

# The Blocks database—a system for protein classification

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## ABSTRACT

**The Blocks Database contains multiple alignments of conserved regions in protein families. The database can be searched by e-mail and World Wide Web (WWW) servers (<http://blocks.fhcr.org/help>) to classify protein and nucleotide sequences.**

## INTRODUCTION

Many known proteins can be grouped into families according to functional and sequence similarities. The similarity of the proteins across the sequences in each family is far from uniform. While some regions are clearly conserved, others display little sequence similarity. Often the conserved regions are crucial to the protein's function, for example enzymatic catalytic sites. Such conserved regions can be used to probe an uncharacterized sequence to indicate its function (1).

The description of a protein family by its conserved regions focuses on the family's characteristic and distinctive sequence features, thus reducing noise. Databases of conserved features of protein families can be utilized to classify sequences from proteins, cDNAs and genomic DNA (2–5). An example is the Blocks Database (3), which consists of ungapped multiple alignments of short regions, called 'blocks' (6). The database was constructed from sequences of protein families using a fully automated method. Searching the Blocks database with a sequence query allows detection of one or more blocks representing a family.

## Block determination

A best set of blocks representing each protein group is found automatically by the two-step PROTOMAT system (3). The first step incorporates a motif finder. Currently we use the MOTIF algorithm (7): MOTIF exhaustively evaluates spaced triplets of amino acids that are common to multiple sequences. We have also implemented a Gibbs sampling motif finder that iteratively optimizes random 'seeds' for blocks (8). The MOTIF and Gibbs algorithms generate similar block sets for the sequences used in the Blocks Database (9). The second step of the PROTOMAT system combines and refines the original blocks and assembles a best set of blocks that is consistently found in most of the sequences in the group. An example of a best set of blocks for the iron-containing alcohol dehydrogenase family is presented in Figure 1. The

two-step procedure is repeated for each protein group and the results are concatenated to make a database of blocks.

## Current database version

Version 8.0 of the Blocks Database consists of 2884 blocks based on 770 protein families documented in PROSITE 12.0 (5), which is keyed to Swiss-Prot 29 (10). PROSITE also supplies the documentation for each family. The distributions of number of blocks and number of sequences per family are shown in Figure 2 for BLOCKS 8.0.

## Searching the Blocks database

The BLIMPS (Blocks IMPROVED Searcher) program searches the Blocks Database (9). BLIMPS transforms each block into a position specific scoring matrix (PSSM), sometimes called a profile (11). Each PSSM column corresponds to a block position and contains values based on the amino acid frequencies in each position.

To prevent domination of the PSSM by a large subgroup of related sequences, each sequence segment in a block is weighted using position-based sequence weights (12). To reduce the effect of small sequence samples, the amino acid frequencies in each PSSM position (observed counts) are supplemented with artificial 'pseudo-counts'. Currently we model pseudo-counts on amino acid substitution probabilities (13; SH and JGH, unpublished results).

BLIMPS compares a query sequence with a block by sliding the PSSM over the sequence (nucleotide sequences are translated in all the frames into six amino acid sequences). For every alignment, each sequence position receives the value of its amino acid in the aligned PSSM column. These scores are summed to obtain the score of the sequence segment. This is repeated with all blocks in the database, and the top scores are saved. In addition to searching a sequence against a database of blocks, BLIMPS can search a block against a database of sequences.

## Block calibration

In order to recognize scores representing genuine relationships, it is necessary to know what scores are expected by chance alone. To accomplish this, each block is calibrated by searching it against the Swiss-Prot sequence database. Two scores specific to the block are noted—the score at the 99.5% level of the true negative scores and the median of the true positive scores (14). True positive scores are scores of blocks optimally aligned with

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ID   ADH_IRON_1; BLOCK
AC   BL0C913A; distance from previous block=(64,516)
DE   Iron-containing alcohol dehydrogenases proteins.
BL   GDK motif; width=38; seqs=11; 99.5%=703; strength=1474
ADH1_CLOAB ( 65) PDPSVETVFKGAEILMRQFEFDWIIAMGGGSPIDAAKAM 50

ADH2_ZYMMO ( 69) FNPTVAVLEGCRIKLDNNSDFVVISLGGSSPHDCAKAI 46
ADH4_YEAST ( 71) FNFMIANVTAGLKVLEENSEIVVSIIGGSAHDNAKAI 58
ADHE_CLOAB ( 516) READLETKKKAPEENSSFPDPTIIALGGTPEKSSAKLM 100
ADHE_ECOLI ( 517) ADPTLSIVRKGAEIANSFKPDVILALGGSSPMDAAKTM 60
FUCO_ECOLI ( 70) FNPTITVWKEGCGVFPQNSGADYLIAGGGSPQDTCKAI 59
GLDA_BACST ( 68) GEASHNEVSRFANTARKABAAIIVIGVGGKTLDTAKAV 73
GLDA_ECOLI ( 79) GECSONEIDRLRGIARETAQCGAILGIGGKTLDTAKAI 82
MLHE_BACMT ( 67) PTPADTQVHEGVVDFKQENC DALVSIIGGSSHDFAKAI 58
ADHA_CLOAB ( 70) FNPRTITVVKGIIEICRENNVDLVLALGGSEALDUSKVI 49
ADHE_CLOAB ( 70) FNPRTVTVKGVKICRENGVEVVLAIIGGSAIDCAKVI 44
//
ID   ADH_IRON_1; BLOCK
AC   BL0C913B; distance from previous block=(43,72)
DE   Iron-containing alcohol dehydrogenases proteins.
BL   FPE motif; width=34; seqs=11; 99.5%=679; strength=1484
ADH1_CLOAB ( 168) PDVAVVDSLEAETMPPRLTAHTGMDALTAIEAY 66

ADH2_ZYMMO ( 169) PMVEVNDPLLNVCMFKGLTAATGMDALTAHAPRAY 56
ADH4_YEAST ( 171) PAVAVNDPSTNFGLPALTAATGLDALTHCIEAY 67
ADHE_CLOAB ( 627) FNMATVDAELNMKMPKGLTAYSGIDALVNSIEAY 85
ADHE_ECOLI ( 628) FDMAIVDANLVDMDFKSLCAFGLDAVTHAPRAY 82
FUCO_ECOLI ( 172) PQVAFIDADMDCGMPKALKAATGVDALTAIEAY 83
GLDA_BACST ( 149) PDLVLDVTKIIANAPRLLASGIADALACTWVEAR 100
GLDA_ECOLI ( 160) FNMVIVDTKIVAGAPARLLAAGIGDALATWFEAR 80
MEDH_BACMT ( 167) PTVRIVDPELNVKPKAGLTIAATGMDALSHAIAY 69
ADHA_CLOAB ( 170) PKFSVLDPTTYFTFVFKICTAAGTADIMSHITPEAY 85
ADHE_CLOAB ( 170) PKFSVLDPTTYFTFVFKICTAAGTADIMSHIPEAY 92
//
ID   ADH_IRON_1; BLOCK
AC   BL0C913C; distance from previous block=(56,76)
DE   Iron-containing alcohol dehydrogenases proteins.
BL   HHG motif; width=22; seqs=11; 99.5%=492; strength=1438
ADHE_CLOAB ( 220) CHSMAIKLSSBHINPISGIANAL 66

FUCO_ECOLI ( 262) VEGMAHFLGAFYNTPHGVANAL 44
GLDA_BACST ( 259) HNGFTALEGEIHHLTHEGEKVAF 100
GLDA_ECOLI ( 269) VHNGLTAIPDAHYYHGEKVAF 100
MREH_BACMT ( 259) VHSISHQVGGVYKLGHGICNSV 78

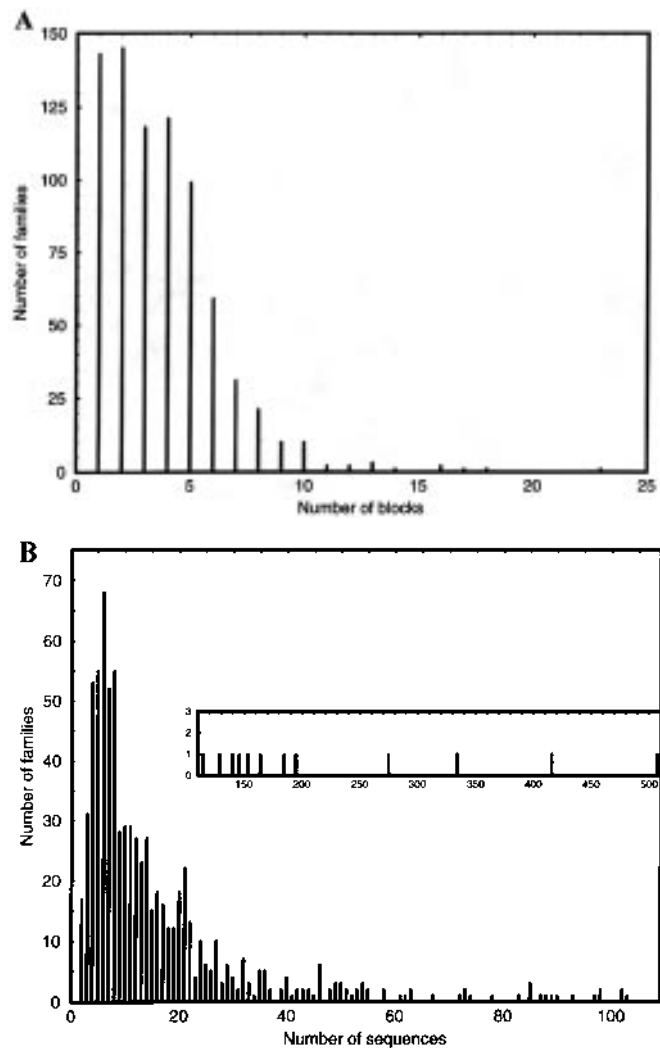
ADH1_CLOAB ( 258) CHSMAHKTFGAVFHIPHGIANAL 47
ADHE_ECOLI ( 721) CHSMAHKLGSQFHIPHGLANAL 47

ADH2_ZYMMO ( 261) VHAMAHQLGCVYVNLPHGVGNVAV 36
ADH4_YEAST ( 263) VHALAHLQLGCGPYHLPHGVGNVAV 41

ADHA_CLOAB ( 266) CHPMEHELSAYYDITHGVLAI 50
ADHE_CLOAB ( 266) VHLMEHELSAYYDITHGVLAI 49
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**Figure 1.** Blocks database format. Each block entry is divided into header and sequences parts. The header part consists of four lines. The ID (identification) line contains the block's family short description and identifies the entry as a block type. The AC (accession) line gives the block's accession code and the minimal and maximal distances of the block from the previous block or the protein N' end. The block accession code is made up of the letters 'BL' followed by the family PROSITE accession number and the individual block's letter code (A for first block, B for second etc.; blocks from single block families have no letter suffix). The DE (description) line contains the long description of the family. The short and long descriptions are taken from PROSITE. The BL (block) line gives the spaced triplet motif of the block, the block's width, number of sequences, 99.5%-level raw score and strength score (median standardized score of known true positive sequences). Each sequence line contains the sequence Swiss-Prot name, the start position of the segment, the sequence segment and the sequence weight (100 being most distant). Segments that are <80% similar are separated by blank lines. Each block entry ends with a '/' line. The block entries are sorted by their accession codes, each family's blocks grouped together and ordered. The figure shows the three blocks of the iron-containing alcohol dehydrogenase family from BLOCKS 8.0.



**Figure 2.** Statistics of Blocks Database version 8.0. (A) Number of blocks per family. (B) Number of sequences per family.

their known family members and all other scores are assumed to be true negatives.

Blocks vary in width and conservation and hence their search scores are variable too. In order to compare scores from different blocks the scores need to be normalized. The 99.5% scores are used to standardize the raw search scores. Each raw score is divided by the 99.5% score of the blocks and multiplied by 1000. Therefore, any standardized score above 1000 is a result better than all but the top 0.5% of the true negatives.

The median of standardized scores for true positive alignments is termed 'strength'. Strong blocks are more effective than weak blocks (standardized scores <1100) at separating true positives from true negatives.

### Interpreting a search result

The Blocks database can be searched with a sequence query using the BLIMPS program on our e-mail and WWW servers. As an example, BLOCKS 8.0 was searched with a bacterial dichlorocatechol oxidase (Swiss-Prot TDFD\_ALCEU) as a



## OTHER USES OF THE BLOCKS DATABASE

The automated construction and extensive data in the Blocks database make it suitable for uses other than protein classification. The local alignments of sequence segments provided data for the BLOSUM series of amino acid substitution matrices (18). These matrices performed very well in sequence database searches (19,20). The Blocks database was also used to test and compare different methods for weighting sequences to reduce redundancy (12).

Many blocks are made up of sequence segments with known functions such as ligand binding regions, catalytic domains and transmembranal domains (SP unpublished observations). This can be a resource for research on specific domains. For example, in studying protein nucleotide binding sites one can search for block families annotated as having such sites or for blocks containing the known signature of the sites. The blocks found can help refine the signature and even reveal unannotated sites.

## OTHER SEARCHABLE DATABASES OF PROTEIN FAMILIES

PROSITE is a compilation of specific sites, patterns and profiles found in protein sequences (5). PRINTS (4), ProDom (21) and SBASE (22) are databases of protein motifs and domains. PRINTS and SBASE have cross references to the Blocks database. All these databases find conserved regions by different methods and may include different groups of proteins. Therefore, different databases can provide complementary information.

## ACCESS

### Anonymous FTP

<u>Location</u>	<u>Address</u>	<u>Directory</u>
USA	ncbi.nlm.nih.gov	/repository/blocks
UK	ftp.ebi.ac.uk	/pub/databases/blocks
Israel	bioinformatics.weizmann.ac.il	/pub/databases/blocks
Japan	ftp.nig.ac.jp	/pub/db/blocks

The Blocks database is distributed as a flat text file containing the individual block entries.

The NCBI site also includes the software that we developed to construct and utilize the Blocks Database, including the BLIMPS search program.

The BlockSearch program developed by R. Fuchs for fast block searches (23) can be found at the UK site in directories pub/software/unix and pub/software/vax.

### E-mail servers

blocks@howard.fhcrc.org (for searching the Blocks Database)  
blockmaker@howard.fhcrc.org (for making blocks from user supplied protein sequences)

Send the word 'help' in the subject line or as the only word in the message body to obtain help files from both servers.

Queries or more information about the BLOCKS database can be obtained by sending an email to: henikoff@howard.fhcrc.org.

### WWW

<http://blocks.fhcrc.org>

This site offers Blocks database searches, block retrievals, block logos, block construction, help files and related bibliography.

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