

InterProScan: protein domains identifier

E. Quevillon, V. Silventoinen, S. Pillai, N. Harte, N. Mulder, R. Apweiler and R. Lopez*

European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK

Received February 11, 2005; Revised and Accepted March 30, 2005

ABSTRACT

InterProScan [E. M. Zdobnov and R. Apweiler (2001) *Bioinformatics*, 17, 847–848] is a tool that combines different protein signature recognition methods from the InterPro [N. J. Mulder, R. Apweiler, T. K. Attwood, A. Bairoch, A. Bateman, D. Binns, P. Bradley, P. Bork, P. Bucher, L. Cerutti *et al.* (2005) *Nucleic Acids Res.*, 33, D201–D205] consortium member databases into one resource. At the time of writing there are 10 distinct publicly available databases in the application. Protein as well as DNA sequences can be analysed. A web-based version is accessible for academic and commercial organizations from the EBI (<http://www.ebi.ac.uk/InterProScan/>). In addition, a standalone Perl version and a SOAP Web Service [J. Snell, D. Tidwell and P. Kulchenko (2001) *Programming Web Services with SOAP*, 1st edn. O'Reilly Publishers, Sebastopol, CA, <http://www.w3.org/TR/soap/>] are also available to the users. Various output formats are supported and include text tables, XML documents, as well as various graphs to help interpret the results.

INTRODUCTION

When carrying out analysis of protein sequences, the aim is to find out as much information as possible about potential relationships with other sequences as well as characterizing their physicochemical properties. The first step usually involves comparing the protein sequence against a non-redundant protein sequence database by using Blast (1) or Fasta (2), which will reveal which sequence(s) are similar to the query sequence alone. To obtain further information about a protein's specific function, searches against secondary databases (also known as pattern or signature databases) are necessary. When such searches return significant matches or hits, these results help in the assignment of a particular function or functional domain to the query protein. The InterPro (<http://www.ebi.ac.uk/interpro>) (3) database was created to unite secondary databases that contain overlapping information on protein

families, domains and functional sites (Table 1). InterPro entries are divided into groups based on the protein families or the domains that the signatures represent. If the structure and function of a protein family is well characterized, searches of the secondary databases offer a fast track into inferring biological function. Searching individually against each of these databases to get the most information is repetitive, time consuming and labour intensive. To search InterPro with a novel protein sequence, a tool, InterProScan (4), has been developed (<http://www.ebi.ac.uk/InterProScan>) that combines the protein function recognition methods of the member databases of InterPro into one application. Since its creation, there have been several releases to improve it, adding functionality as well as new databases to the system.

InterProScan TOOL

Here, we describe the use of the web browser based version of InterProScan available from the EBI at <http://www.ebi.ac.uk/InterProScan> (Figure 1). This service is free to all academic and commercial organizations and offers interactive as well as email job submission. Direct email submissions should be directed to interproscan@ebi.ac.uk. Instructions and documentation are available when sending an email to the above address that contains the word 'help' in the message body. Users requiring high-throughput use of the application or who wish to carry out analysis using other databases can download

Table 1. Database members and their applications

Database	Application
ProDom (6)	BlastProDom (Blastall) (4)
PRINTS (7)	FingerPrintScan (8)
SMART (9)	Hmmpfam (http://hmmer.wustl.edu/)
TIGRFAMs (10)	Hmmpfam (http://hmmer.wustl.edu/)
Pfam (11)	Hmmpfam (http://hmmer.wustl.edu/)
PROSITE (12)	ScanRegExp + ProfileScan (13)
PIRSF (14)	Hmmpfam (http://hmmer.wustl.edu/)
SUPERFAMILY (15)	Hmmpfam (http://hmmer.wustl.edu/)
CATH (16)	Hmmpfam (http://hmmer.wustl.edu/)
PANTHER (17)	Hmmsearch (http://hmmer.wustl.edu/)
SignalPHMM	SignalPHMM (18)
Transmembrane	TMHMM2.0 (19)

*To whom correspondence should be addressed at: EMBL Outstation – The European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK. Tel: +44 1223 494423; Fax: +44 1223 494468; Email: rls@ebi.ac.uk

EMBL-EBI
European Bioinformatics Institute

Get Nucleotide sequences for Site search

EBI Database Queries

EBI Home About EBI Research Services **Toolbox** Databases Downloads Submissions
PROTEIN FUNCTIONAL ANALYSIS

InterProScan Sequence Search

This form allows you to query your sequence against InterPro. For more detailed information see the documentation for the perl stand-alone InterProScan package ([Readme file](#) or [FAQs](#)), or the InterPro [user manual](#) or [help pages](#).

[Download Software](#)

YOUR EMAIL	RESULTS
<input type="text"/>	interactive ▾
APPLICATIONS TO RUN <input type="radio"/> Clear all <input type="radio"/> Check all	
<input checked="" type="checkbox"/> BlastProDom	<input checked="" type="checkbox"/> FPrintScan
<input checked="" type="checkbox"/> HMMPiR	<input checked="" type="checkbox"/> HMMPfam
<input checked="" type="checkbox"/> HMMSmart	<input checked="" type="checkbox"/> HMMTigr
<input checked="" type="checkbox"/> ProfileScan	<input checked="" type="checkbox"/> ScanRegExp
<input checked="" type="checkbox"/> SuperFamily	<input checked="" type="checkbox"/> SignalPHMM
<input checked="" type="checkbox"/> TMHMM	
TRANSLATION TABLE (DNA/RNA only)	MIN. OPEN READING FRAME SIZE
None ▾	100 ▾

Enter or Paste a **PROTEIN** ▾ Sequence in any format:

Upload a file:

Figure 1. EBI's InterProScan job submission page.

a standalone version from <ftp://ftp.ebi.ac.uk/pub/databases/interpro/iprscan>. Users requiring programmatic access to the InterProScan can do so using a SOAP-based Web Service called WSInterProScan (5), which is described at <http://www.ebi.ac.uk/Tools/webservices/WSInterProScan.html>. All of these make use of a centrally maintained core version of InterProScan version 4.0.

The job input form

The first section of the input form consists of the users email address and how the results are to be displayed. The first thing a user needs to decide when using the InterProScan submission form (Figure 1) is how he/she wants to see the results. This is carried out by making a selection on the RESULTS menu. Two options are available: 'interactive', which will return the results to the browser once the job is completed, and 'email', which will return the results to the email specified in the YOUR EMAIL text dialog.

The next section has a set of check boxes that either choose all or clears all the methods available. Each method can be

ticked on or off, according to the user's requirements. For example, users interested only in signal peptide cleavage sites or the transmembrane domains described in InterPro entries may choose the corresponding methods individually.

The third section of the submission form is specific for DNA as the sequence input. DNA sequences will be translated to protein according to the translation rules specified in the TRANSLATION TABLE menu. The default is the standard code. Each translation will generate peptide sequences in six frames and all will be searched. The minimum length of an open reading frame produced after translation can be specified in the MIN. OPEN READING FRAME SIZE menu. This dictates that only peptides above the selected value will be searched by the methods chosen in the second section.

The fourth section of the input form consists of the sequence input panel. The components of this panel include a selection menu for the molecule type. This one can be DNA or protein. The default is protein. When DNA is selected it enables the TRANSLATION TABLE menu in the third section of the form. Help is available by clicking on the HELP image. This will open a new browser window that contains comprehensive

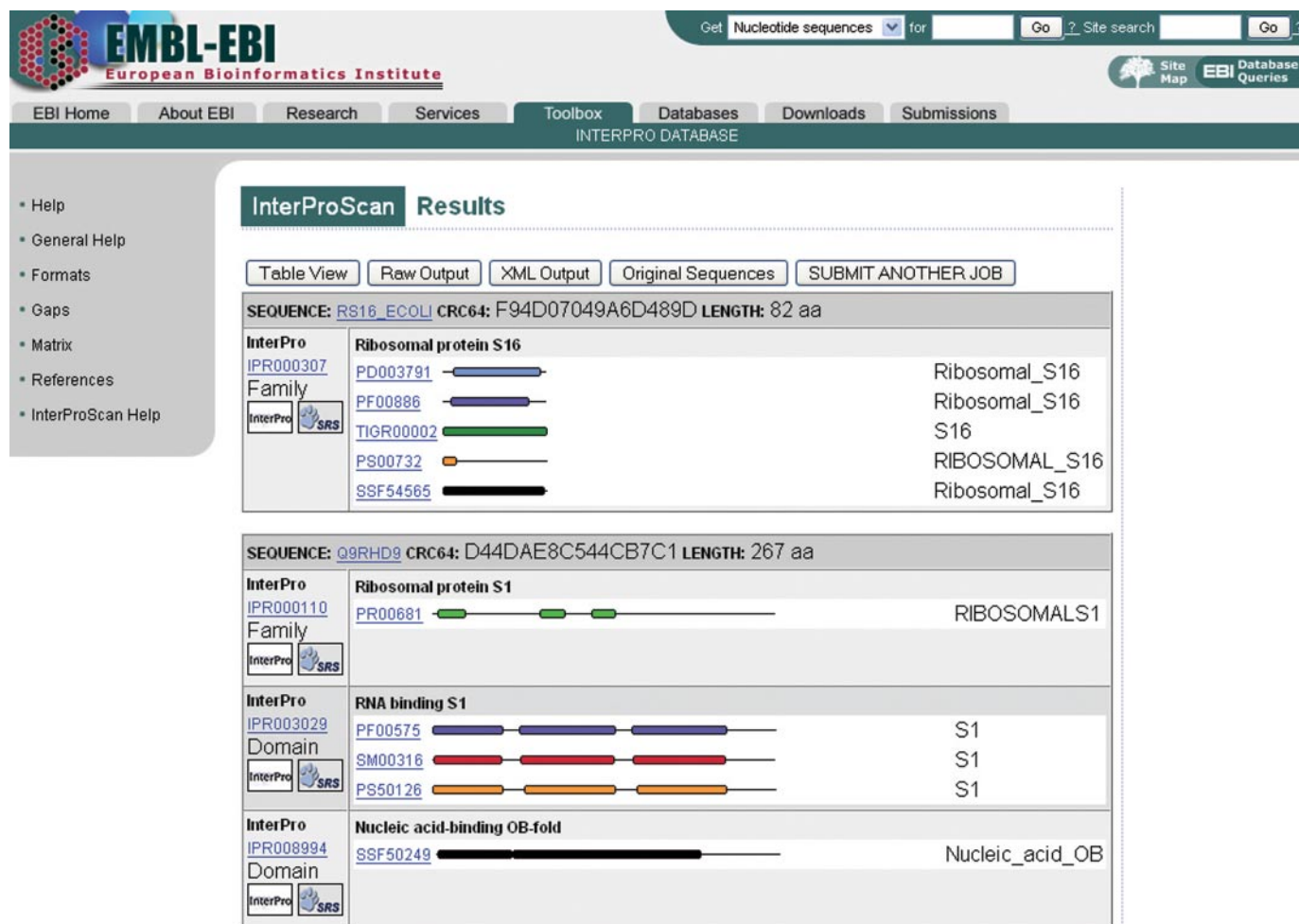


Figure 2. InterProScan graphical results view.

information about InterProScan. There is also an UPLOAD dialog that can be used instead of cutting and pasting sequences into the input window. Finally, there are the Submit and Reset buttons. The sequence input text dialog will accept protein or DNA sequence in any of the standard sequence formats in use today. These include EMBL, SWISS, GenBank, NBRF/PIR, CODATA, Fasta, GCG and RAW text. Primary or secondary identifiers (accession number or identifier) of a protein sequence in the databases can also be used. In this case, the user will type a database name followed by a colon and the identifier. For example, 'UNIPROT:INSR_HUMAN'. It is not possible to submit more than 10 protein input sequences at the same time. Each protein sequence must be at least five amino acids long. Only one nucleic acid sequence may be used at a time and the length for this sequence must be ≤ 5000 bases.

InterProScan output

Before InterProScan launches each of the protein sequence analysis applications, it takes advantage of pre-computed results whenever possible. It calculates a checksum (CRC64) for the query sequence and compares it with the checksums of the protein sequences that are present in a database called

IPRMATCHES. This is a database that lists all the entries from UniProt/Swiss-Prot and UniProt/TrEMBL that match one or more InterPro entries. If the checksum calculated for the query sequence does not match any checksums found in the IPRMATCHES database, the protein sequence analysis applications are launched in parallel; otherwise the IPRMATCHES entry is returned.

Once a job is completed, the output of each of the applications is individually parsed to produce a merged results file. This file is in the tab-delimited format. A converter is called onto generate, on the fly, an XML document, which is used to generate the HTML output. This consists of two views: a picture or a graphical view (Figure 2) that displays a cartoon of the sequence with highlighted domains or functional sites corresponding to the matches in the InterPro databases. Each match contains hypertext links to the InterPro database main web resource as well as to the individual member databases' websites where the matches are further described. A table view (Figure 3) is also available by clicking on the 'table view' button. This one consists of complete database names, hyperlinked match identifiers, the sequence coordinates (start-stop pairs) where the match occurs, *E*-values and the status of the match in InterPro (e.g. 'T' for true or '?' for unknown). Parent-child relationships are displayed if they

EMBL-EBI
European Bioinformatics Institute

Get Nucleotide sequences for [] Go ? Site search [] Go ?

Site Map EBI Database Queries

EBI Home About EBI Research Services Toolbox Databases Downloads Submissions

INTERPRO DATABASE

InterProScan Results

Picture View Raw Output XML Output Original Sequences SUBMIT ANOTHER JOB

SEQUENCE: [RS16_ECOLI](#) CRC64: F94D07049A6D489D LENGTH: 82 aa

InterPro IPR000307 Family SRS	Ribosomal protein S16		
	PRODOM	PD003791	<i>Ribosomal_S16</i> 4.0E-33 [10-77]T
	PFAM	PF00886	<i>Ribosomal_S16</i> 2.7000000000000004E-33 [8-68]T
	TIGRFAMs	TIGR00002	<i>S16</i> 117.16 [2-81]T
	PROFILE	PS00732	<i>RIBOSOMAL_S16</i> 8.0E-5 [2-11]T
	SUPERFAMILY	SSF54565	<i>Ribosomal_S16</i> 1.81E-8 [1-79]T
Parent	no parent		
Children	no children		
Found in	no entries		
Contains	no entries		
GO terms	Molecular Function: structural constituent of ribosome (GO:0003735) Cellular Component: intracellular (GO:0005622) Cellular Component: ribosome (GO:0005840) Biological Process: protein biosynthesis (GO:0006412)		

SEQUENCE: [Q9RHD9](#) CRC64: D44DAE8C544CB7C1 LENGTH: 267 aa

InterPro IPR000110 Family SRS	Ribosomal protein S1		
	PRINTS	PR00681	<i>RIBOSOMALS1</i> 1.5E-17 [6-27]T 1.5E-17 [85-104]T 1.5E-17 [125-143]T
	Parent	no parent	

Figure 3. InterProScan table results view.

exist in an InterPro entry. GO annotation is also shown if available. Other options in the HTML results page include the raw output in the tab-delimited format, the XML document and the sequences used as input (original sequences). The results for each job are stored at the EBI for at least 24 h.

THE STANDALONE VERSION OF InterProScan

For users who wish to run their own installation of InterProScan, there is a free standalone version available from the EBI's ftp server (<ftp://ftp.ebi.ac.uk/pub/databases/interpro/iprscan/RELEASE/latest>). This version can run from the command line or as a CGI through a web interface. Additional features that have been developed for this version include the use of the Perl indexing library, which indexes all data files, input sequences and the applications results for easy querying and retrieval of primary identifiers, names, job results and their status. From the results page, users can access each input sequence individually or the full input file, and also access the original outputs from each application.

The standalone version of InterProScan has been designed to run on either a single machine or a cluster of machines. It supports the use of various queuing systems such as LSF, OpenPBS and SGE.

The standalone version is composed of three different packages:

- (i) Perl core package containing all the scripts and modules to run InterProScan.
- (ii) Data package, which contains all the data needed by each application to run (~4 GB unzipped).
- (iii) Binary package precompiled for six different platforms (Linux, OSF1, AIX, Sun, IRIX and MacOSX).

CONCLUSIONS

We describe here the current state of the EBI InterProScan server, along with much of its unique flexibility, which is freely available to the public. Questions, comments and suggestions from users are encouraged and may be addressed to <http://www.ebi.ac.uk/support/>.

ACKNOWLEDGEMENTS

InterPro is funded by the award of grant number QLRI-CT-2000-00517 and in part by grant number QLRI-CT-2001000015 from the European Union under the RTD program 'Quality of Life and Management of Living Resources'. InterPro is a member database of the MRC-funded eFamily project. Funding to pay the Open Access publication charges for this article was provided by The European Molecular Biology Laboratory (EMBL).

Conflict of interest statement. None declared.

REFERENCES

- 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.
- Pearson,W.R. and Lipman,D.J. (1988) Improved tools for biological sequence analysis. *Proc. Natl Acad. Sci. USA*, **85**, 2444–2448.
- Mulder,N.J., Apweiler,R., Attwood,T.K., Bairoch,A., Bateman,A., Binns,D., Bradley,P., Bork,P., Bucher,P., Cerutti,L. *et al.* (2005) InterPro, progress and status in 2005. *Nucleic Acids Res.*, **33**, D201–D205.
- Zdobnov,E.M. and Apweiler,R. (2001) InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics*, **17**, 847–848.
- Snell,J., Tidwell,D. and Kulchenko,P. (2001) *Programming Web Services with SOAP, 1st edn.* O'Reilly & Associates, Sebastopol, CA.
- Bru,C., Courcelle,E., Carrère,S., Beausse,Y., Dalmar,S. and Kahn,D. (2005) The ProDom database of protein domain families: more emphasis on 3D. *Nucleic Acids Res.*, **33**, D212–D215.
- Attwood,T.K., Bradley,P., Flower,D.R., Gaulton,A., Maudling,N., Mitchell,A.L., Moulton,G., Nordle,A., Paine,K., Taylor,P. *et al.* (2003) PRINTS and its automatic supplement, prePRINTS. *Nucleic Acids Res.*, **31**, 400–402.
- Scordis,P., Flower,D.R. and Attwood,T.K. (1999) FingerPRINTScan: intelligent searching of the PRINTS motif database. *Bioinformatics*, **15**, 799–806.
- Letunic,I., Goodstadt,L., Dickens,N.J., Doerks,T., Schultz,J., Mott,R., Ciccarelli,F., Copley,R.R., Ponting,C.P. and Bork,P. (2002) Recent improvements to the SMART domain-based sequence annotation resource. *Nucleic Acids Res.*, **30**, 242–244.
- Haft,D.H., Selengut,J.D. and White,O. (2003) The TIGRFAMs database of protein families. *Nucleic Acids Res.*, **31**, 371–373.
- Bateman,A., Coin,L., Durbin,R., Finn,R.D., Hollich,V., Griffiths-Jones,S., Khanna,A., Marshall,M., Moxon,S., Sonnhammer,E.L. *et al.* (2004) The Pfam protein families database. *Nucleic Acids Res.*, **32**, D138–D141.
- Hulo,N., Sigrist,C.J., Le Saux,V., Langendijk-Genevaux,P.S., Bordoli,L., Gattiker,A., De Castro,E., Bucher,P. and Bairoch,A. (2004) Recent improvements to the PROSITE database. *Nucleic Acids Res.*, **32**, D134–D137.
- Thompson,J.D., Higgins,D.G. and Gibson,T.J. (1994) Improved sensitivity of profile searches through the use of sequence weights and gap excision. *Comput. Appl. Biosci.*, **10**, 19–29.
- Wu,C.H., Nikolskaya,A., Huang,H., Yeh,L.S., Natale,D.A., Vinayaka,C.R., Hu,Z.Z., Mazumder,R., Kumar,S., Kourtesis,P. *et al.* (2004) PIRSF: family classification system at the Protein Information Resource. *Nucleic Acids Res.*, **32**, D112–D114.
- 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.
- Pearl,F.M., Lee,D., Bray,J.E., Sillitoe,I., Todd,A.E., Harrison,A.P., Thornton,J.M. and Orengo,C.A. (2000) Assigning genomic sequences to CATH. *Nucleic Acids Res.*, **28**, 277–282.
- Mi,H., Lazareva-Ulitsky,B., Loo,R., Kejariwal,A., Vandergriff,J., Rabkin,S., Guo,N., Muruganujan,A., Doremioux,O., Campbell,M.J. *et al.* (2005) The PANTHER database of protein families, subfamilies, functions and pathways. *Nucleic Acids Res.*, **33**, D284–D288.
- Bendtsen,J.D., Nielsen,H., von Heijne,G. and Brunak,S. (2004) Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.*, **340**, 783–795.
- Sonnhammer,E.L., von Heijne,G. and Krogh,A. (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc. Int. Conf. Intell. Syst. Mol. Biol.*, **6**, 175–182.