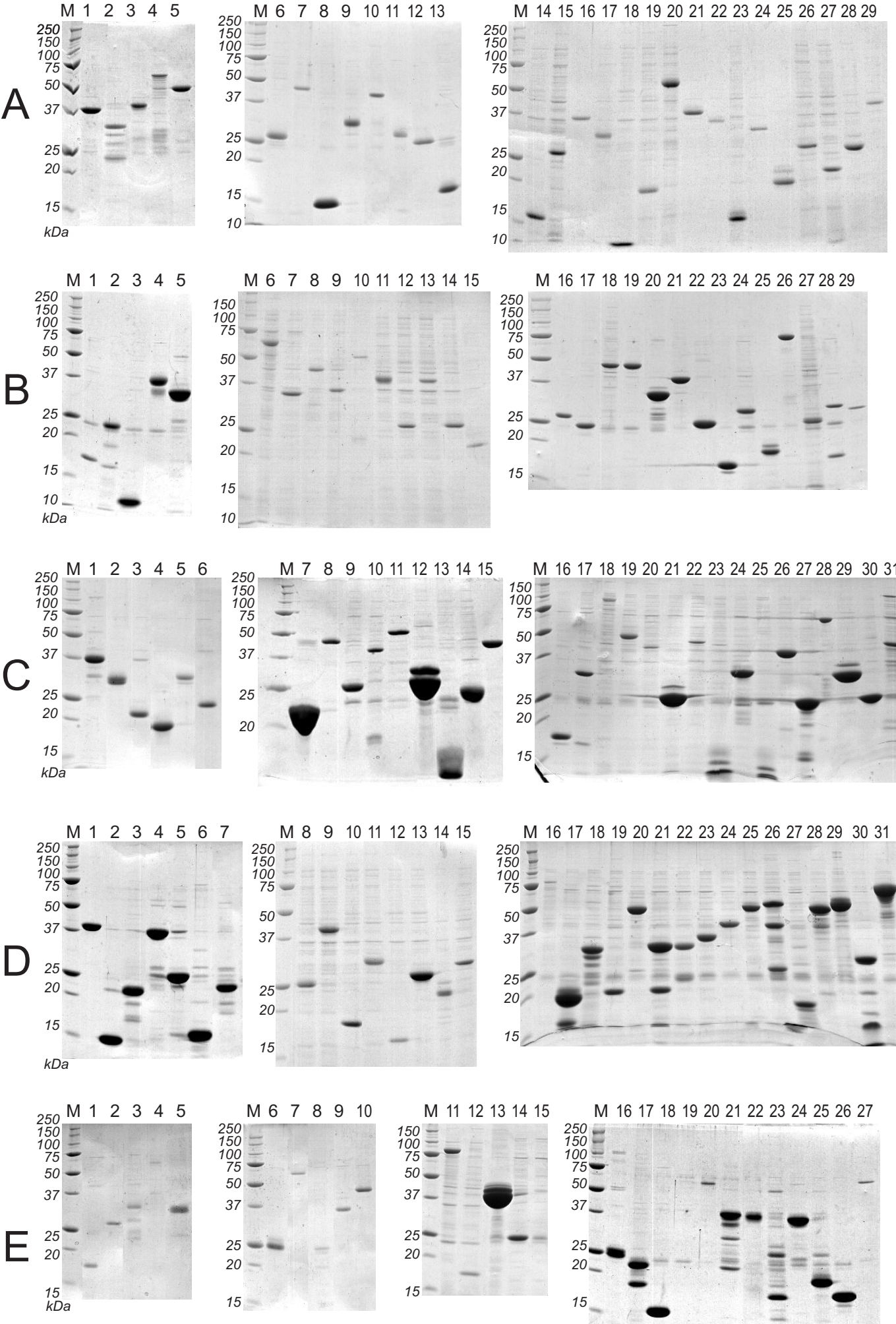
90 100 110 120 130 140 150 160
M K H H H H H H H S D Y D I P T T
His7-tag
BamHI
SalI
BglII
KpnI
NotI
HindIII
GAGAATCTTTATTTTCAGGGATCCGGGTCGACTGTTGATAGATCTCGGTACCGCGGCCGCTCGACCTGCAGCCAA
170 180 190 200 210 220 230 240
E N L Y F Q G S G S T V D R S R Y R G R S T C S Q
TEV protease cleavage site


PvuI (155)
PstI (281)
HincII (666)
argU
PstI (773)
kan
pSE111
5.1 kb
p15A ori
EcoRI
(3400)
lacIQ
EcoRI (2232)

Figure(1


A
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250 150 100 75 50 37 25 20 15
kDa
M 6 7 8 9 10 11 12 13
250 150 100 75 50 37 25 20 15 10
M 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
250 150 100 75 50 37 25 20 15 10
B
M 1 2 3 4 5
250 150 100 75 50 37 25 20 15 10
kDa
M 6 7 8 9 10 11 12 13 14 15
150 100 75 50 37 25 20 15 10
M 16 17 18 19 20 21 22 23 24 25 26 27 28 29
250 150 100 75 50 37 25 20 15
C
M 1 2 3 4 5 6
250 150 100 75 50 37 25 20 15
kDa
M 7 8 9 10 11 12 13 14 15
250 150 100 75 50 37 25 20
M 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
150 100 75 50 37 25 20 15
D
M 1 2 3 4 5 6 7
250 150 100 75 50 37 25 20 15
kDa
M 8 9 10 11 12 13 14 15
250 150 100 75 50 37 25 20 15
M 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
250 150 100 75 50 37 25 20 15
E
M 1 2 3 4 5
250 150 100 75 50 37 25 20 15
kDa
M 6 7 8 9 10
250 150 100 75 50 37 25 20 15
M 11 12 13 14 15
150 100 75 50 37 25 20 15
M 16 17 18 19 20 21 22 23 24 25 26 27
250 150 100 75 50 37 25 20 15 10

Figure 2

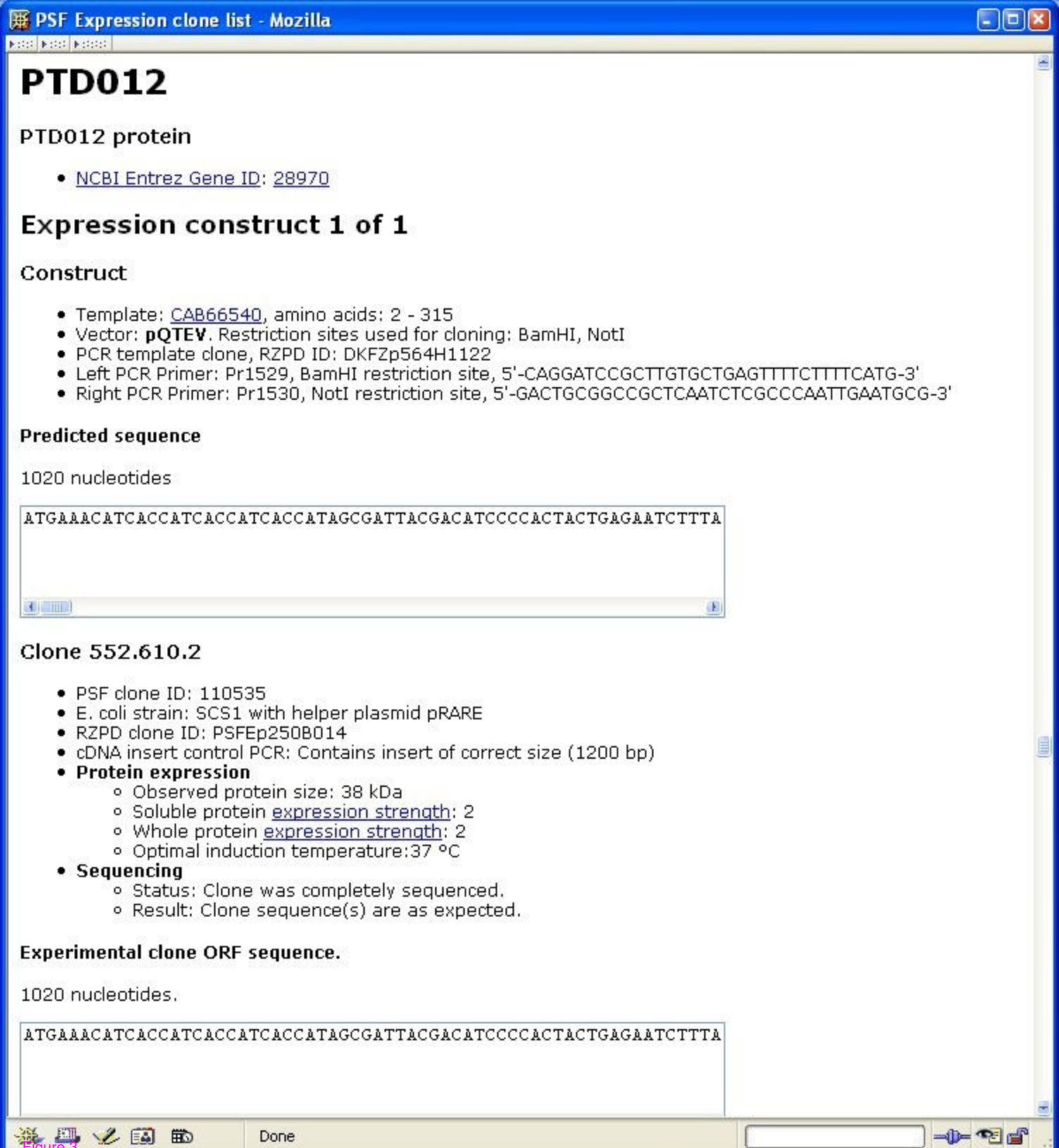
PSF Expression clone list - Mozilla
PTD012
PTD012 protein
NCBI Entrez Gene ID: 28970
Expression construct 1 of 1
Construct
Template: CAB66540, amino acids: 2 - 315
Vector: pQTEV. Restriction sites used for cloning: BamHI, NotI
PCR template clone, RZPD ID: DKFZp564H1122
Left PCR Primer: Pr1529, BamHI restriction site, 5'-CAGGATCCGCTTGTGCTGAGTTTTCTTTTCATG-3'
Right PCR Primer: Pr1530, NotI restriction site, 5'-GACTGCGGCCGCTCAATCTCGCCCAATTGAATGCG-3'
Predicted sequence
1020 nucleotides
ATGAAACATCACCATCACCATCACCATAGCGATTACGACATCCCCACTACTGAGAATCTTTA
Clone 552.610.2
PSF clone ID: 110535
E. coli strain: SCS1 with helper plasmid pRARE
RZPD clone ID: PSFEp250B014
cDNA insert control PCR: Contains insert of correct size (1200 bp)
Protein expression
Observed protein size: 38 kDa
Soluble protein expression strength: 2
Whole protein expression strength: 2
Optimal induction temperature:37 °C
Sequencing
Status: Clone was completely sequenced.
Result: Clone sequence(s) are as expected.
Experimental clone ORF sequence.
1020 nucleotides.
ATGAAACATCACCATCACCATCACCATAGCGATTACGACATCCCCACTACTGAGAATCTTTA
Done

Figure 3

Additional files provided with this submission:

Additional file 2 : psfToHtml.xsl : 5Kb
http://www.microbialcellfactories.com/imedia/1341799466904486/sup2.XSL
Additional file 1 : psfClones.xml : 2237Kb
http://www.microbialcellfactories.com/imedia/1400371927737960/sup1.XML