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SUPERFAMILY: HMMs representing all proteins of known structure. SCOP sequence searches, alignments and genome assignments

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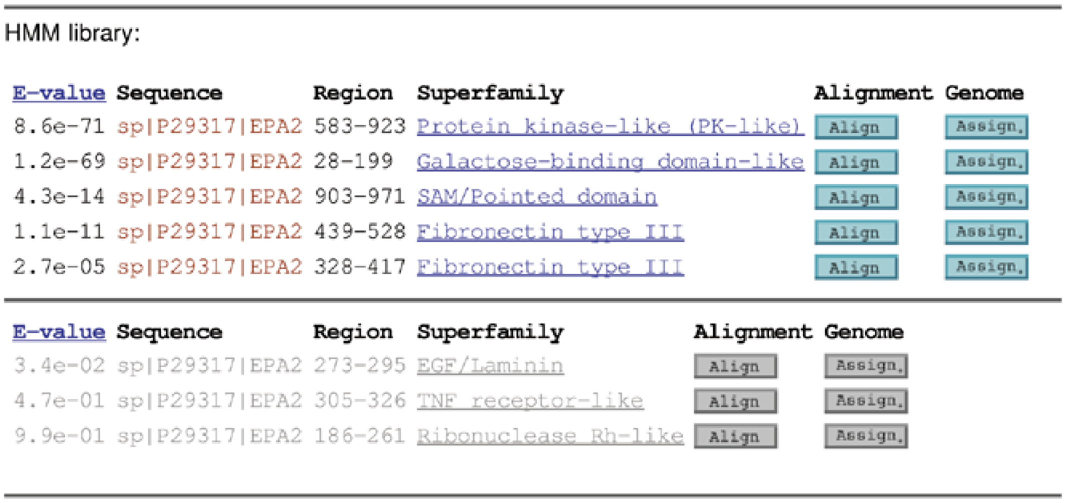
ABSTRACT

The SUPERFAMILY database contains a library of hidden Markov models representing all proteins of known structure. The database is based on the SCOP 'superfamily' level of protein domain classification which groups together the most distantly related proteins which have a common evolutionary ancestor. There is a public server at http://supfam.org which provides three services: sequence searching, multiple alignments to sequences of known structure, and structural assignments to all complete genomes. Given an amino acid or nucleotide query sequence the server will return the domain architecture and SCOP classification. The server produces alignments of the query sequences with sequences of known structure, and includes multiple alignments of genome and PDB sequences. The structural assignments are carried out on all complete genomes (currently 59) covering approximately half of the soluble protein domains. The assignments, superfamily breakdown and statistics on them are available from the server. The database is currently used by this group and others for genome annotation, structural genomics, gene prediction and domain-based genomic studies.

INTRODUCTION

The SUPERFAMILY database is based on the SCOP (1) classification of protein domains. SCOP is a structural domain-based heirarchical classification with several levels including the 'superfamily' level. Proteins grouped together at the superfamily level are defined as having structural, functional and sequence evidence for a common evolutionary ancestor. It is at this level, as the name suggests, that SUPERFAMILY operates because it is the level with the most distantly related protein domains. The level below is the 'family' level which groups more closely related domains, and the level above is the 'fold'



HMM library:

E-value	Sequence	Region	Superfamily	Alignment	Genome
8.6e-71	sp|P29317|EPA2	583-923	Protein kinase-like (PK-like)	Align	Assign.
1.2e-69	sp|P29317|EPA2	28-199	Galactose-binding domain-like	Align	Assign.
4.3e-14	sp|P29317|EPA2	903-971	SAM/Pointed domain	Align	Assign.
1.1e-11	sp|P29317|EPA2	439-528	Fibronectin type III	Align	Assign.
2.7e-05	sp|P29317|EPA2	328-417	Fibronectin type III	Align	Assign.

E-value	Sequence	Region	Superfamily	Alignment	Genome
3.4e-02	sp|P29317|EPA2	273-295	EGF/Laminin	Align	Assign.
4.7e-01	sp|P29317|EPA2	305-326	TNF receptor-like	Align	Assign.
9.9e-01	sp|P29317|EPA2	186-261	Ribonuclease Rh-like	Align	Assign.

Figure 1. An example of the result of a sequence query. The protein (sp|P2931|EPA2_HUMAN) is a multi-domain protein with five structural domains predicted with confidence, and shown in grey, three non-significant predictions. Each domain covers a different region of the query sequence and may be classified in a different SCOP superfamily with a different score. The 'Align' button links to a sequence alignment, and the 'Assign.' button links to all genome assignments for the given superfamily.

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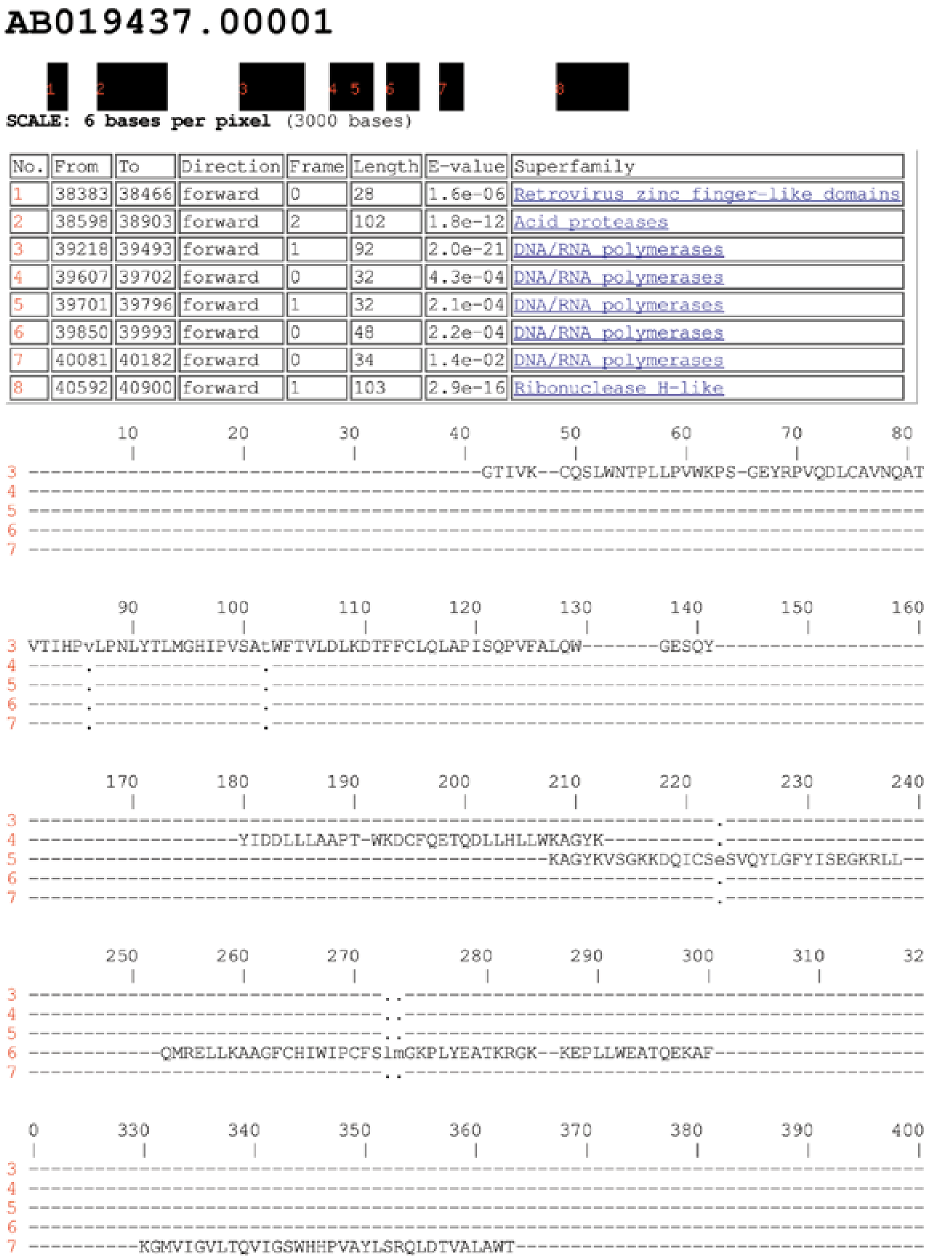

AB019437.00001

SCALE: 6 bases per pixel (3000 bases)

No.	From	To	Direction	Frame	Length	E-value	Superfamily
1	38383	38466	forward	0	28	1.6e-06	Retrovirus zinc finger-like domains
2	38598	38903	forward	2	102	1.8e-12	Acid proteases
3	39218	39493	forward	1	92	2.0e-21	DNA/RNA polymerases
4	39607	39702	forward	0	32	4.3e-04	DNA/RNA polymerases
5	39701	39796	forward	1	32	2.1e-04	DNA/RNA polymerases
6	39850	39993	forward	0	48	2.2e-04	DNA/RNA polymerases
7	40081	40182	forward	0	34	1.4e-02	DNA/RNA polymerases
8	40592	40900	forward	1	103	2.9e-16	Ribonuclease H-like

```
          10        20        30        40        50        60        70        80
           |         |         |         |         |         |         |         |
3 ----------------------------------------GTIVK--CQSLWNTPLLPVWKPS-GEYRPVQDLCAVNQAT
4 --------------------------------------------------------------------------------
5 --------------------------------------------------------------------------------
6 --------------------------------------------------------------------------------
7 --------------------------------------------------------------------------------

          90       100       110       120       130       140       150       160
           |         |         |         |         |         |         |         |
3 VTIHPvLPNLYTLMGHIPVSAtWFTVLDLKDTFFCLQLAPISQPVFALQW-------GESQY------------------
4 -----.---------------.----------------------------------------------------------
5 -----.---------------.----------------------------------------------------------
6 -----.---------------.----------------------------------------------------------
7 -----.---------------.----------------------------------------------------------

         170       180       190       200       210       220       230       240
           |         |         |         |         |         |         |         |
3 -------------------------------------------------------------.------------------
4 ------------------YIDDLLLAAPT-WKDCFQETQDLLHLLWKAGYK----------.------------------
5 ----------------------------------------------KAGYKVSGKKDQICSeSVQYLGFYISEGKRLL--
6 -------------------------------------------------------------.------------------
7 -------------------------------------------------------------.------------------

         250       260       270         280       290       300       310       32
           |         |         |           |         |         |         |
3 -------------------------------..-----------------------------------------------
4 -------------------------------..-----------------------------------------------
5 -------------------------------..-----------------------------------------------
6 -----------QMRELLKAAGFCHIWIPCFSlmGKPLYEATKRGK--KEPLLWEATQEKAF-------------------
7 -------------------------------..-----------------------------------------------

  0        330       340       350       360       370       380       390       400
  |          |         |         |         |         |         |         |         |
3 --------------------------------------------------------------------------------
4 --------------------------------------------------------------------------------
5 --------------------------------------------------------------------------------
6 --------------------------------------------------------------------------------
7 ----------KGMVIGVLTQVIGSWHHPVAYLSRQLDTVALAWT------------------------------------
```

Figure 2. A section of a result of a nucleotide search of human contig AB019437.00001 clearly showing a DNA/RNA polymerase domain consisting of exons 3–7. The alignment shows how the exons combine in order to make up a complete domain.

level which groups domains with similar topology which are not necessarily related.

The database uses hidden Markov models (HMMs) which are profiles based on multiple sequence alignments designed to represent a protein family (or superfamily) which can be used to search sequence databases for homologues. The SAM-T99 HMM software (2) is one of the best methods for the detection of remote protein homologues. The SAM software was used to build a library of models (3) representing all proteins of known structure, which forms the core of the SUPERFAMILY database. These models have added value by expert curation and tuning designed to detect and classify SCOP domains at the superfamily level.

There are existing databases which use HMMs representing protein domains such as Pfam (4), SMART (5) and others. There are also unifying databases which have several of these methods included, e.g. InterPro (6) and CDD (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). There are two main differences to SUPERFAMILY: these other databases span all proteins whereas SUPERFAMILY only covers those with a known structural representative, and they also group domains into families based on sequence similarity alone leading to a level of

Table 1. The genome assignments for 56 genomes using the model library and assignment procedure

Genome	A	B	C	D	E	F	G	H
Arabidopsis thaliana	at	E	25 470	13 320	52	38	17 957	564
Homo sapiens	hs	E	23 867	11 661	49	37	21 201	595
Caenorhabditis elegans	ce	E	19 705	7851	40	29	12 628	537
Drosophila melanogaster	dm	E	14 331	6851	48	34	11 479	554
Mesorhizobium loti	mk	B	6752	3552	53	44	4631	433
Saccharomyces cerevisiae	sc	E	6297	2770	44	33	3760	461
Pseudomonas aeruginosa	pa	B	5570	3079	55	45	4261	439
Escherichia coli o157	eo	B	5283	2502	47	41	3346	454
Escherichia coli	ec	B	4289	2292	53	45	3097	453
Mycobacterium tuberculosis CDC1551	mu	B	4187	1911	46	41	2594	391
Bacillus subtilis	bs	B	4100	2027	49	44	2754	417
Bacillus halodurans	bh	B	4066	2000	49	43	2688	415
Mycobacterium tuberculosis	mb	B	3918	1959	50	41	2650	392
Vibrio cholerae	vc	B	3835	1852	48	42	2527	424
Caulobacter crescentus	cc	B	3737	1997	53	46	2663	404
Clostridium acetobutylicum	ca	B	3672	1819	50	41	2382	401
Cyanobacterium synechocystis	cs	B	3169	1589	50	42	2164	379
Deinococcus radiodurans	dr	B	3102	1561	50	42	2007	379
Sulfolobus solfataricus	ss	A	2977	1412	47	40	1790	323
Xylella fastidiosa	xf	B	2766	1097	40	41	1477	359
Aeropyrum pernix	ap	A	2694	836	31	33	1067	289
Staphylococcus aureus	sa	B	2594	1313	51	43	1728	368
Archaeoglobus fulgidus	af	A	2407	1238	51	45	1664	320
Lactococcus lactis	ll	B	2266	1170	52	43	1514	334
Streptococcus pneumoniae	sr	B	2094	1044	50	43	1351	330
Neisseria meningitidis A	nn	B	2065	958	46	42	1266	342
Pyrococcus horikoshii	ph	A	2064	904	44	40	1175	294
Halobacterium	hb	A	2058	1023	50	42	1351	306
Neisseria meningitidis	nm	B	2025	941	46	43	1264	342
Pasteurella multocida	pm	B	2014	1112	55	46	1467	359
Methanobacterium thermoautotrophicum	mt	A	1869	971	52	44	1297	307
Thermotoga maritima	tm	B	1846	1003	54	46	1335	343
Pyrococcus abyssi	pb	A	1765	957	54	45	1231	298
Methanococcus jannaschii	mj	A	1715	872	51	45	1132	288
Haemophilus influenzae	hi	B	1709	943	55	48	1243	341
Streptococcus pyogenes	sq	B	1696	887	52	44	1189	328
Campylobacter jejuni	cj	B	1634	845	52	43	1095	329
Mycobacterium leprae	ml	B	1605	844	53	48	1215	327
Helicobacter pylori	hp	B	1553	670	43	38	882	295
Aquifex aeolicus	aa	B	1522	902	59	49	1203	334
Thermoplasma volcanium	tv	A	1499	795	53	45	1034	284
Helicobacter pylori J99	hq	B	1491	681	46	38	896	287
Thermoplasma acidophilum	ta	A	1478	795	54	45	1051	286
Chlamydophila pneumoniae AR39	cq	B	1110	443	40	36	625	243
Chlamydophila pneumoniae J138	cp	B	1070	446	42	36	628	243

Table 1. *Continued*

Genome	A	B	C	D	E	F	G	H
Chlamydophila pneumoniae	cr	B	1052	443	42	36	625	242
Treponema pallidum	tp	B	1031	467	45	38	655	235
Chlamydia muridarum	cm	B	909	423	47	39	604	234
Chlamydia trachomatis	ct	B	894	419	47	40	597	235
Borrelia burgdorferi	bb	B	850	415	49	42	574	225
Rickettsia prowazekii	rp	B	834	437	52	44	605	248
Mycoplasma pulmonis	mq	B	782	363	46	34	485	186
Mycoplasma pneumoniae	mp	B	677	308	45	35	414	179
Ureaplasma urealyticum	uu	B	611	267	44	33	367	170
Buchnera sp.	bn	B	564	380	67	56	560	248
Mycoplasma genitalium	mg	B	480	261	54	41	362	172

For each genome the table shows in order: the name of the species of the genome; a two-character code (A); the domain, where ‘E’ is for eukaryota, ‘A’ is for archea and ‘B’ is for bacteria (B); the number of genes comprising the genome (C); the number of genes which have at least one SCOP domain assigned (D); the percentage of genes with at least one domain assigned (E); the percentage of the actual sequence covered by SCOP domains because multi-domain genes may have some domains assigned but not others (F); the total number of domains assigned (G); the total number (out of a possible 859) of superfamilies represented by at least one domain in the genome (H).

classification more similar to the family than the superfamily level. Structural assignments have been carried out using PSI-BLAST (7) based on the CATH (8) database but are much less extensive (http://www.biochem.ucl.ac.uk/bsm/cath_new/Gene3D).

DATABASE CONTENTS

The database may be accessed directly via a public server at http://stash.mrc-lmb.cam.ac.uk/SUPERFAMILY or via a link from each domain entry in SCOP at http://scop.mrc-lmb.cam.ac.uk/scop. There are also links from some genome databases, for example, Ensembl at http://www.ensembl.org. The underlying machinery of the database consists of a library of HMMs, a relational database and some programs. All of these are also available for download.

Structural assignments to sequences

The HMM library representing all proteins of known structure may be used to assign structural domains to sequences of unknown structure. An amino acid or nucleotide sequence may be queried against the library, and then the domain architecture and SCOP classification is returned (Fig. 1). The procedure has been optimised for remote homology detection retaining an estimated error rate of <1%. Three-dimensional models can be generated and these have been used to compare the method to other automatic structure prediction servers at LiveBench (http://bioinfo.pl). The server’s specificity is one of the best, especially for hard targets.

Nucleotide searches are carried out by translating sequences into the six reading frames and splitting across stop codons. Thus, the resulting structural assignments do not require any prior gene prediction and can be used to locate possible genes from raw DNA (Fig. 2). This does not provide gene prediction on its own, but is useful if no gene prediction is available and may suggest possible coding regions which gene prediction algorithms may not have identified.

Multiple sequence alignments

The models are used to generate multiple sequence alignments to sequences of known structure. A sequence with structural domains assigned (as above) can be aligned to a known sequence of the structural domain in question. On the public server there is a link to the alignment from the result page from a sequence query (Fig. 1). The server contains all PDB sequences and all complete genome sequences, which can be added to obtain a multiple alignment; users can also upload their own sequences for addition to the multiple alignment.

In the absence of a sequence query, the multiple alignments can be reached via links from SCOP or a keyword search on the server.

Genome assignments

The SUPERFAMILY procedure has been used to carry out structural assignments to all complete genomes (Tables 1 and 2). The assignments cover ~35% of eukaryote and 45% of prokaryote sequence, which is estimated as half of the soluble protein domains. This coverage is expected to increase as structural genomics projects solve more novel structures, giving a more complete structural picture of the genomes.

The SCOP classification of the structural domains in genomes provides a framework for comparing superfamilies within and across genomes. The public server provides statistics, and the breakdown of the genomes into superfamilies of different sizes. Within each superfamily of a given genome the individual genes may be displayed, with links to their domain architecture and sequence alignments.

A growing number of genome assignments are served via a distributed annotation system (DAS) server at http://supfam.org:8080/das allowing people to view the annotation from different sources in a single browser. To use this service a DAS client is required which can be obtained from http://www.biodas.org.

Table 2. The assignments for 11 miscellaneous sequence sets including, amongst other things, five alternative human gene sets and some incomplete genomes

Genome	A	B	C	D	E	F	G	H
Viridiplantae sequences from GenPept	sp	E	46 369	31 232	67	58	64 711	546
Softberry human gene predictions	hv	E	38 170	15 235	40	31	28 223	613
Ensembl 0.8 human gene predictions	hx	E	29 303	14 437	49	39	25 558	597
Ensembl 1.0 human gene predictions	hs	E	27 615	13 210	48	37	23 402	595
Affymetrix human gene predictions	hu	E	21 111	10 339	49	37	19 876	581
Mus musculus cDNAs	mm	E	21 076	6223	30	29	8047	496
Human known genes	ht	E	8243	4995	61	41	9769	531
Mus musculus incomplete genome	mn	E	6978	3463	50	39	5599	391
Oryza sativa incomplete genome	os	E	2425	759	31	28	987	177
Guillardia theta nucleomorph genome	gt	E	485	203	42	33	261	92
Rhizobium plasmid	pn	P	417	202	48	40	250	77

In Table 1 the current Ensembl (version 1.1) is used for *Homo sapiens*.

APPLICATIONS

The most straightforward application is a simple sequence search, of which the public server currently (pre-publication) receives over 1000 per month. Many larger sets of sequences have been run as special requests for specific studies; the database is used on several structural genomics projects' targets (e.g. SPiNE at http://spine.mbb.yale.edu/spine).

Although the assignments to nucleotide sequence do not provide complete gene predictions, they can be used as information contributing to a gene prediction. Current work is generating the data for the human genome for this purpose.

The genome assignments provide annotation of the genes, much of which is novel. This information is not just accessed by users of the database but is also used by several genome projects (including all completed large eukaryotes) to aid their annotation efforts, or verbatim as annotation in its own right.

The SUPERFAMILY data provides a framework which already forms the basis of several ongoing genomic studies (9,10). The data is also used by the HIGH database (http://genomesapiens.org) of immunoglobulin genes in human.

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REFERENCES

1. Murzin,A.G., Brenner,S.E., Hubbard,T. and Chothia,C. (1995) SCOP: a structural classification of proteins database for the investigation of sequences and structures. *J. Mol. Biol.*, **247**, 536–540.
2. Karplus,K., Barrett,C. and Hughey,R. (1998) Hidden Markov models for detecting remote protein homologies. *Bioinformatics*, **14**, 846–856.
3. Gough,J., Karplus,K., Hughey,R. and Chothia,C. (2001) Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. *J. Mol. Biol.*, **313**, 903–919.
4. Bateman,A., Birney,E., Durbin,R., Eddy,S.E., Lowe,K.L. and Sonnhammer,E.L. (2000) The Pfam protein families database. *Nucleic Acids Res.*, **28**, 263–266. Updated article in this issue: *Nucleic Acids Res.* (2002), **30**, 276–280.
5. Schultz,J., Copley,R.R., Doerks,T., Ponting,C.P. and Bork,P. (2000) SMART: a web-based tool for the study of genetically mobile domains. *Nucleic Acids Res.*, **28**, 231–234. Updated article in this issue: *Nucleic Acids Res.* (2002), **30**, 242–244.
6. Apweiler,R., Attwood,T.K., Bairoch,A., Bateman,A., Birney,E., Biswas,M., Bucher,P., Cerutti,L., Corpet,F., Croning,M.D.R. *et al.* (2000) InterPro—an integrated documentation resource for protein families, domains and functional sites. *Bioinformatics*, **16**, 1145–1150.
7. Altschul,S.F., Madden,T.L., Schaffer,A.A., Zhang,J., Zhang,Z., Miller,W. and Lipman,D.J. (1997) Gapped blast and psi-blast: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.
8. Orengo,C.A., Michie,A.D., Jones,S., Jones,D.T., Swindells,M.B. and Thornton,J.M. (1997) CATH—a hierarchic classification of protein domain structures. *Structure*, **5**, 1093–1108.
9. Apic,G., Gough,J. and Teichmann,S.A. (2001) Domain combinations in archael, eubacterial and eukaryotic proteins. *J. Mol. Biol.*, **310**, 311–325.
10. Teichmann,S.A., Rison,S.C.G., Thornton,J.M., Riley,M., Gough,J. and Chothia,C. (2001) The evolution and structural anatomy of the small molecule metabolic pathways in *Escherichia coli*. *J. Mol. Biol.*, **311**, 693–708.