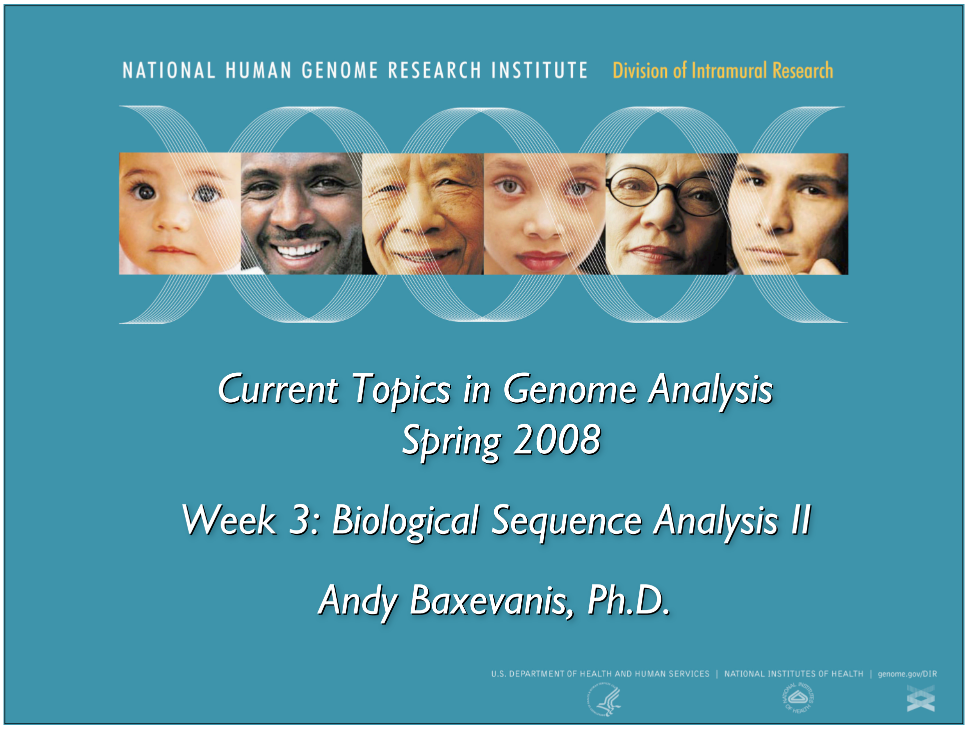


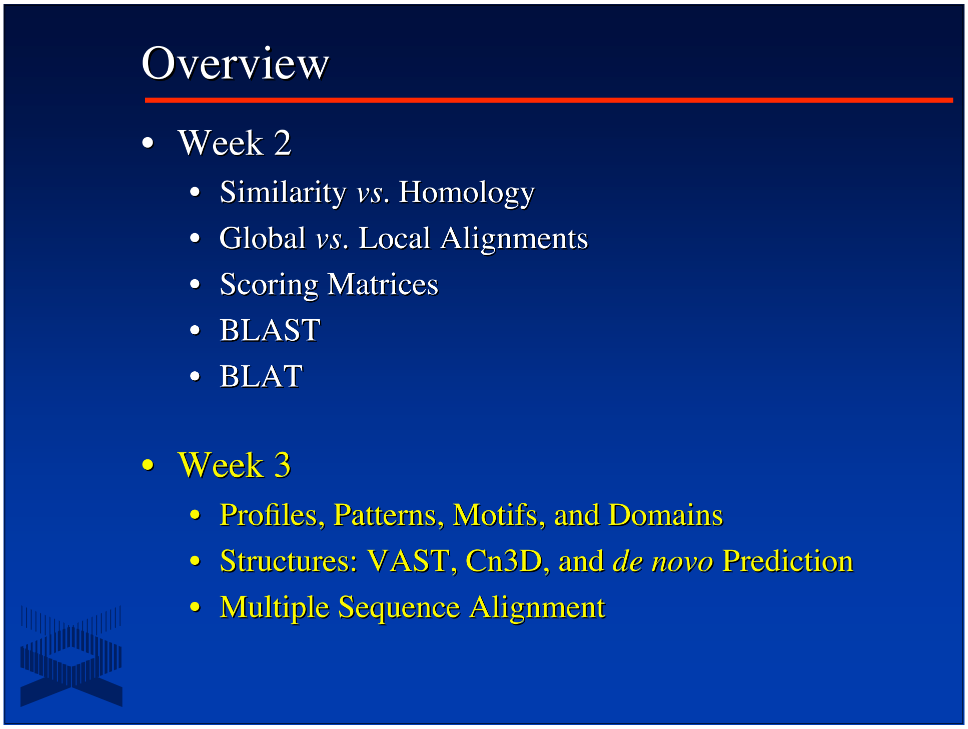
NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

Current Topics in Genome Analysis
Spring 2008

Week 3: Biological Sequence Analysis II

Andy Baxevanis, Ph.D.

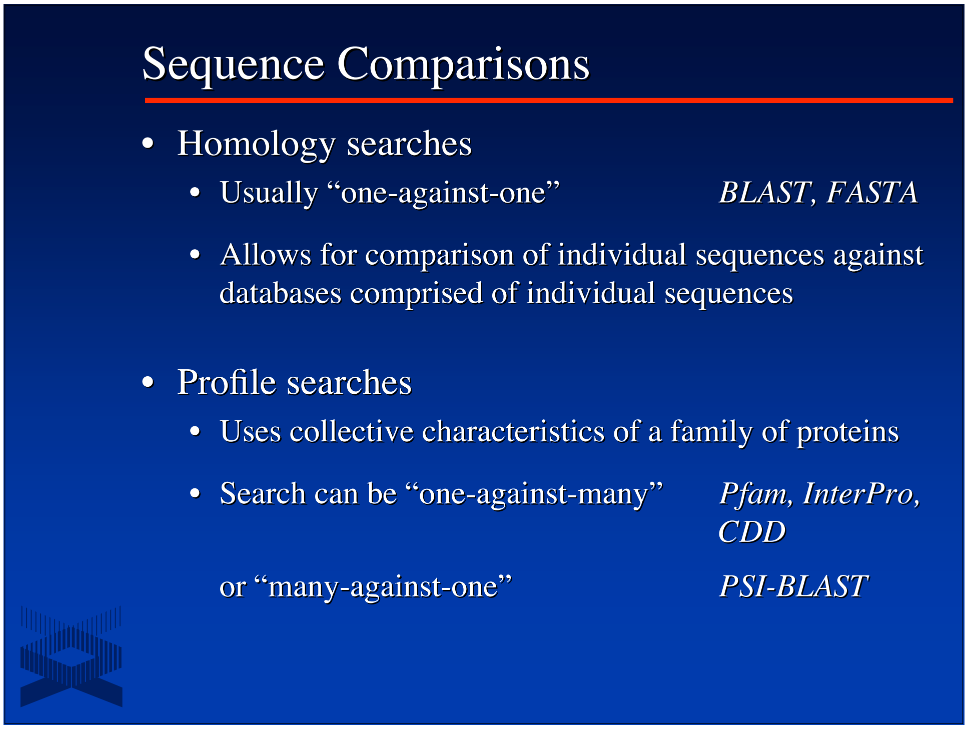
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR



Overview

- Week 2
  - Similarity *vs*. Homology
  - Global *vs*. Local Alignments
  - Scoring Matrices
  - BLAST
  - BLAT

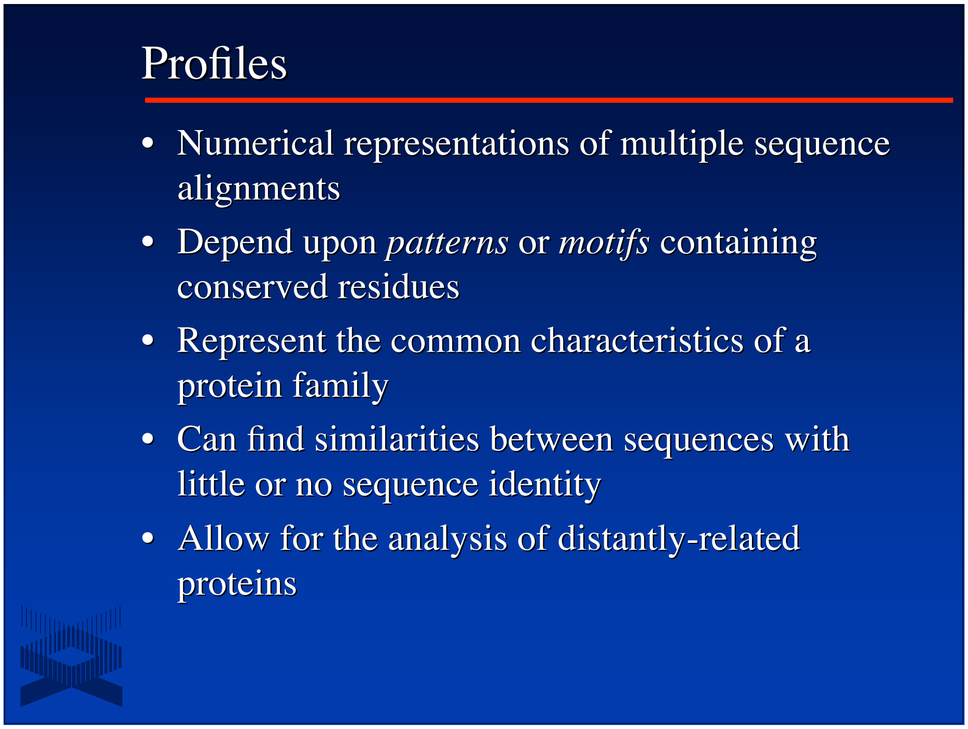
- Week 3
  - Profiles, Patterns, Motifs, and Domains
  - Structures: VAST, Cn3D, and *de novo* Prediction
  - Multiple Sequence Alignment



Sequence Comparisons

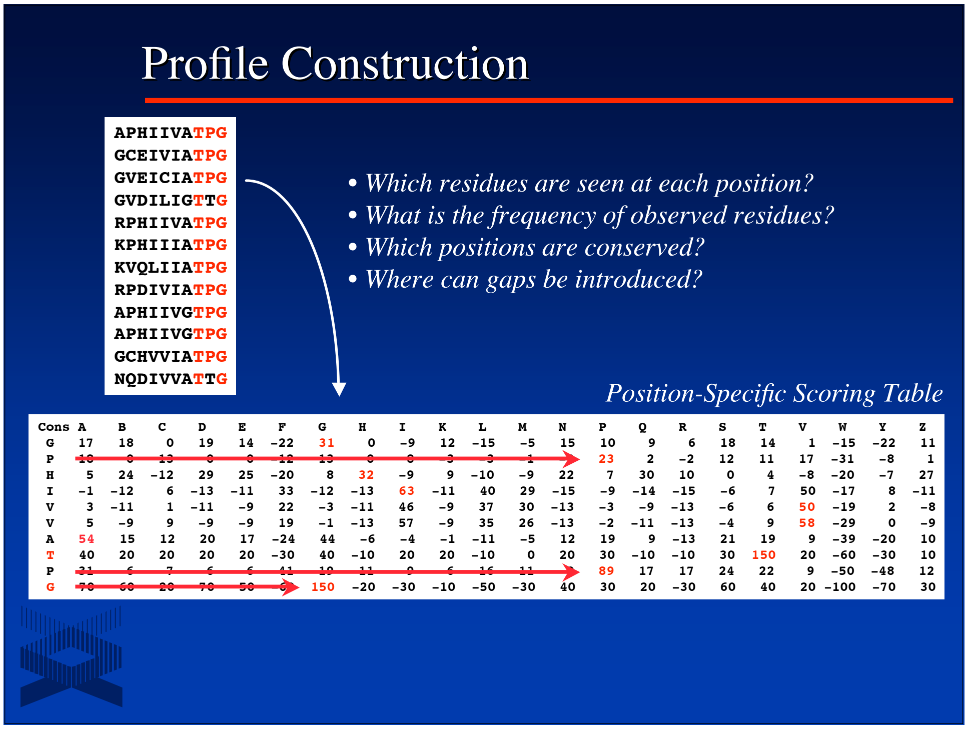
- Homology searches
  - Usually "one-against-one" *BLAST, FASTA*
  - Allows for comparison of individual sequences against databases comprised of individual sequences
- Profile searches
  - Uses collective characteristics of a family of proteins
  - Search can be "one-against-many" *Pfam, InterPro, CDD*

    or "many-against-one" *PSI-BLAST*



Profiles

- Numerical representations of multiple sequence alignments
- Depend upon *patterns* or *motifs* containing conserved residues
- Represent the common characteristics of a protein family
- Can find similarities between sequences with little or no sequence identity
- Allow for the analysis of distantly-related proteins



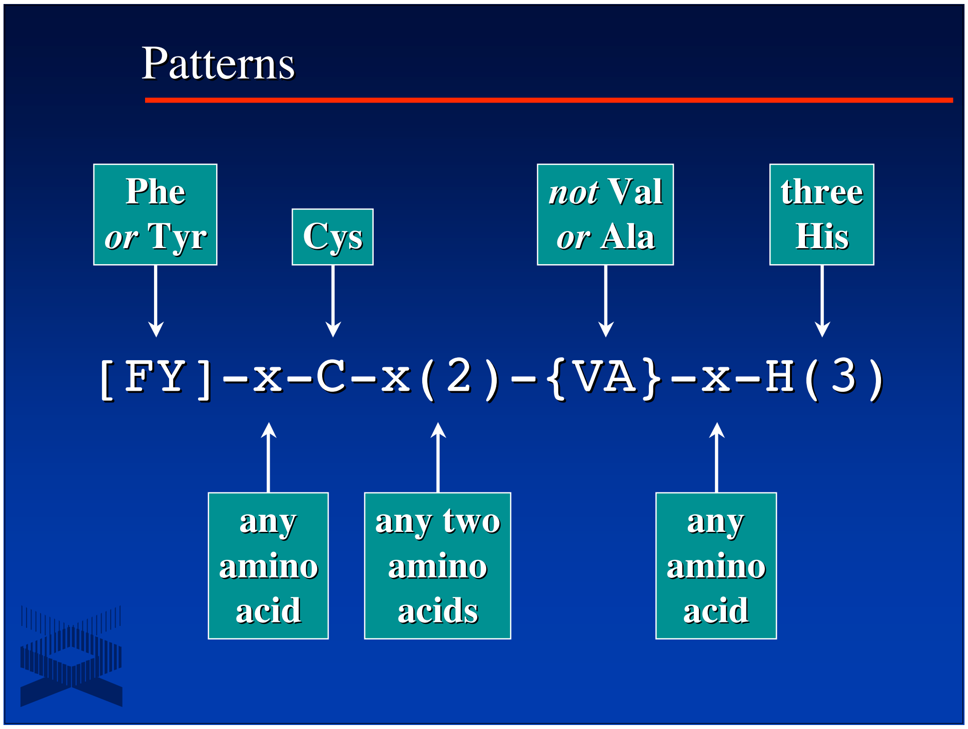
Profile Construction

```
APHIIVATPG
GCEIVIATPG
GVEICIATPG
GVDILIGTTG
RPHIIVATPG
KPHIIIATPG
KVQLIIATPG
RPDIVIATPG
APHIIVGTPG
APHIIVGTPG
GCHVVIATPG
NQDIVVATTG
```

- *Which residues are seen at each position?*
- *What is the frequency of observed residues?*
- *Which positions are conserved?*
- *Where can gaps be introduced?*

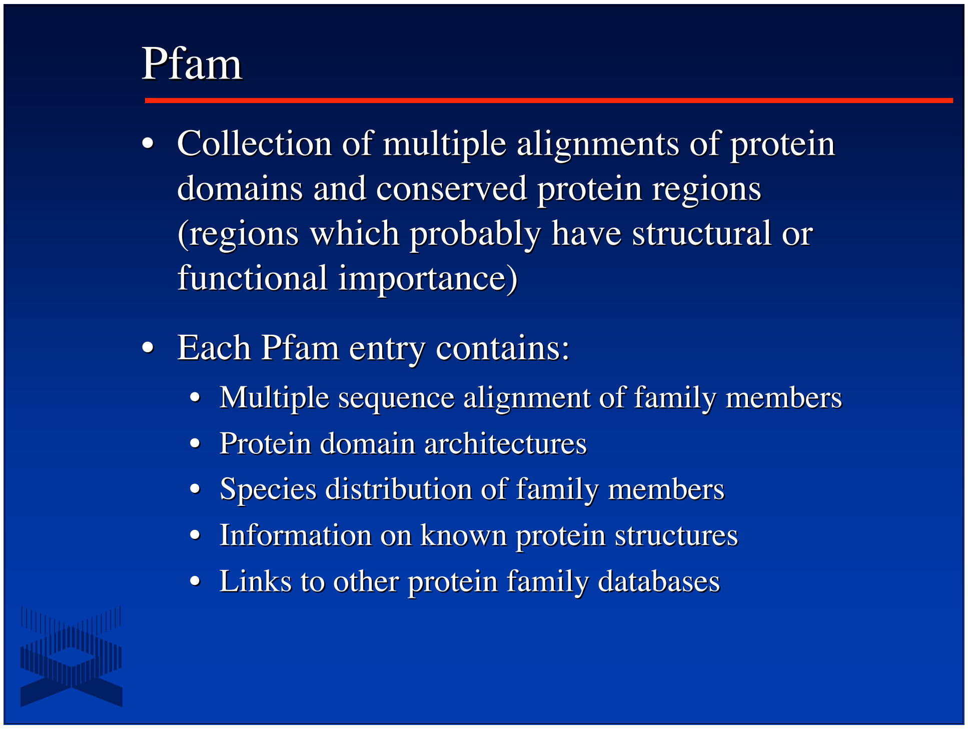
Position-Specific Scoring Table

Cons	A	B	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Z
G	17	18	0	19	14	-22	31	0	-9	12	-15	-5	15	10	9	6	18	14	1	-15	-22	11
P	~~18~~	~~0~~	~~13~~	~~0~~	~~0~~	~~-12~~	~~13~~	~~0~~	~~8~~	~~-3~~	~~-3~~	~~-1~~	[illegible]	23	2	-2	12	11	17	-31	-8	1
H	5	24	-12	29	25	-20	8	32	-9	9	-10	-9	22	7	30	10	0	4	-8	-20	-7	27
I	-1	-12	6	-13	-11	33	-12	-13	63	-11	40	29	-15	-9	-14	-15	-6	7	50	-17	8	-11
V	3	-11	1	-11	-9	22	-3	-11	46	-9	37	30	-13	-3	-9	-13	-6	6	50	-19	2	-8
V	5	-9	9	-9	-9	19	-1	-13	57	-9	35	26	-13	-2	-11	-13	-4	9	58	-29	0	-9
A	54	15	12	20	17	-24	44	-6	-4	-1	-11	-5	12	19	9	-13	21	19	9	-39	-20	10
T	40	20	20	20	20	-30	40	-10	20	20	-10	0	20	30	-10	-10	30	150	20	-60	-30	10
P	~~31~~	~~6~~	~~7~~	~~6~~	~~6~~	~~41~~	~~19~~	~~11~~	~~9~~	~~6~~	~~16~~	~~11~~	[illegible]	89	17	17	24	22	9	-50	-48	12
G	~~70~~	~~60~~	~~20~~	~~70~~	~~50~~	[illegible]	150	-20	-30	-10	-50	-30	40	30	20	-30	60	40	20	-100	-70	30



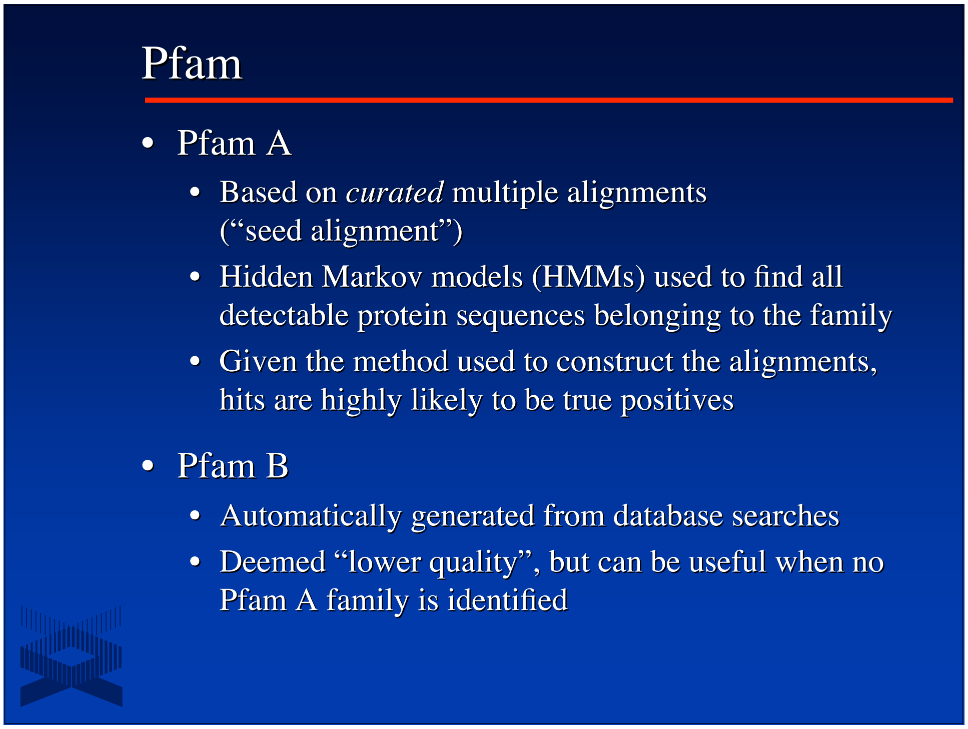
Patterns

[FY]-x-C-x(2)-{VA}-x-H(3)



Pfam

- Collection of multiple alignments of protein domains and conserved protein regions (regions which probably have structural or functional importance)
- Each Pfam entry contains:
    - Multiple sequence alignment of family members
    - Protein domain architectures
    - Species distribution of family members
    - Information on known protein structures
    - Links to other protein family databases

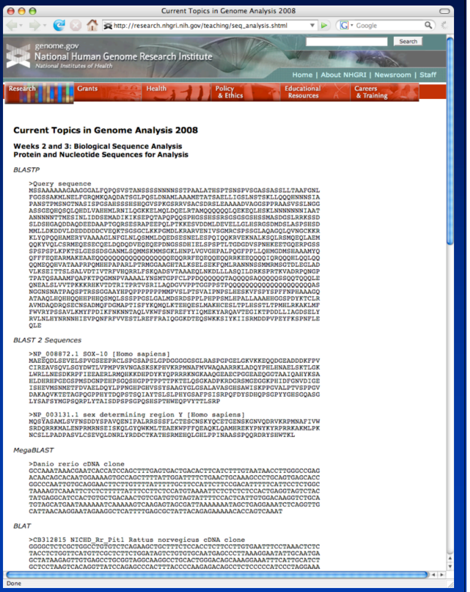


Pfam

- Pfam A
    - Based on *curated* multiple alignments (“seed alignment”)
    - Hidden Markov models (HMMs) used to find all detectable protein sequences belonging to the family
    - Given the method used to construct the alignments, hits are highly likely to be true positives
- Pfam B
    - Automatically generated from database searches
    - Deemed “lower quality”, but can be useful when no Pfam A family is identified

Sequences Used in Examples

http://research.nhgri.nih.gov/teaching/seq_analysis.shtml


Current Topics in Genome Analysis 2008
http://research.nhgri.nih.gov/teaching/seq_analysis.shtml
Google
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Health
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Educational Resources
Careers & Training
Done

Current Topics in Genome Analysis 2008

Weeks 2 and 3: Biological Sequence Analysis
Protein and Nucleotide Sequences for Analysis

BLASTP

```
>Query sequence
MSSAAAAAAGAAGGGALFQPQSVSTANSSSSNNNNSSTPAALATHSPTSNSPVSGASSASSLLTAAFGNL
FGGSSAKMLNELFGRQMKQAQDATSGLPQSLDNAMLAAAMETATSAELLIGSLNSTSKLLQQQHNNNSIA
PANSTPMSNGTNASISPGSAHSSSHSHQGVSPKGSRRVSACSDRSLEAAAADVAGGSPPRAASVSSLNGG
ASSGEQHQSQLQHDLVAHHMLRNILQGKKELMQLDQELRTAMQQQQQQLQEKEQLHSKLNNNNNNNIAAT
ANNNNNTTMESINLIDDSEMADIKIKSEPQTAPQPQQSPHGSSHSSRSGSGSGSHSSMASDGSLRRKSSD
SLDSHGAQDDAQDEEDAAPTGQRSESRAPEEPQLPTKKESVDDMLDEVELLGLHSRGSDMDSLASPSHSD
MMLLDKDDVLDEDDDDDCVEQKTSGSGCLKKPGMDLKRARVENIVSGMRCSPSSGLAQAGQLQVNGCKKR
KLYQPQQHAMERYVAAAAGLNFGLNLQSMMLDQEDSESNELESPQIQQKRVEKNALKSQLRSMQEQLAEM
QQKYVQLCSRMEQESECQELDQDQDVEQEQEPDNGSSDHIELSPSPTLTGDGDVSPNHKEETGQERPGSS
SPSPSPLKPKTSLGESSDSGANMLSQMMSKMMSGKLHNPLVGVGHPALPQGFPPLLQHMGDMSHAAAMYQ
QFFFEQEARMAKEAAEQQQQQQQQQQQQQQQQQQEQQRRFEQEQQEQQRRKEEQQQQIQRQQQHLQQLQQ
QQMEQQHVATAAPRPQMHHPAPARLPTRMGGAAGHTALKSELSEKFQMLRANNNSSMMRMSGTDLEGLAD
VLKSEITTSLSALVDTIVTRFVHQRRLFSKQADSVTAAAEQLNKDLLLASQILDRKSPRTKVADRPQNGP
TPATQSAAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDQQQQQTAQQQQSAQQQQSSQQTQQQLE
QNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDGVVPPTGGPPSTPQQQQQQQQQQQQQQQQQQQQAS
NGGNSNATPAQSPTRSSGGAAYHPQPPPPPPPMMPVSLPTSVAIPNPSLHESKVFSPYSPFFNPHAAAGQ
ATAAQLHQHHQQHHPHHQSMQLSSSPPGSLGALMDSRDSPPLPHPPSMLHPALLAAAHHGGSPDYKTCLR
AVMDAQDRQSECNSADMQFDGMAPTISFYKQMQLKTEHQESLMAKHCESLTPLHSSTLTPMHLRKAKLMF
FWVRYPSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAGDSELY
RVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIYKIISRMDDPVPEYFKSPNFLE
QLE
```

BLAST 2 Sequences

```
>NP_008872.1 SOX-10 [Homo sapiens]
MAEEQDLSEVELSPVGSEEPRCLSPGSAPSLGPDGGGGGSGLRASPGPGELGKVKKEQQDGEADDDKFPV
CIREAVSQVLSGYDWTLVPMPVRVNGASKSKPHVKRPMNAFMVWAQAARRKLADQYPHLHNAELSKTLGK
LWRLLNESDKRPFIEEAERLRMQHKKDHPDYKYQPRRRKNGKAAQGEAECPGGEAEQGGTAAIQAHYKSA
HLDHRHPGEGSPMSDGNPEHPSGQSHGPPTPPTTPKTELQSGKADPKRDGRSMGEGGKPHIDFGNVDIGE
ISHEVMSNMETFDVAELDQYLPPNGHPGHVSSYSAAGYGLGSALAVASGHSAWISKPPGVALPTVSPPGV
DAKAQVKTETAGPQGPPHYTDQPSTSQIAYTSLSLPHYGSAFPSISRPQFDYSDHQPSGPYYGHSGQASG
LYSAFSYMGPSQRPLYTAISDPSPSGPQSHSPTHWEQPVYTTLSRP

>NP_003131.1 sex determining region Y [Homo sapiens]
MQSYASAMLSVFNSDDYSPAVQENIPALRRSSSFLCTESCNSKYQCETGENSKGNVQDRVKRPMNAFIVW
SRDQRRKMALENPRMRNSEISKQLGYQWKMLTEAEKWPFFQEAQKLQAMHREKYPNYKYRPRRKAKMLPK
NCSLLPADPASVLCSEVQLDNRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWTKL
```

MegaBLAST

```
>Danio rerio cDNA clone
GCCAAATAAACGAATCACCATCCAGCTTTGAGTGACTGACACTTCATCTTTGTAATAACCTTGGGCCGAG
ACAACAGCACAATGGAAAAGTGCCAGCTTTTATTGGATTTTCTGAACTGCAAAGCCCTGCAGTGAGCACC
GGCCCAATTGTGCAGGAACTTCTTGTTTTATTTTTGCTTCCATTCTTCCGACATTTTTCATTCCTCTGGC
TAAAAGTCAAATTCTCTCTTTTTATTTCCTTCTCCATGTAAAATTCTCTCTCTCCACTGAGGTAGTCTAC
TATGAGGCATCCACTGTGCTGACAACTGTCGATGTGTAGTATTTTCCACTCATTGTGGACAAGGTCTGCA
TGTAGCATGAATAAAAAATCAAAAAGTCAAGAGTAGCGATTAAAAAAATAGCTGAGGAAATCTCAGGTTG
CATTAACAAGGAATAGAAGGCTCATTTTGAGCGCTATTACAGAGAAAAACACCAGTCAAAT
```

BLAT

```
>CB312815 NICHD_Rr_Pit1 Rattus norvegicus cDNA clone
GGGGCTCTCGCTGGCCTGTGTCTCAGAAGCTGCTTTCTCCACCTCTTCCTTGTGAATTTCCTAAACTCTC
TACCTCTGGTTCATGTTCGCTCTTCTGGATAGTCTGTGTGCAATGAGCCCTTAAAGGAATATTGCAATGA
GCTATAAGAGTTGTGAGCCTGCGGTAGGCAAGGCCTGCACTGGGACAGCAAAGGAAATTTCATTGCATCT
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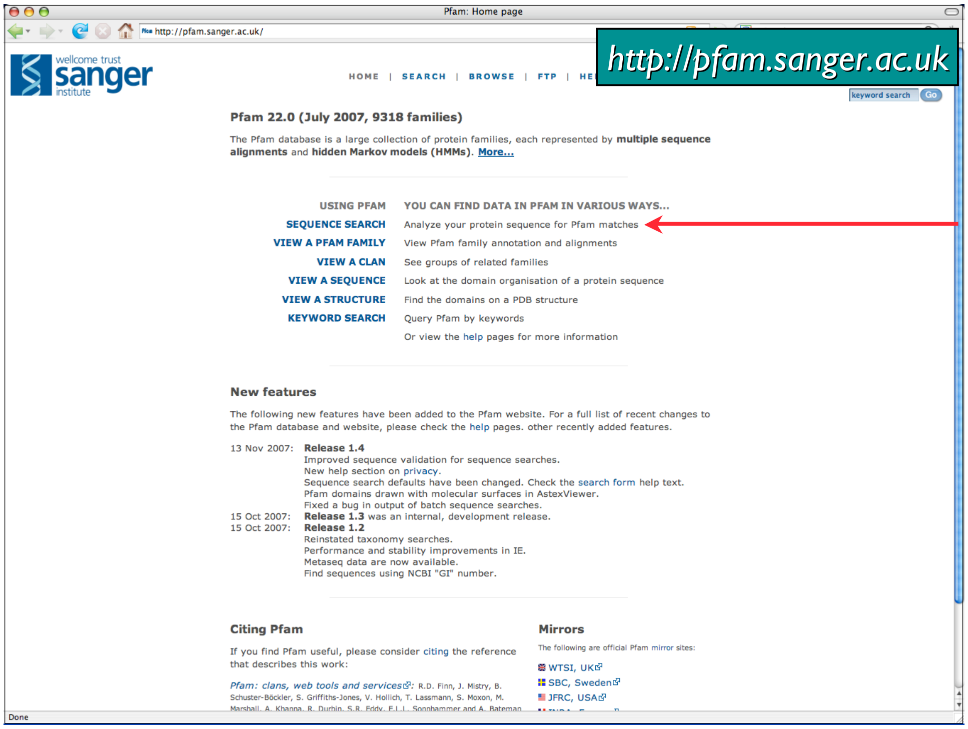


http://pfam.sanger.ac.uk

Pfam 22.0 (July 2007, 9318 families)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. **More...**

USING PFAM	YOU CAN FIND DATA IN PFAM IN VARIOUS WAYS...
SEQUENCE SEARCH	Analyze your protein sequence for Pfam matches
VIEW A PFAM FAMILY	View Pfam family annotation and alignments
VIEW A CLAN	See groups of related families
VIEW A SEQUENCE	Look at the domain organisation of a protein sequence
VIEW A STRUCTURE	Find the domains on a PDB structure
KEYWORD SEARCH	Query Pfam by keywords
	Or view the help pages for more information

New features

The following new features have been added to the Pfam website. For a full list of recent changes to the Pfam database and website, please check the help pages. other recently added features.

13 Nov 2007:	**Release 1.4** Improved sequence validation for sequence searches. New help section on privacy. Sequence search defaults have been changed. Check the search form help text. Pfam domains drawn with molecular surfaces in AstexViewer. Fixed a bug in output of batch sequence searches.
15 Oct 2007:	**Release 1.3** was an internal, development release.
15 Oct 2007:	**Release 1.2** Reinstated taxonomy searches. Performance and stability improvements in IE. Metaseq data are now available. Find sequences using NCBI "GI" number.

Citing Pfam

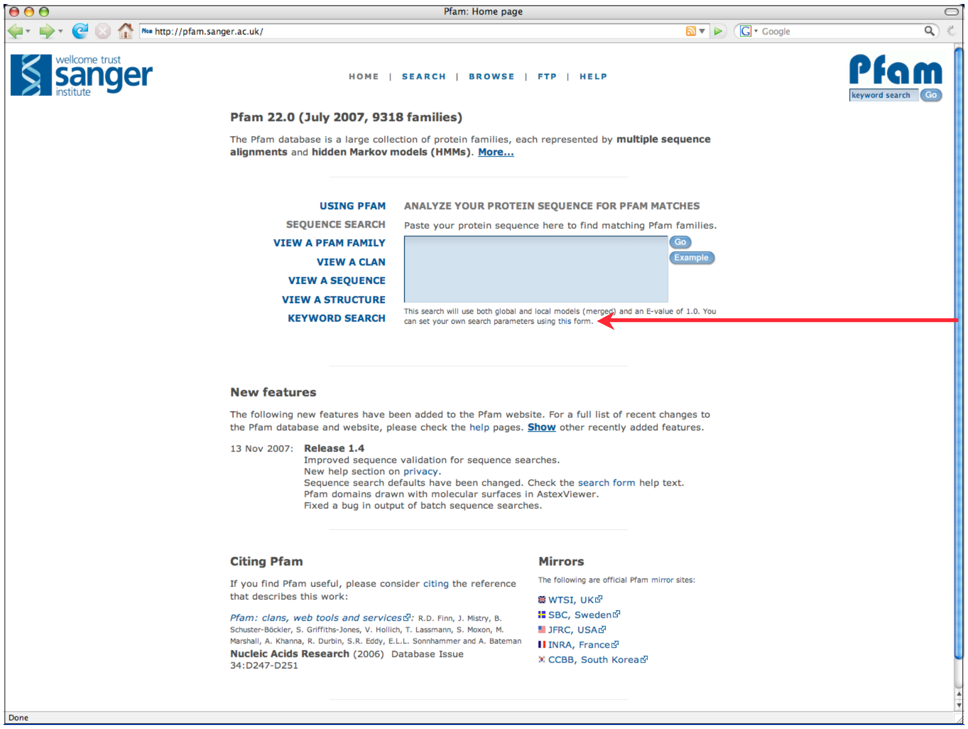
If you find Pfam useful, please consider citing the reference that describes this work:

Pfam: clans, web tools and services: R.D. Finn, J. Mistry, B. Schuster-Böckler, S. Griffiths-Jones, V. Hollich, T. Lassmann, S. Moxon, M. Marshall, A. Khanna, R. Durbin, S.R. Eddy, E.L.L. Sonnhammer and A. Bateman

Mirrors

The following are official Pfam mirror sites:

- WTSI, UK
- SBC, Sweden
- JFRC, USA


Pfam: Home page
http://pfam.sanger.ac.uk/
wellcome trust sanger institute
HOME | SEARCH | BROWSE | FTP | HELP
Pfam
keyword search
Go
Go
Example
Done

Pfam 22.0 (July 2007, 9318 families)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. More...

USING PFAM
SEQUENCE SEARCH
VIEW A PFAM FAMILY
VIEW A CLAN
VIEW A SEQUENCE
VIEW A STRUCTURE
KEYWORD SEARCH

ANALYZE YOUR PROTEIN SEQUENCE FOR PFAM MATCHES

Paste your protein sequence here to find matching Pfam families.

This search will use both global and local models (merged) and an E-value of 1.0. You can set your own search parameters using this form.

New features

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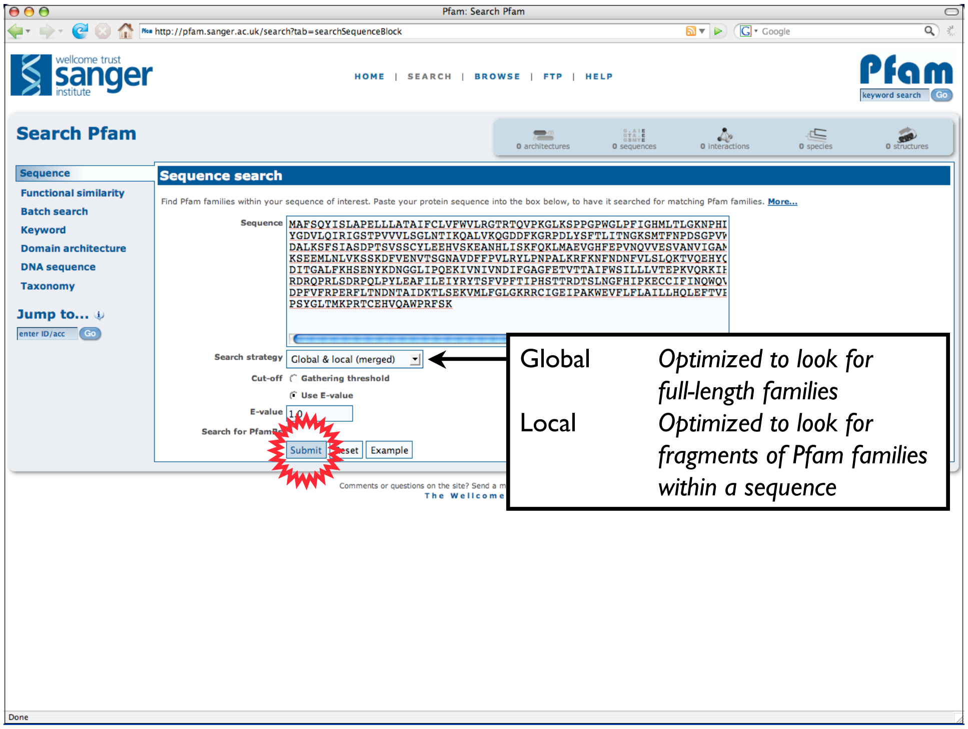
Mirrors

The following are official Pfam mirror sites:

- WTSI, UK
- SBC, Sweden
- JFRC, USA
- INRA, France
- CCBB, South Korea



Search Pfam

Sequence search

Find Pfam families within your sequence of interest. Paste your protein sequence into the box below, to have it searched for matching Pfam families. More...

Sequence

```
MAFSQYISLAPELLLATAIFCLVFWVLRGTRTQVPKGLKSPPGPWGLPFIGHMLTLGKNPHI
YGDVLQIRIGSTPVVVLSGLNTIKQALVKQGDDFKGRPDLYSFTLITNGKSMTFNPDSGPVW
DALKSFSIASDPTSVSSCYLEEHVSKEANHLISKFQKLMAEVGHFEPVNQVVESVANVIGAM
KSEEMLNLVKSSKDFVENVTSGNAVDFFPVLRYLPNPALKRFKNFNDNFVLSLQKTVQEHYQ
DITGALFKHSENYKDNGGLIPQEKIVNIVNDIFGAGFETVTTAIFWSILLLVTEPKVQRKIH
RDRQPRLSDRPQLPYLEAFILEIYRYTSFVPFTIPHSTTRDTSLNGFHIPKECCIFINQWQV
DPFVFRPERFLTNDNTAIDKTLSEKVMLFGLGKRRCIGEIPAKWEVFLFLAILLHQLEFTVP
PSYGLTMKPRTCEHVQAWPRFSK
```

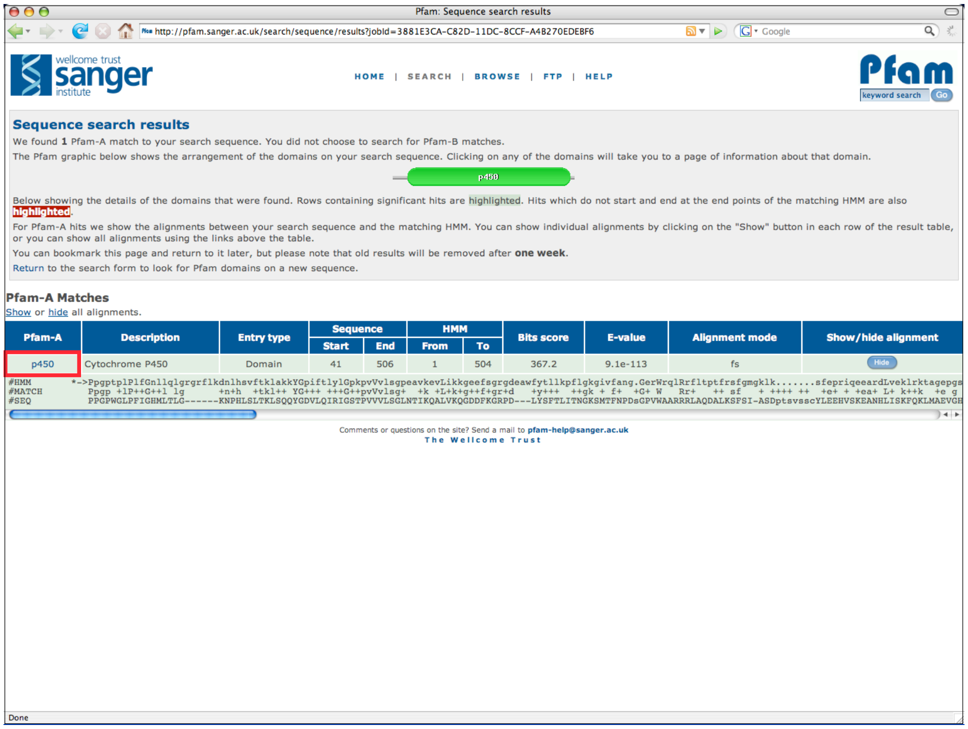
Search strategy: Global & local (merged)

Cut-off: ○ Gathering threshold ● Use E-value

E-value: 1.0

Search for PfamB

Global	*Optimized to look for full-length families*
Local	*Optimized to look for fragments of Pfam families within a sequence*


Pfam: Sequence search results
http://pfam.sanger.ac.uk/search/sequence/results?jobId=3881E3CA-C82D-11DC-8CCF-A4B270EDEBF6
Google
wellcome trust sanger institute
HOME | SEARCH | BROWSE | FTP | HELP
Pfam
keyword search
Go
p450
Done

Sequence search results

We found **1** Pfam-A match to your search sequence. You did not choose to search for Pfam-B matches.

The Pfam graphic below shows the arrangement of the domains on your search sequence. Clicking on any of the domains will take you to a page of information about that domain.

Below showing the details of the domains that were found. Rows containing significant hits are highlighted. Hits which do not start and end at the end points of the matching HMM are also **highlighted**.

For Pfam-A hits we show the alignments between your search sequence and the matching HMM. You can show individual alignments by clicking on the "Show" button in each row of the result table, or you can show all alignments using the links above the table.

You can bookmark this page and return to it later, but please note that old results will be removed after **one week**.

Return to the search form to look for Pfam domains on a new sequence.

Pfam-A Matches

Show or hide all alignments.

Pfam-A	Description	Entry type	Sequence		HMM		Bits score	E-value	Alignment mode	Show/hide alignment
			Start	End	From	To				
p450	Cytochrome P450	Domain	41	506	1	504	367.2	9.1e-113	fs	Hide

```
#HMM       *->PpgptplPlfGnllqlgrgrflkdnlhsvftklakkYGpiftlylGpkpvVvlsgpeavkevLikkgeefsgrgdeawfytllkpflgkgivfang.GerWrqlRrfltptfrsfgmklk.......sfepriqeeardLveklrktagepgs
#MATCH        Ppgp +lP++G++l lg      +n+h  +tkl++ YG+++ +++G++pvVvlsg+  +k +L+k+g++f+gr+d    +y+++  ++gk + f+  +G+ W    Rr+    ++ sf    + ++++ ++   +e+ + +ea+ L+ k++k  +e g
#SEQ          PPGPWGLPFIGHMLTLG------KNPHLSLTKLSQQYGDVLQIRIGSTPVVVLSGLNTIKQALVKQGDDFKGRPD---LYSFTLITNGKSMTFNPDsGPVWAARRRLAQDALKSFSI-ASDptsvsscYLEEHVSKEANHLISKFQKLMAEVGH
```

Comments or questions on the site? Send a mail to **pfam-help@sanger.ac.uk**

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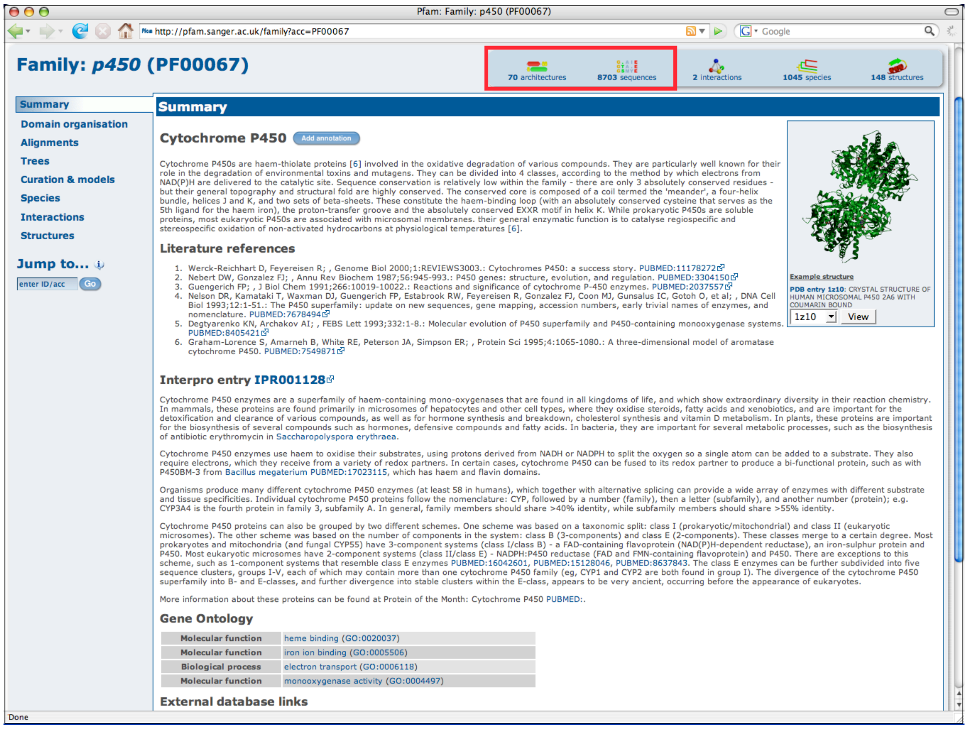


Family: *p450* (PF00067)

Summary

Cytochrome P450

Cytochrome P450s are haem-thiolate proteins [6] involved in the oxidative degradation of various compounds. They are particularly well known for their role in the degradation of environmental toxins and mutagens. They can be divided into 4 classes, according to the method by which electrons from NAD(P)H are delivered to the catalytic site. Sequence conservation is relatively low within the family - there are only 3 absolutely conserved residues - but their general topography and structural fold are highly conserved. The conserved core is composed of a coil termed the 'meander', a four-helix bundle, helices J and K, and two sets of beta-sheets. These constitute the haem-binding loop (with an absolutely conserved cysteine that serves as the 5th ligand for the haem iron), the proton-transfer groove and the absolutely conserved EXXR motif in helix K. While prokaryotic P450s are soluble proteins, most eukaryotic P450s are associated with microsomal membranes. their general enzymatic function is to catalyse regiospecific and stereospecific oxidation of non-activated hydrocarbons at physiological temperatures [6].

PDB entry 1z10: CRYSTAL STRUCTURE OF HUMAN MICROSOMAL P450 2A6 WITH COUMARIN BOUND

Literature references

1. Werck-Reichhart D, Feyereisen R; , Genome Biol 2000;1:REVIEWS3003.: Cytochromes P450: a success story. PUBMED:11178272
2. Nebert DW, Gonzalez FJ; , Annu Rev Biochem 1987;56:945-993.: P450 genes: structure, evolution, and regulation. PUBMED:3304150
3. Guengerich FP; , J Biol Chem 1991;266:10019-10022.: Reactions and significance of cytochrome P-450 enzymes. PUBMED:2037557
4. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, et al; , DNA Cell Biol 1993;12:1-51.: The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. PUBMED:7678494
5. Degtyarenko KN, Archakov AI; , FEBS Lett 1993;332:1-8.: Molecular evolution of P450 superfamily and P450-containing monooxygenase systems. PUBMED:8405421
6. Graham-Lorence S, Amarneh B, White RE, Peterson JA, Simpson ER; , Protein Sci 1995;4:1065-1080.: A three-dimensional model of aromatase cytochrome P450. PUBMED:7549871

Interpro entry IPR001128

Cytochrome P450 enzymes are a superfamily of haem-containing mono-oxygenases that are found in all kingdoms of life, and which show extraordinary diversity in their reaction chemistry. In mammals, these proteins are found primarily in microsomes of hepatocytes and other cell types, where they oxidise steroids, fatty acids and xenobiotics, and are important for the detoxification and clearance of various compounds, as well as for hormone synthesis and breakdown, cholesterol synthesis and vitamin D metabolism. In plants, these proteins are important for the biosynthesis of several compounds such as hormones, defensive compounds and fatty acids. In bacteria, they are important for several metabolic processes, such as the biosynthesis of antibiotic erythromycin in Saccharopolyspora erythraea.

Cytochrome P450 enzymes use haem to oxidise their substrates, using protons derived from NADH or NADPH to split the oxygen so a single atom can be added to a substrate. They also require electrons, which they receive from a variety of redox partners. In certain cases, cytochrome P450 can be fused to its redox partner to produce a bi-functional protein, such as with P450BM-3 from Bacillus megaterium PUBMED:17023115, which has haem and flavin domains.

Organisms produce many different cytochrome P450 enzymes (at least 58 in humans), which together with alternative splicing can provide a wide array of enzymes with different substrate and tissue specificities. Individual cytochrome P450 proteins follow the nomenclature: CYP, followed by a number (family), then a letter (subfamily), and another number (protein); e.g. CYP3A4 is the fourth protein in family 3, subfamily A. In general, family members should share >40% identity, while subfamily members should share >55% identity.

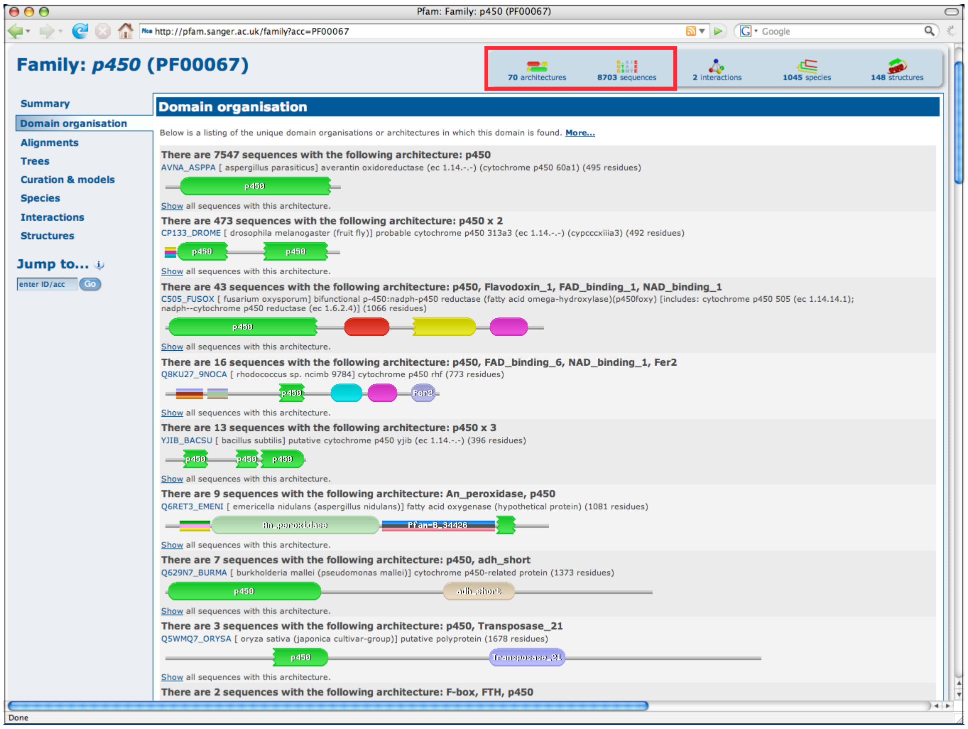
Cytochrome P450 proteins can also be grouped by two different schemes. One scheme was based on a taxonomic split: class I (prokaryotic/mitochondrial) and class II (eukaryotic microsomes). The other scheme was based on the number of components in the system: class B (3-components) and class E (2-components). These classes merge to a certain degree. Most prokaryotes and mitochondria (and fungal CYP55) have 3-component systems (class I/class B) - a FAD-containing flavoprotein (NAD(P)H-dependent reductase), an iron-sulphur protein and P450. Most eukaryotic microsomes have 2-component systems (class II/class E) - NADPH:P450 reductase (FAD and FMN-containing flavoprotein) and P450. There are exceptions to this scheme, such as 1-component systems that resemble class E enzymes PUBMED:16042601, PUBMED:15128046, PUBMED:8637843. The class E enzymes can be further subdivided into five sequence clusters, groups I-V, each of which may contain more than one cytochrome P450 family (eg, CYP1 and CYP2 are both found in group I). The divergence of the cytochrome P450 superfamily into B- and E-classes, and further divergence into stable clusters within the E-class, appears to be very ancient, occurring before the appearance of eukaryotes.

More information about these proteins can be found at Protein of the Month: Cytochrome P450 PUBMED:.

Gene Ontology

Molecular function	heme binding (GO:0020037)
Molecular function	iron ion binding (GO:0005506)
Biological process	electron transport (GO:0006118)
Molecular function	monooxygenase activity (GO:0004497)

External database links



Family: *p450* (PF00067)

Domain organisation

Below is a listing of the unique domain organisations or architectures in which this domain is found. More...

There are 7547 sequences with the following architecture: p450
AVNA_ASPPA [aspergillus parasiticus] averantin oxidoreductase (ec 1.14.-.-) (cytochrome p450 60a1) (495 residues)

Show all sequences with this architecture.

There are 473 sequences with the following architecture: p450 x 2
CP133_DROME [drosophila melanogaster (fruit fly)] probable cytochrome p450 313a3 (ec 1.14.-.-) (cypcccxiiia3) (492 residues)

Show all sequences with this architecture.

There are 43 sequences with the following architecture: p450, Flavodoxin_1, FAD_binding_1, NAD_binding_1
C505_FUSOX [fusarium oxysporum] bifunctional p-450:nadph-p450 reductase (fatty acid omega-hydroxylase)(p450foxy) [includes: cytochrome p450 505 (ec 1.14.14.1); nadph--cytochrome p450 reductase (ec 1.6.2.4)] (1066 residues)

Show all sequences with this architecture.

There are 16 sequences with the following architecture: p450, FAD_binding_6, NAD_binding_1, Fer2
Q8KU27_9NOCA [rhodococcus sp. ncimb 9784] cytochrome p450 rhf (773 residues)

Show all sequences with this architecture.

There are 13 sequences with the following architecture: p450 x 3
YJIB_BACSU [bacillus subtilis] putative cytochrome p450 yjib (ec 1.14.-.-) (396 residues)

Show all sequences with this architecture.

There are 9 sequences with the following architecture: An_peroxidase, p450
Q6RET3_EMENI [emericella nidulans (aspergillus nidulans)] fatty acid oxygenase (hypothetical protein) (1081 residues)

Show all sequences with this architecture.

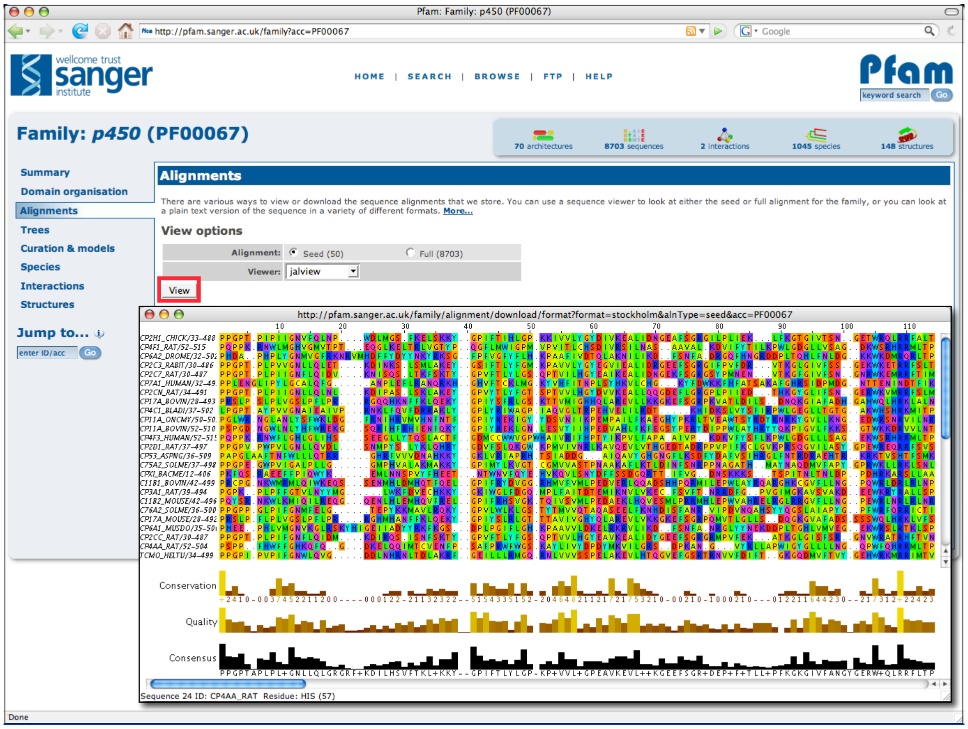
There are 7 sequences with the following architecture: p450, adh_short
Q629N7_BURMA [burkholderia mallei (pseudomonas mallei)] cytochrome p450-related protein (1373 residues)

Show all sequences with this architecture.

There are 3 sequences with the following architecture: p450, Transposase_21
Q5WMQ7_ORYSA [oryza sativa (japonica cultivar-group)] putative polyprotein (1678 residues)

Show all sequences with this architecture.

There are 2 sequences with the following architecture: F-box, FTH, p450



Family: *p450* (PF00067)

Alignments

There are various ways to view or download the sequence alignments that we store. You can use a sequence viewer to look at either the seed or full alignment for the family, or you can look at a plain text version of the sequence in a variety of different formats. More...

View options

Alignment: ● Seed (50) ○ Full (8703)

Viewer: jalview

View

http://pfam.sanger.ac.uk/family/alignment/download/format?format=stockholm&alnType=seed&acc=PF00067

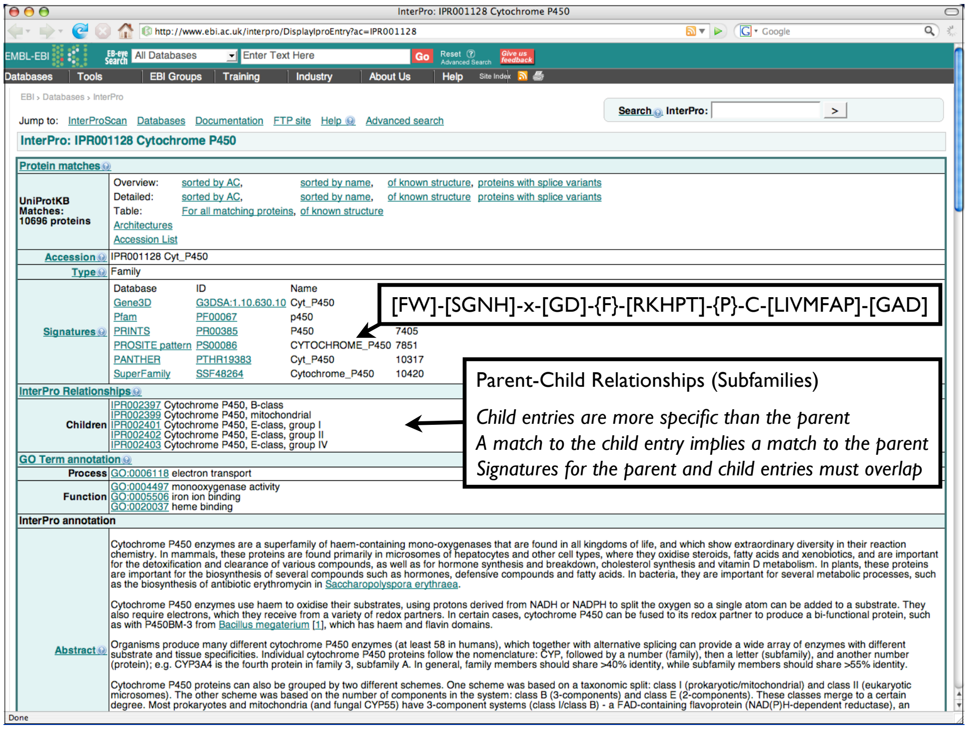
```
                            10        20        30        40        50        60        70        80        90       100       110
CP2H1_CHICK/33-488 PPGPT.PIPIIGNVFQLNP....WDLMGS.FKELSKKY..GPIFTIHLGP.KKIVVLYGYDIVKEALIDNGEAFSGRGILPLIEK...LFKGTGIVTSN..GETWRQLRRFALT
CP4F1_RAT/52-515   PQPPK.RNWLMGHVGMVTPT...EQGLKELTRLVGTYP..QGFLMWIGPM.VPVITLCHSDIVRSILNAS..AAVAL.KDVIFYTILKPWLGDGLLVSAG..DKWSRHRRMLTP
CP6A2_DROME/32-50  PHDA..PHPLYGNMVGFRKNRVMHDFFYDYYNKYRKSG..FPFVGFYFLH.KPAAFIVDTQLAKNILIKD..FSNFA.DRGQFHNGRDDPLTQHLFNLDG..KKWKDMRQRLTP
CP2C3_RABIT/30-486 PPGPT.PLPVVGNLLQLET....KDINKS.LSMLAKEY..GSIFTLYFGM.KPAVVLYGYEGVIEALIDRGEEFSGRGIFPVFDR...VTKGLGIVFSS..GEKWKETRRFSLT
CP2C7_RAT/30-487   PPGPT.PLPIIGNFLQIDV....KNISQS.LTKFSKTY..GPVFTLYLGS.QPTVILHGYEAIKEALIDNGEKFSGRGSYPMNEN...VTKGFGIVFSN..GNRWKEMRRFTIM
CP7A1_HUMAN/32-49. PPLENGLIPYLGCALQFG.....ANPLEFLRANQRKH..GHVFTCKLMG.KYVHFITNPLSYHKVLCHG....KYFDWKKFHFATSAKAFGHRSIDPMDG..NTTENINDTFIK
CP2CN_RAT/34-491   PPGPT.PLPIIGNLLQLNL....KDIPAS.LSKLAKEY..GPVYTLYFGT.SPTVVLHGYDVVKEALLQQGDEFLGRGPLPIIED...THKGYGLIFSN..GERWKVMRRFSLM
CP17A_BOVIN/28-493 PRSLP.SLPLVGSLPFLPR....RGQQHKNFFKLQEKY..GPIYSFRLGS.KTTVMIGHHQLAREVLLKKGKEFSGRPKVATLDILS..DNQKGIAFADH.GAHWQLHRKLALN
CP4C1_BLADI/37-502 LPGPT.AYPVVGNAIEAIVP...RNKLFQVFDRRAKLY..GPLYRIWAGP.IAQVGLTRPEHVELILRDT.....KHIDKSLVYSFIRPWLGEGLLTGTG..AKWHSHRKMITP
CP11A_ONCMY/50-50. PGLWR.NGLANLYSFWKLDG...FRNIHRVMVHNFNTF..GPIYREKIGY.YDSVNIIKPEMPAILFKAEGHYPKRLTVEAWTSYRDYRNRKYGVLLKNG..EDWRSNRVILNR
CP11A_BOVIN/52-510 PSPGD.NGWLNLYHFWREKG...SQRIHFRHIENFQKY..GPIYREKLGN.LESVYIIHPEDVAHLFKFEGSYPERYDIPPWLAYHRYYQKPIGVLFKKS..GTWKKDRVVLNT
CP4F3_HUMAN/52-51  PQPPK.RNWFLGHLGLIHS....SEEGLLYTQSLACTF..GDMCCWWVGPWHAIVRIFHPTYIKPVLFAPA.AIVP...KDKVFYSFLKPWLGDGLLLSAG..EKWSRHRRMLTP
CP2D1_RAT/37-497   PPGPV.PWPVLGNLLQVDL....SNMPYS.LYKLQHRY..GDVFSLQKGW.KPMVIVNRLKAVQEVLVTHGEDTADRPPVPIFKCLGVKPRSQGVILASY.GPEWREQRRFSVS
CP53_ASPNG/36-509  PAPGLAAFTNFWLLLQTRR.....GHRFVVVDNAHKKY..GKLVRIAPRH.TSIADDG....AIQAVYGHGNGFLKSDFYDAFVSIHRGLFNTRDRAEHTR..KRKTVSHTFSMK
C75A2_SOLME/37-498 PPGPE.GWPVIGALPLLG.....GMPHVALAKMAKKY..GPIMYLKVGT.CGMVVASTPNAAKAFLKTLDINFSNRPPNAGATH..MAYNAQDMVFAPY.GPRWKLLRKLSNL
CPXI_BACME/12-406  PKFQS.RAEEFFPIQWYK......EMLNNSPVYFHEET...NTWNVFQYE.HVKQVLSNYDFFSSDGQRTT.IFVG..DNSKKKS...TSPITNLTNLDP..PDHRKARSLLAA
C11B1_BOVIN/42-499 PRCPG.NKWMRMLQIWKEQS...SENMHLDMHQTFQEL..GPIFRYDVGG.RHMVFVMLPEDVERLQQADSHHPQRMILEPWLAYRQARGHKCGVFLLNG..PQWRLDRLRLNP
CP3A1_RAT/39-494   PGPK..PLPFFGTVLNYYMG......LWKFDVECHKKY..GKIWGLFDGQ.MPLFAITDTEMIKNVLVKEC.FSVFT.NRRDFG..PVGIMGKAVSVAKD..EEWKRYRALLSP
C11B2_MOUSE/42-496 PQYSR.NKWLKMIQILREQG...QENLHLEMHQVFREL..GPIFRHSVGK.TQIVSVMLPEDAEKLHQVESMLPRRMHLEPWVAHRELRGLRRGVFLLNG..PEWRLNRLRLNR
C76A2_SOLME/36-500 PPGPP.GLPIFGNMFELG.....TEPYKKMAVLRQKY..GPVLWLKLGS.TYTMVVQTAQASEELFKNHDISFANR.VIPDVNQAHSYYQGSLAIAPYG..PFWRFQRRICTI
CP17A_MOUSE/28-492 PRSLP.FLPLVGSLPFLPR....RGHMHANFFKLQEKY..GPIYSLRLGT.TTAVIVGHYQLAREVLVKKGKEFSGRPQMVTLGLLS..DQGKGVAFADS.SSSWQLHRKLVFS
CP6A1_MUSDO/35-50  PHEE..PHLVMGNVKGLRSKYHIGEIIADYYRKFKGS...DPLPGIFLGH.KPAAVVLDKELRKRVLIKD..FSNFA.NRGLYYNEKDDPLTGHLVMVEG..EKWRSLRTKLSP
CP2CC_RAT/30-487   PPGPT.PLPIFGNFLQIDM....KDIRQS.ISNFSKTY..GPVFTLYFGS.QPTVVLHGYEAVKEALIDYGEEFSGRGRMPVFEK...ATKGLGISFSR..GNVWRATRHFTVN
CP4AA_RAT/52-504   PSPP..FHWFFGHKQFQ..G...DKELQQIMTCVENFP..SAFPRWFWGS.KAYLIVYDPDYMKVILGRS..DPKAN.G...VYRLLAPWIGYGLLLLNG..QPWFQHRRMLTP
TCMO_HELTU/34-499  PPGPI.PVPIFGNWLQVG.....DDLNHRNLTDLAKRF..GEILLLRMGQ.RNLVVVSSPELAKEVLHTQGVEFGSRTRNVVFDIFT..GKGQDMVFTVY.GEHWRKMRRIMTV

Conservation       +2410-0037452211200----000122-21132322--5154335152-204648211217217532 10-00210-1000210---0122116442 30--217312+22423

Quality

Consensus          PPGPTAPLPL+GNLLQLGRGRF+KDILHSVFTKL+KKY--GPIFTLYLGP-KP+VVL+GPEAVKEVL++KGEEFSGR+DEP+F+TLL+PFKGKGIVFANGYGERW+QLRRFLTP
```

Sequence 24 ID: CP4AA_RAT Residue: HIS (57)



InterPro: IPR001128 Cytochrome P450

http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001128

InterPro: IPR001128 Cytochrome P450

Protein matches

UniProtKB Matches: 10696 proteins

Overview: sorted by AC, sorted by name, of known structure, proteins with splice variants
Detailed: sorted by AC, sorted by name, of known structure proteins with splice variants
Table: For all matching proteins, of known structure
Architectures
Accession List

Accession IPR001128 Cyt_P450

Type Family

Signatures

Database	ID	Name	
Gene3D	G3DSA:1.10.630.10	Cyt_P450	
Pfam	PF00067	p450	
PRINTS	PR00385	P450	7405
PROSITE pattern	PS00086	CYTOCHROME_P450	7851
PANTHER	PTHR19383	Cyt_P450	10317
SuperFamily	SSF48264	Cytochrome_P450	10420

[FW]-[SGNH]-x-[GD]-{F}-[RKHPT]-{P}-C-[LIVMFAP]-[GAD]

InterPro Relationships

Children
IPR002397 Cytochrome P450, B-class
IPR002399 Cytochrome P450, mitochondrial
IPR002401 Cytochrome P450, E-class, group I
IPR002402 Cytochrome P450, E-class, group II
IPR002403 Cytochrome P450, E-class, group IV

Parent-Child Relationships (Subfamilies)

Child entries are more specific than the parent
A match to the child entry implies a match to the parent
Signatures for the parent and child entries must overlap

GO Term annotation

Process GO:0006118 electron transport

Function GO:0004497 monooxygenase activity
GO:0005506 iron ion binding
GO:0020037 heme binding

InterPro annotation

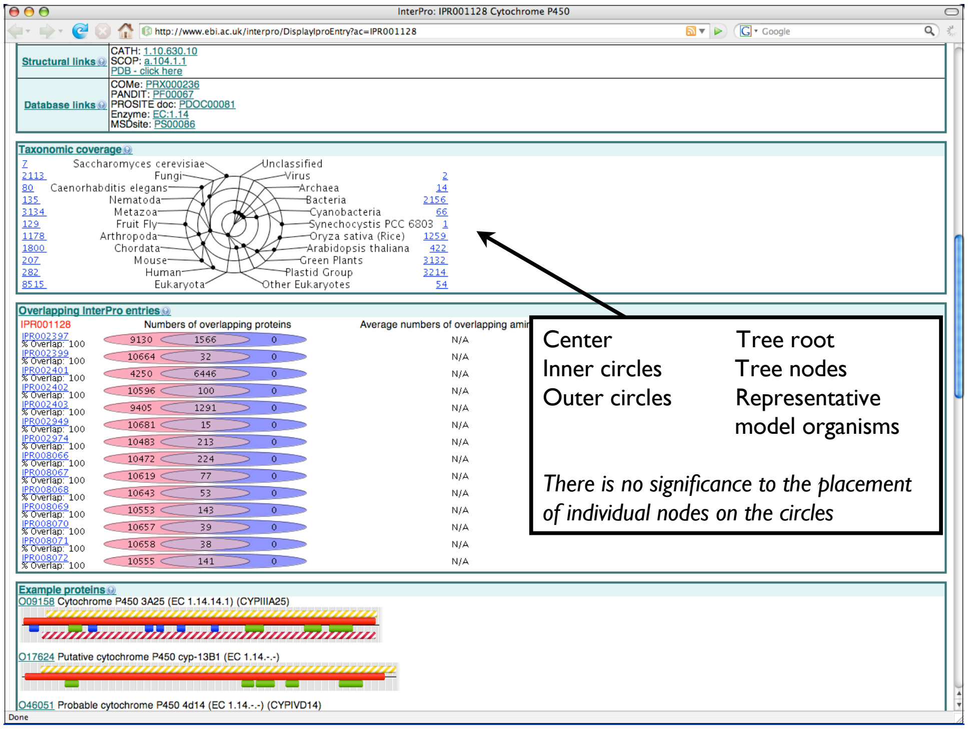
Abstract

Cytochrome P450 enzymes are a superfamily of haem-containing mono-oxygenases that are found in all kingdoms of life, and which show extraordinary diversity in their reaction chemistry. In mammals, these proteins are found primarily in microsomes of hepatocytes and other cell types, where they oxidise steroids, fatty acids and xenobiotics, and are important for the detoxification and clearance of various compounds, as well as for hormone synthesis and breakdown, cholesterol synthesis and vitamin D metabolism. In plants, these proteins are important for the biosynthesis of several compounds such as hormones, defensive compounds and fatty acids. In bacteria, they are important for several metabolic processes, such as the biosynthesis of antibiotic erythromycin in Saccharopolyspora erythraea.

Cytochrome P450 enzymes use haem to oxidise their substrates, using protons derived from NADH or NADPH to split the oxygen so a single atom can be added to a substrate. They also require electrons, which they receive from a variety of redox partners. In certain cases, cytochrome P450 can be fused to its redox partner to produce a bi-functional protein, such as with P450BM-3 from Bacillus megaterium [1], which has haem and flavin domains.

Organisms produce many different cytochrome P450 enzymes (at least 58 in humans), which together with alternative splicing can provide a wide array of enzymes with different substrate and tissue specificities. Individual cytochrome P450 proteins follow the nomenclature: CYP, followed by a number (family), then a letter (subfamily), and another number (protein); e.g. CYP3A4 is the fourth protein in family 3, subfamily A. In general, family members should share >40% identity, while subfamily members should share >55% identity.

Cytochrome P450 proteins can also be grouped by two different schemes. One scheme was based on a taxonomic split: class I (prokaryotic/mitochondrial) and class II (eukaryotic microsomes). The other scheme was based on the number of components in the system: class B (3-components) and class E (2-components). These classes merge to a certain degree. Most prokaryotes and mitochondria (and fungal CYP55) have 3-component systems (class I/class B) - a FAD-containing flavoprotein (NAD(P)H-dependent reductase), an



InterPro: IPR001128 Cytochrome P450

http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001128

Structural links CATH: 1.10.630.10
SCOP: a.104.1.1
PDB - click here

Database links COMe: PRX000236
PANDIT: PF00067
PROSITE doc: PDOC00081
Enzyme: EC:1.14
MSDsite: PS00086

Taxonomic coverage

7	Saccharomyces cerevisiae	Unclassified	
2113	Fungi	Virus	2
80	Caenorhabditis elegans	Archaea	14
135	Nematoda	Bacteria	2156
3134	Metazoa	Cyanobacteria	66
129	Fruit Fly	Synechocystis PCC 6803	1
1178	Arthropoda	Oryza sativa (Rice)	1259
1800	Chordata	Arabidopsis thaliana	422
207	Mouse	Green Plants	3132
282	Human	Plastid Group	3214
8515	Eukaryota	Other Eukaryotes	54

Center	Tree root
Inner circles	Tree nodes
Outer circles	Representative model organisms

There is no significance to the placement of individual nodes on the circles

Overlapping InterPro entries

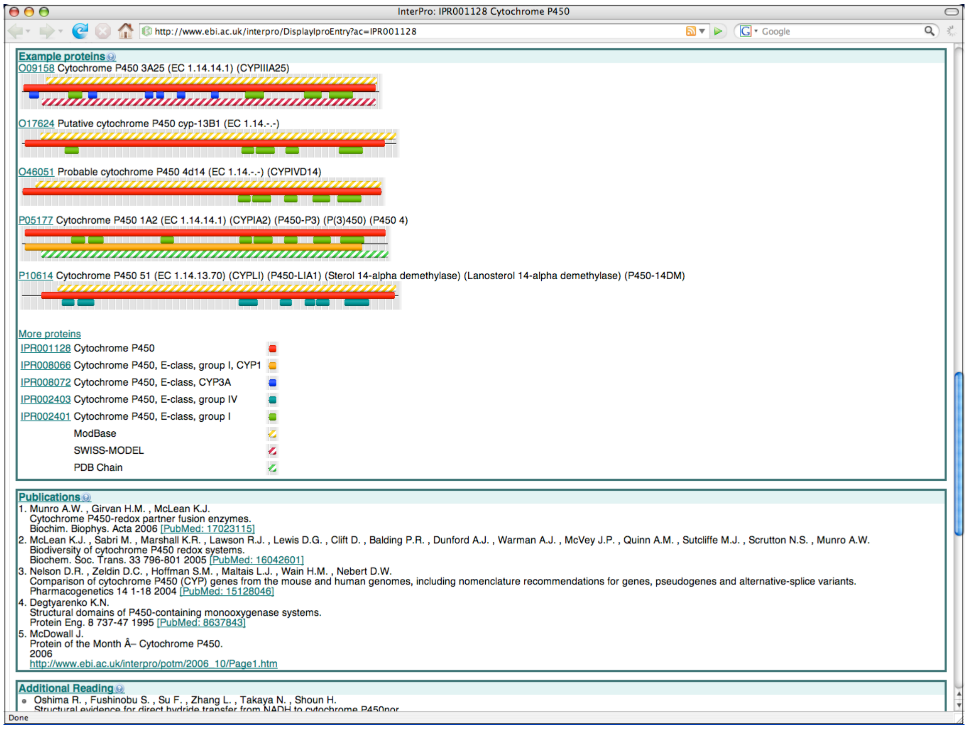
IPR001128	Numbers of overlapping proteins			Average numbers of overlapping amir
IPR002397 % Overlap: 100	9130	1566	0	N/A
IPR002399 % Overlap: 100	10664	32	0	N/A
IPR002401 % Overlap: 100	4250	6446	0	N/A
IPR002402 % Overlap: 100	10596	100	0	N/A
IPR002403 % Overlap: 100	9405	1291	0	N/A
IPR002949 % Overlap: 100	10681	15	0	N/A
IPR002974 % Overlap: 100	10483	213	0	N/A
IPR008066 % Overlap: 100	10472	224	0	N/A
IPR008067 % Overlap: 100	10619	77	0	N/A
IPR008068 % Overlap: 100	10643	53	0	N/A
IPR008069 % Overlap: 100	10553	143	0	N/A
IPR008070 % Overlap: 100	10657	39	0	N/A
IPR008071 % Overlap: 100	10658	38	0	N/A
IPR008072 % Overlap: 100	10555	141	0	N/A

Example proteins

O09158 Cytochrome P450 3A25 (EC 1.14.14.1) (CYPIIIA25)

O17624 Putative cytochrome P450 cyp-13B1 (EC 1.14.-.-)

O46051 Probable cytochrome P450 4d14 (EC 1.14.-.-) (CYPIVD14)



InterPro: IPR001128 Cytochrome P450

http://www.ebi.ac.uk/interpro/DisplayIproEntry?ac=IPR001128

Example proteins

O09158 Cytochrome P450 3A25 (EC 1.14.14.1) (CYPIIIA25)

O17624 Putative cytochrome P450 cyp-13B1 (EC 1.14.-.-)

O46051 Probable cytochrome P450 4d14 (EC 1.14.-.-) (CYPIVD14)

P05177 Cytochrome P450 1A2 (EC 1.14.14.1) (CYPIA2) (P450-P3) (P(3)450) (P450 4)

P10614 Cytochrome P450 51 (EC 1.14.13.70) (CYPLI) (P450-LIA1) (Sterol 14-alpha demethylase) (Lanosterol 14-alpha demethylase) (P450-14DM)

More proteins

IPR001128	Cytochrome P450	🟥
IPR008066	Cytochrome P450, E-class, group I, CYP1	🟧
IPR008072	Cytochrome P450, E-class, CYP3A	🟦
IPR002403	Cytochrome P450, E-class, group IV	🟦
IPR002401	Cytochrome P450, E-class, group I	🟩
	ModBase	▨
	SWISS-MODEL	▨
	PDB Chain	▨

Publications

1. Munro A.W. , Girvan H.M. , McLean K.J.
   Cytochrome P450-redox partner fusion enzymes.
   Biochim. Biophys. Acta 2006 [PubMed: 17023115]
2. McLean K.J. , Sabri M. , Marshall K.R. , Lawson R.J. , Lewis D.G. , Clift D. , Balding P.R. , Dunford A.J. , Warman A.J. , McVey J.P. , Quinn A.M. , Sutcliffe M.J. , Scrutton N.S. , Munro A.W.
   Biodiversity of cytochrome P450 redox systems.
   Biochem. Soc. Trans. 33 796-801 2005 [PubMed: 16042601]
3. Nelson D.R. , Zeldin D.C. , Hoffman S.M. , Maltais L.J. , Wain H.M. , Nebert D.W.
   Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants.
   Pharmacogenetics 14 1-18 2004 [PubMed: 15128046]
4. Degtyarenko K.N.
   Structural domains of P450-containing monooxygenase systems.
   Protein Eng. 8 737-47 1995 [PubMed: 8637843]
5. McDowall J.
   Protein of the Month Â– Cytochrome P450.
   2006
   http://www.ebi.ac.uk/interpro/potm/2006_10/Page1.htm

Additional Reading

- Oshima R. , Fushinobu S. , Su F. , Zhang L. , Takaya N. , Shoun H.
  Structural evidence for direct hydride transfer from NADH to cytochrome P450nor

Done

Current Protocols in Bioinformatics

CPBI Unit 2.5
Pfam

CPBI Unit 2.7
InterPro

Identifying Protein Domains with the Pfam Database

UNIT 2.5

Hundreds of thousands of protein sequences are now known and the deluge of data shows no signs of slowing. The sequence analysis of proteins may seem like a perpetual task. However, the majority of protein sequences appear to fall into a few thousand protein families (Chothia, 1992). Very often these families are representative of proteins at the domain level, where domains are discrete structural units that are frequently found in different protein contexts. Pfam is a database of such protein domain families (Sonnhammer et al., 1997; Bateman et al., 2002), with each family represented by multiple sequence alignments and profile hidden Markov models (HMMs). In addition, each family has associated annotation, literature references, and links to other databases. The entries in Pfam are available via the Web and in flatfile format.

The use of Pfam by molecular biologists as a protein information resource and analysis tool is widespread. Genome sequencing projects, including human and fly, have used Pfam extensively for large-scale functional annotation of genomic data, while smaller groups devoted to a single protein or biochemical pathway frequently use Pfam for their analyses. The multiple sequence alignments around which Pfam families are built are important for understanding both protein structure and function, and form the basis for techniques such as secondary structure prediction, fold recognition, and phylogenetic analysis, and can aid mutation design. This unit contains detailed information on how to access and utilize the information present in the Pfam database, namely the families, multiple alignments, and annotation. Details on running Pfam, both remotely (see Basic Protocol) and locally (see Alternate Protocol 1) are presented. The unit also includes a brief discussion of analyzing genomic DNA with Pfam (see Alternate Protocol 2). Information on underlying flatfiles is also presented (see Appendix at the end of this unit).

ANALYZING A PROTEIN SEQUENCE WITH Pfam VIA THE WEB

BASIC PROTOCOL

A primary use of the Pfam database is to determine what domains are in a protein of interest. This section describes several approaches for carrying out this analysis. In a typical session any one of them or several in various combinations may be used, depending on the kinds of questions asked and the types of information sought. A later section describes more advanced analyses that would typically follow these basic ones (see Suggestions for Further Analysis).

Necessary Resources

Hardware

Workstation with network connection

Software

Javascript-capable browser (e.g., Netscape 4.0, Internet Explorer 4.0)

Files

Protein sequence of interest in appropriate format (e.g. FASTA; APPENDIX 1B)

The example sequences used below are available at the Current Protocols Web site (http://www3.interscience.wiley.com/c_p/cpbi_sampledatafiles.htm).

Contributed by Robert Finn, Sam Griffiths-Jones, and Alex Bateman
Current Protocols in Bioinformatics (2003) 2.5.1-2.5.19
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Recognizing Functional Domains
2.5.1
Supplement 1

The InterPro Database and Tools for Protein Domain Analysis

UNIT 2.7

InterPro (Apweiler et al., 2001a) is an integrated documentation resource of protein families, domains, and functional sites that rationalizes data from the major protein-signature databases Pfam (UNIT 2.5; Bateman et al., 2002), PROSITE (UNIT 3.6; Falquet et al., 2002), PRINTS (Attwood et al., 2002), ProDom (Corpet et al., 2000), SMART (Letunic et al., 2002), PIR Superfamily (Wu et al., 2003), and TIGRFAMs (Haft et al., 2001). These databases, summarized in Table 2.7.1 along with other useful sites (also see Internet Resources), use a variety of methods for creation of diagnostic signatures, including regular expressions, profiles, fingerprints, hidden Markov models (HMMs), and sequence-clustering algorithms. In InterPro, signatures describing the same domain, family, repeat, or post-translational modification are grouped into single entries with unique accession numbers. Entries that are subsets of others are associated by parent/child (family and subfamily) or contains/found-in (domain composition) relationships. The associated software package, InterProScan (Zdobnov and Apweiler, 2001), combines the search methods from each of the member databases into one package and provides an output with all results in a single format, namely HTML, XML, or text. The software has been used for annotation of numerous completely sequenced genomes, notably human (The International Human Genome Consortium, 2001).

InterPro provides a one-stop shop for protein-sequence classification, freeing the user from having to go to each database separately and rationalize the different results in varying formats. The high-quality manual annotation provides useful information on each InterPro entry and, where possible, InterPro entries are mapped to Gene Ontology (GO) terms (UNIT 7.2; The Gene Ontology Consortium, 2001), facilitating automatic mapping of large sets of proteins to GO. The first two methods describe how a sequence may be submitted to InterProScan via a Web (see Basic Protocol 1) or E-mail server (see Alternate Protocol 1). As an alternative, a method describing how to install InterProScan for local running is also described (see Alternate Protocol 2). In addition, details on browsing InterPro families and domains of interest using the InterPro Web server (see Basic Protocol 2) and sequence retrieval system (SRS; see Alternate Protocols 3 and 4) are provided. Where necessary, the protocols are illustrated with examples.

PROTEIN SEQUENCE CLASSIFICATION USING INTERPROSCAN VIA THE INTERNET

BASIC PROTOCOL 1

A query protein sequence may be run against all the protein signatures in InterPro to identify protein domains or diagnostic features of protein families. The sequence-based search, InterProScan, uses tools provided by the member databases, including ScanRegExp for PROSITE patterns, pfscan for PROSITE profiles (Falquet et al., 2002), hmmpfam (Eddy, 1998; *http://hmmer.wustl.edu*) for Pfam (Bateman et al., 2002), SMART, PIR Superfamily, and TIGRFAM's HMMs, fingerPRINTScan for PRINTS fingerprints (Scordis et al., 1999), and BlastProDom for ProDom domains (Corpet et al., 2000). Independent researchers may submit their sequences using a Web interface at *http://www.ebi.ac.uk/interpro/scan.html* and obtain results of hits in InterPro in either a graphical or tabular view. The choice of output format is HTML or XML. The results from all submissions, including interactive searches, are also received by E-mail, which provides the XML-formatted results and a link to the HTML output. A mail server is available for sequence searches at *interproscan@ebi.ac.uk*. The mail and Web server for InterProScan are based on SRS (Etzold et al., 1996), which indexes the InterPro matches and data XML files. The advantage of this is the access to additional features in SRS.

Contributed by Nicola J. Mulder and Rolf Apweiler
Current Protocols in Bioinformatics (2003) 2.7.1-2.7.19
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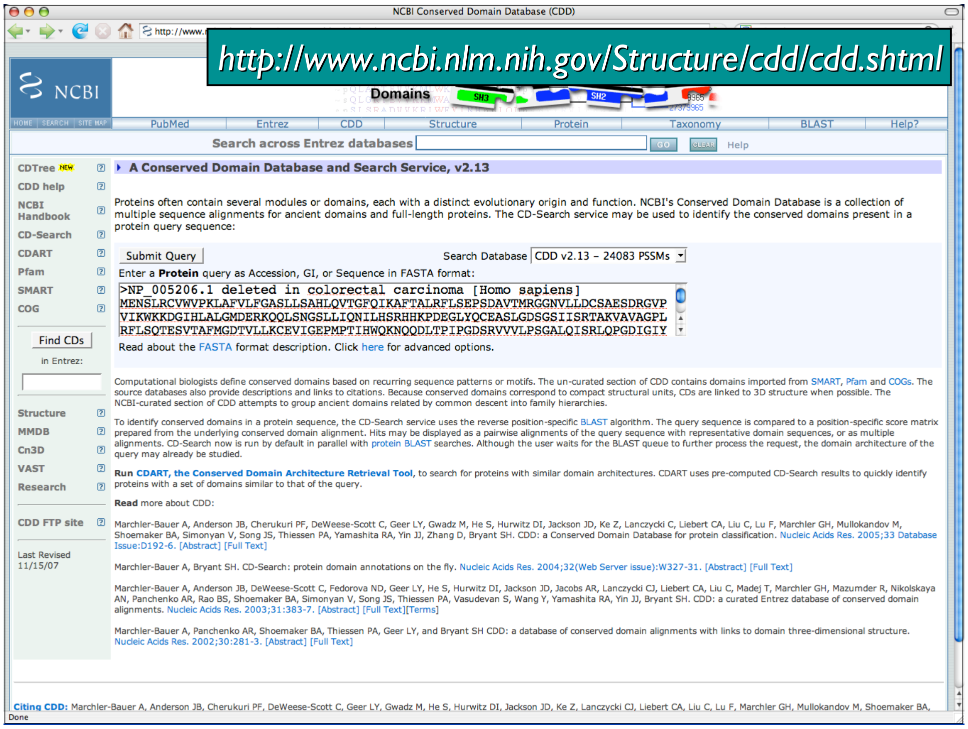
Recognizing Functional Domains
2.7.1
Supplement 2

http://nihlibrary.nih.gov
Search "Online Journals" for "Current Protocols in Bioinformatics"

Conserved Domain Database (CDD)

- Identify conserved domains in a protein sequence
- "Secondary database"
  - Pfam A and B
  - Simple Modular Architecture Research Tool (SMART)
  - Clusters of Orthologous Groups
- Search performed using RPS-BLAST
  - Query sequence is used to search a database of precalculated position-specific scoring tables
  - *Not* the same method used by Pfam or InterPro



http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml

Search across Entrez databases

in Entrez:

Last Revised
11/15/07

A Conserved Domain Database and Search Service, v2.13

Proteins often contain several modules or domains, each with a distinct evolutionary origin and function. NCBI's Conserved Domain Database is a collection of multiple sequence alignments for ancient domains and full-length proteins. The CD-Search service may be used to identify the conserved domains present in a protein query sequence:

Search Database: CDD v2.13 – 24083 PSSMs

Enter a **Protein** query as Accession, GI, or Sequence in FASTA format:

```
>NP_005206.1 deleted in colorectal carcinoma [Homo sapiens]
MENSLRCVWVPKLAFVLFGASLLSAHLQVTGFQIKAFTALRFLSEPSDAVTMRGGNVLLDCSAESDRGVP
VIKWKKDGIHLALGMDERKQQLSNGSLLIQNILHSRHHKPDEGLYQCEASLGDSGSIISRTAKVAVAGPL
RFLSQTESVTAFMGDTVLLKCEVIGEPMPTIHWQKNQQDLTPIPGDSRVVVLPSGALQISRLQPGDIGIY
```

Read about the FASTA format description. Click here for advanced options.

Computational biologists define conserved domains based on recurring sequence patterns or motifs. The un-curated section of CDD contains domains imported from SMART, Pfam and COGs. The source databases also provide descriptions and links to citations. Because conserved domains correspond to compact structural units, CDs are linked to 3D structure when possible. The NCBI-curated section of CDD attempts to group ancient domains related by common descent into family hierarchies.

To identify conserved domains in a protein sequence, the CD-Search service uses the reverse position-specific BLAST algorithm. The query sequence is compared to a position-specific score matrix prepared from the underlying conserved domain alignment. Hits may be displayed as a pairwise alignments of the query sequence with representative domain sequences, or as multiple alignments. CD-Search now is run by default in parallel with protein BLAST searches. Although the user waits for the BLAST queue to further process the request, the domain architecture of the query may already be studied.

Run CDART, the Conserved Domain Architecture Retrieval Tool, to search for proteins with similar domain architectures. CDART uses pre-computed CD-Search results to quickly identify proteins with a set of domains similar to that of the query.

Read more about CDD:

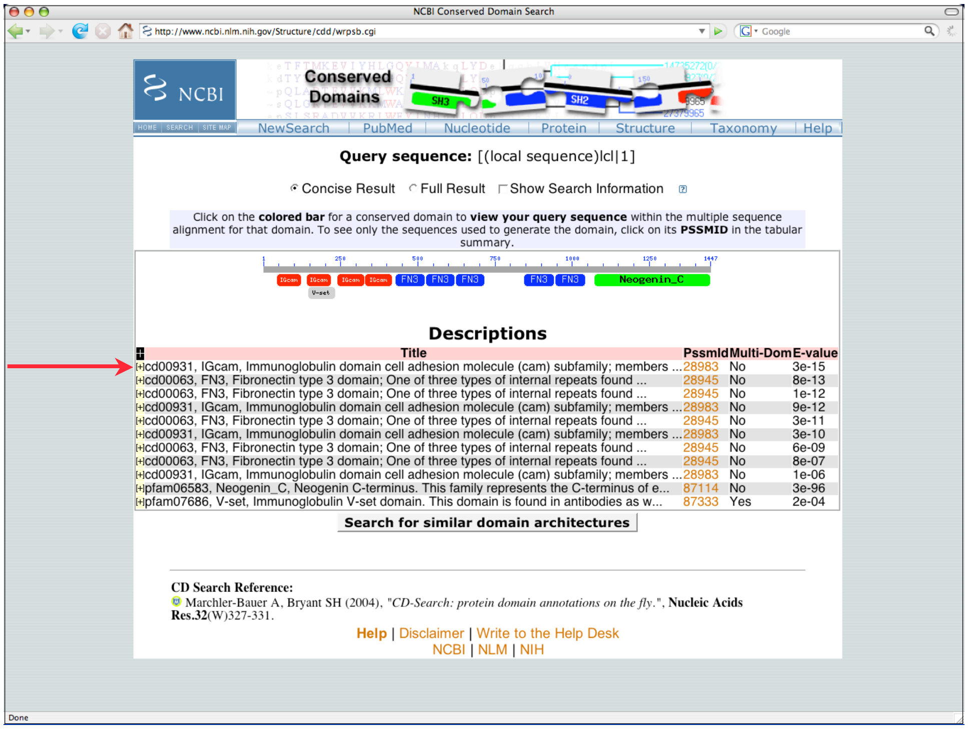
Marchler-Bauer A, Anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD, Ke Z, Lanczycki C, Liebert CA, Liu C, Lu F, Marchler GH, Mullokandov M, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Yamashita RA, Yin JJ, Zhang D, Bryant SH. CDD: a Conserved Domain Database for protein classification. Nucleic Acids Res. 2005;33 Database Issue:D192-6. [Abstract] [Full Text]

Marchler-Bauer A, Bryant SH. CD-Search: protein domain annotations on the fly. Nucleic Acids Res. 2004;32(Web Server issue):W327-31. [Abstract] [Full Text]

Marchler-Bauer A, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler GH, Mazumder R, Nikolskaya AN, Panchenko AR, Rao BS, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH. CDD: a curated Entrez database of conserved domain alignments. Nucleic Acids Res. 2003;31:383-7. [Abstract] [Full Text][Terms]

Marchler-Bauer A, Panchenko AR, Shoemaker BA, Thiessen PA, Geer LY, and Bryant SH CDD: a database of conserved domain alignments with links to domain three-dimensional structure. Nucleic Acids Res. 2002;30:281-3. [Abstract] [Full Text]

Citing CDD: Marchler-Bauer A, Anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Liebert CA, Liu C, Lu F, Marchler GH, Mullokandov M, Shoemaker BA,


NCBI Conserved Domain Search
http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
Google
NCBI
Conserved Domains
SH3
SH2
HOME | SEARCH | SITE MAP
NewSearch | PubMed | Nucleotide | Protein | Structure | Taxonomy | Help
1 250 500 750 1000 1250 1447
IGcam IGcam IGcam IGcam FN3 FN3 FN3 FN3 FN3 Neogenin_C
V-set
Done

Query sequence: [(local sequence)lcl|1]

● Concise Result ○ Full Result □ Show Search Information

Click on the **colored bar** for a conserved domain to **view your query sequence** within the multiple sequence alignment for that domain. To see only the sequences used to generate the domain, click on its **PSSMID** in the tabular summary.

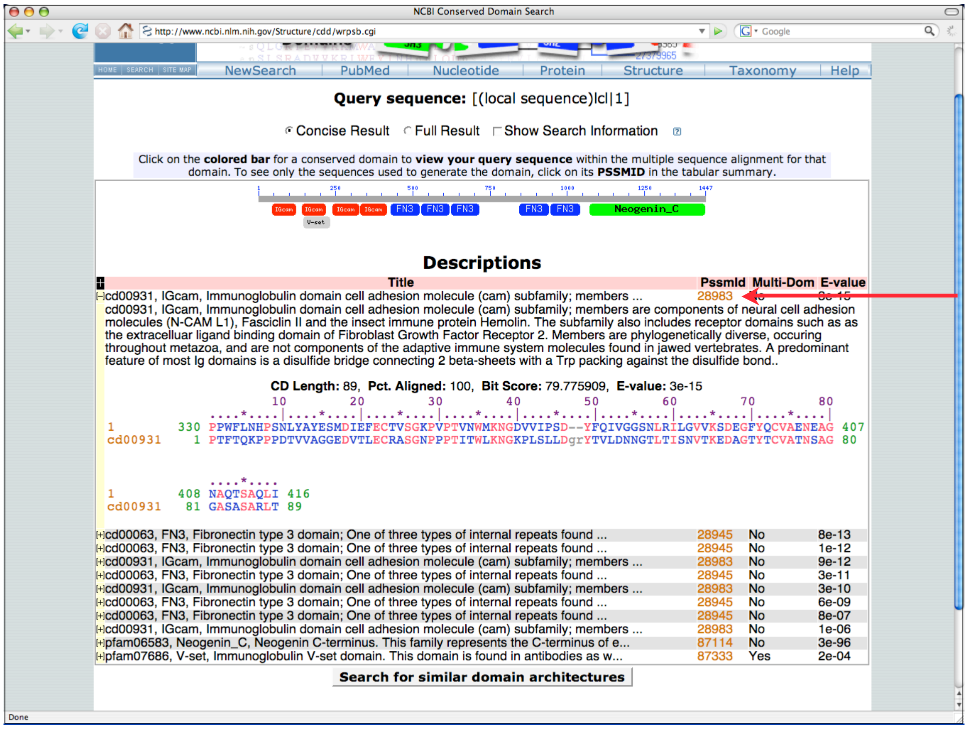
Descriptions

Title	PssmId	Multi-Dom	E-value
[+]cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	3e-15
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	8e-13
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	1e-12
[+]cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	9e-12
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	3e-11
[+]cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	3e-10
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	6e-09
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	8e-07
[+]cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	1e-06
[+]pfam06583, Neogenin_C, Neogenin C-terminus. This family represents the C-terminus of e...	87114	No	3e-96
[+]pfam07686, V-set, Immunoglobulin V-set domain. This domain is found in antibodies as w...	87333	Yes	2e-04

Search for similar domain architectures

CD Search Reference:
Marchler-Bauer A, Bryant SH (2004), "*CD-Search: protein domain annotations on the fly.*", **Nucleic Acids Res.32**(W)327-331.

Help | Disclaimer | Write to the Help Desk
NCBI | NLM | NIH


NCBI Conserved Domain Search
http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
Google
HOME | SEARCH | SITE MAP
NewSearch | PubMed | Nucleotide | Protein | Structure | Taxonomy | Help
1 250 500 750 1000 1250 1447
IGcam IGcam IGcam IGcam FN3 FN3 FN3 FN3 FN3 Neogenin_C
V-set
Done

Query sequence: [(local sequence)lcl|1]

● Concise Result ○ Full Result □ Show Search Information

Click on the **colored bar** for a conserved domain to **view your query sequence** within the multiple sequence alignment for that domain. To see only the sequences used to generate the domain, click on its **PSSMID** in the tabular summary.

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CD Length: 89, **Pct. Aligned:** 100, **Bit Score:** 79.775909, **E-value:** 3e-15

```
                    10        20        30        40        50        60        70        80
               ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1         330  PPWFLNHPSNLYAYESMDIEFECTVSGKPVPTVNWMKNGDVVIPSD--YFQIVGGSNLRILGVVKSDEGFYQCVAENEAG  407
cd00931     1  PTFTQKPPPDTVVAGGEDVTLECRASGNPPPTITWLKNGKPLSLLDgrYTVLDNNGTLTISNVTKEDAGTYTCVATNSAG  80


               ....*....
1         408  NAQTSAQLI  416
cd00931    81  GASASARLT  89
```

Title	PssmId	Multi-Dom	E-value
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	8e-13
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[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	3e-11
[+]cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	3e-10
[+]cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	6e-09
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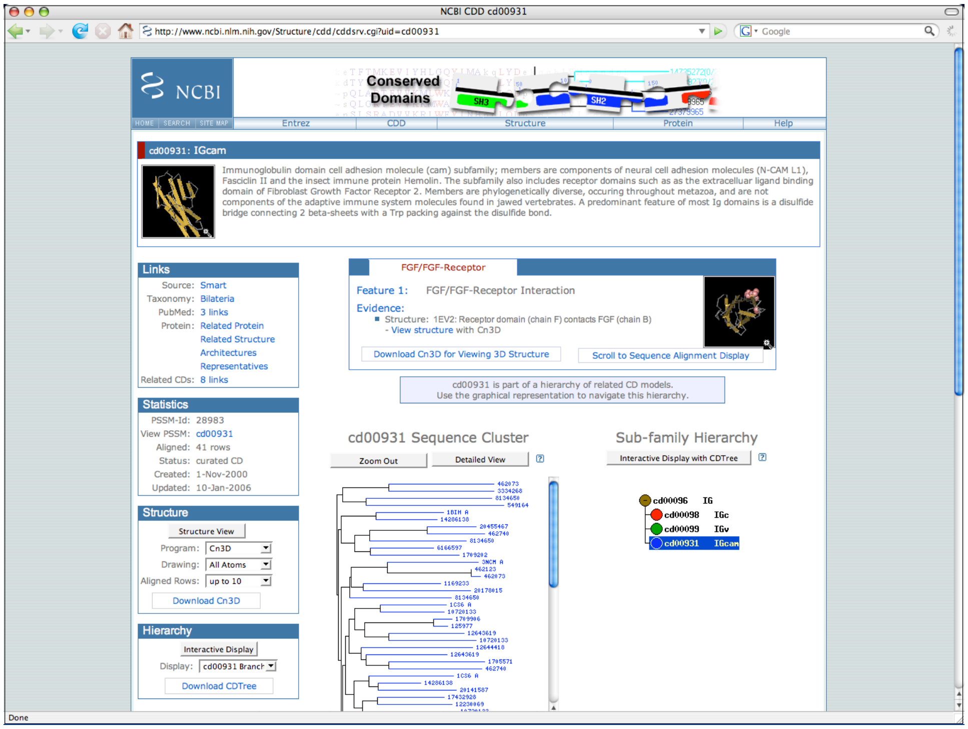
[+]pfam06583, Neogenin_C, Neogenin C-terminus. This family represents the C-terminus of e...	87114	No	3e-96
[+]pfam07686, V-set, Immunoglobulin V-set domain. This domain is found in antibodies as w...	87333	Yes	2e-04

Search for similar domain architectures



NCBI CDD cd00931

http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=cd00931

cd00931: IGcam

Immunoglobulin domain cell adhesion molecule (cam) subfamily; members are components of neural cell adhesion molecules (N-CAM L1), Fasciclin II and the insect immune protein Hemolin. The subfamily also includes receptor domains such as as the extracelluar ligand binding domain of Fibroblast Growth Factor Receptor 2. Members are phylogenetically diverse, occuring throughout metazoa, and are not components of the adaptive immune system molecules found in jawed vertebrates. A predominant feature of most Ig domains is a disulfide bridge connecting 2 beta-sheets with a Trp packing against the disulfide bond.

Links

Source: Smart
Taxonomy: Bilateria
PubMed: 3 links
Protein: Related Protein
Related Structure
Architectures
Representatives
Related CDs: 8 links

Statistics

PSSM-Id: 28983
View PSSM: cd00931
Aligned: 41 rows
Status: curated CD
Created: 1-Nov-2000
Updated: 10-Jan-2006

Structure

Structure View
Program: Cn3D
Drawing: All Atoms
Aligned Rows: up to 10
Download Cn3D

Hierarchy

Interactive Display
Display: cd00931 Branch
Download CDTree

FGF/FGF-Receptor

Feature 1: FGF/FGF-Receptor Interaction

Evidence:
- Structure: 1EV2: Receptor domain (chain F) contacts FGF (chain B)
  - View structure with Cn3D

Download Cn3D for Viewing 3D Structure | Scroll to Sequence Alignment Display

cd00931 is part of a hierarchy of related CD models.
Use the graphical representation to navigate this hierarchy.

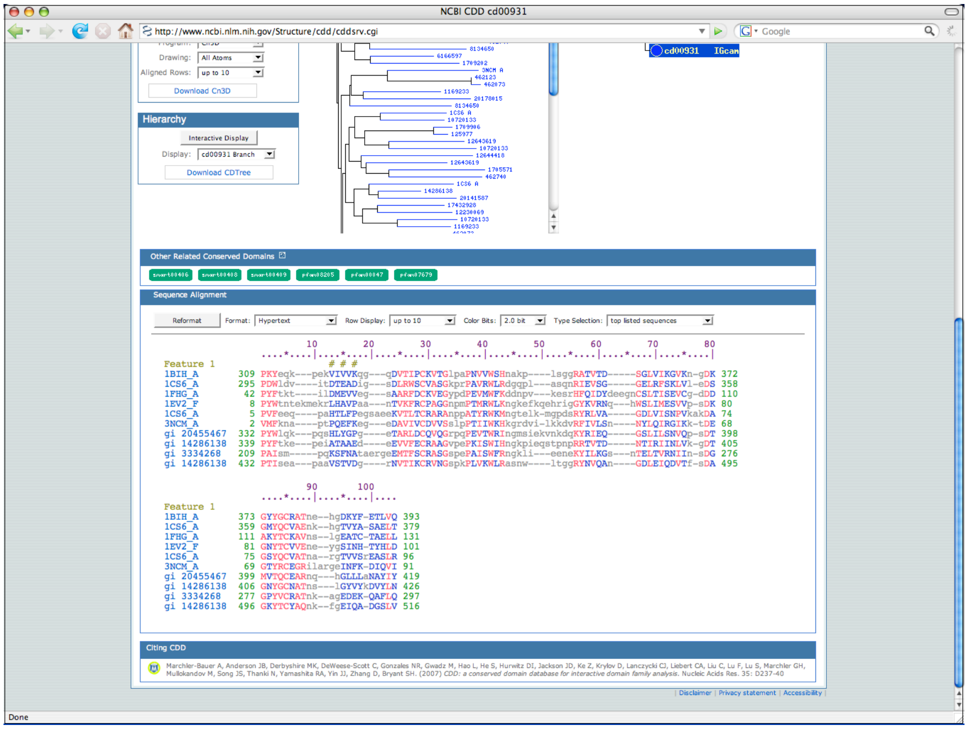
cd00931 Sequence Cluster

Zoom Out | Detailed View

Sub-family Hierarchy

Interactive Display with CDTree

Done



NCBI CDD cd00931

http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi

Drawing: All Atoms
Aligned Rows: up to 10
Download Cn3D

Hierarchy

Interactive Display
Display: cd00931 Branch
Download CDTree

Other Related Conserved Domains

smart00406 | smart00408 | smart00409 | pfam08205 | pfam00047 | pfam07679

Sequence Alignment

Reformat | Format: Hypertext | Row Display: up to 10 | Color Bits: 2.0 bit | Type Selection: top listed sequences

```
                          10        20        30        40        50        60        70        80
                   ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Feature 1                     # # #
1BIH_A        309 PKYeqk---pekVIVVKqg---qDVTIPCKVTGlpaPNVVWSHnakp----lsggRATVTD-----SGLVIKGVKn-gDK 372
1CS6_A        295 PDWldv----itDTEADig---sDLRWSCVASGkprPAVRWLRdgqpl---asqnRIEVSG-----GELRFSKLVl-eDS 358
1FHG_A         42 PYFtkt----ilDMEVVeg---sAARFDCKVEGypdPEVMWFKddnpv---kesrHFQIDYdeegnCSLTISEVCg-dDD 110
1EV2_F          8 PYWtntekmekrLHAVPaa---nTVKFRCPAGGnpmPTMRWLKngkefkqehrigGYKVRNq---hWSLIMESVVp-sDK 80
1CS6_A          5 PVFeeq----paHTLFPegsaeeKVTLTCRARAnppATYRWKMngtelk-mgpdsRYRLVA-----GDLVISNPVkakDA 74
3NCM_A          2 VMFkna----ptPQEFKeg---eDAVIVCDVVSslpPTIIWKHkgrdvi-lkkdvRFIVLSn----NYLQIRGIKk-tDE 68
gi 20455467   332 PYWlqk---pqsHLYGPg----eTARLDCQVQGrpqPEVTWRIngmsiekvnkdqKYRIEQ-----GSLILSNVQp-sDT 398
gi 14286138   339 PYFtke---peiATAAEd----eEVVFECRAAGvpePKISWIHngkpieqstpnpRRTVTD-----NTIRIINLVk-gDT 405
gi 3334268    209 PAIsm-----pqKSFNAtaergeEMTFSCRASGspePAISWFRngkli---eeneKYILKGs---nTELTVRNIIn-sDG 276
gi 14286138   432 PTIsea---paaVSTVDg----rNVTIKCRVNGspkPLVKWLRasnw----ltggRYNVQAn----GDLEIQDVTf-sDA 495

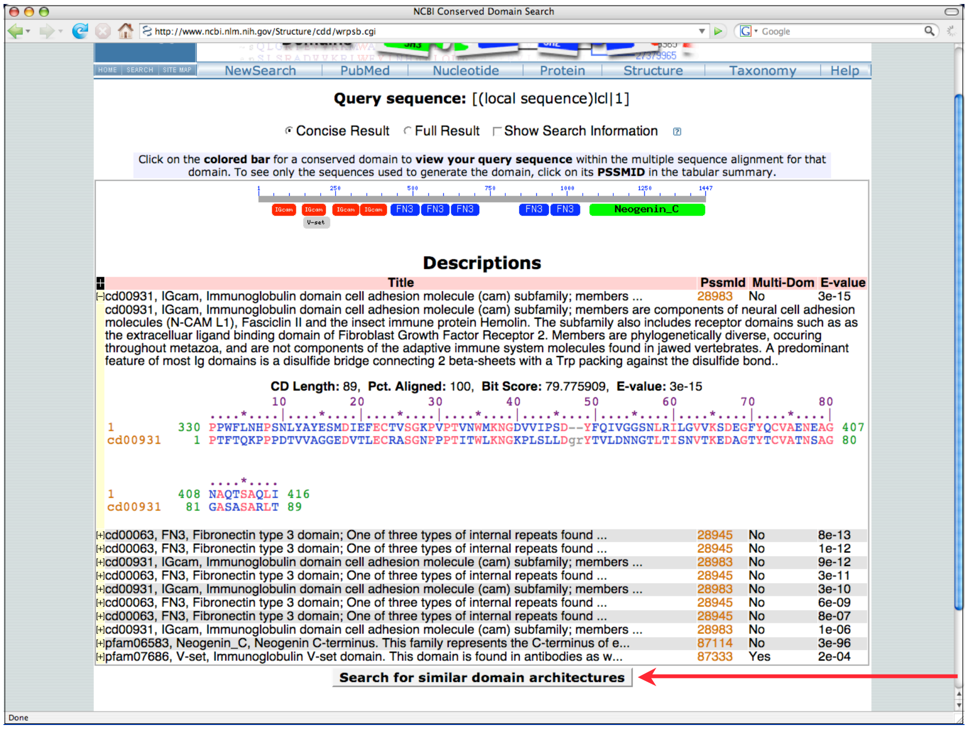

                          90       100
                   ....*....|....*....|....
Feature 1
1BIH_A        373 GYYGCRATne--hgDKYF-ETLVQ 393
1CS6_A        359 GMYQCVAEnk--hgTVYA-SAELT 379
1FHG_A        111 AKYTCKAVns--lgEATC-TAELL 131
1EV2_F         81 GNYTCVVEne--ygSINH-TYHLD 101
1CS6_A         75 GSYQCVATna--rgTVVSrEASLR 96
3NCM_A         69 GTYRCEGRilargeINFK-DIQVI 91
gi 20455467   399 MVTQCEARnq---hGLLLaNAYIY 419
gi 14286138   406 GNYGCNATns---lGYVYkDVYLN 426
gi 3334268    277 GPYVCRATnk--agEDEK-QAFLQ 297
gi 14286138   496 GKYTCYAQnk--fgEIQA-DGSLV 516
```

Citing CDD

Marchler-Bauer A, Anderson JB, Derbyshire MK, DeWeese-Scott C, Gonzales NR, Gwadz M, Hao L, He S, Hurwitz DI, Jackson JD, Ke Z, Krylov D, Lanczycki CJ, Liebert CA, Liu C, Lu F, Lu S, Marchler GH, Mullokandov M, Song JS, Thanki N, Yamashita RA, Yin JJ, Zhang D, Bryant SH. (2007) *CDD: a conserved domain database for interactive domain family analysis*. Nucleic Acids Res. 35: D237-40

| Disclaimer | Privacy statement | Accessibility |

Done



Query sequence: [(local sequence)lcl|1]

● Concise Result ○ Full Result □ Show Search Information

Click on the **colored bar** for a conserved domain to **view your query sequence** within the multiple sequence alignment for that domain. To see only the sequences used to generate the domain, click on its **PSSMID** in the tabular summary.

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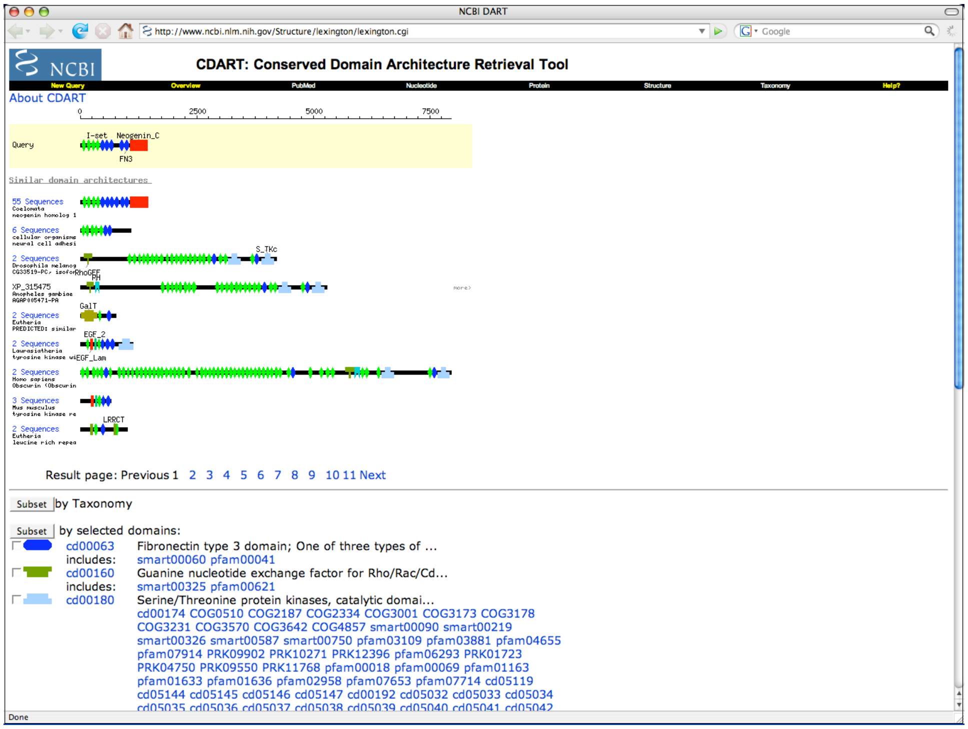
```
                   10        20        30        40        50        60        70        80
           ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1       330 PPWFLNHPSNLYAYESMDIEFECTVSGKPVPTVNWMKNGDVVIPSD--YFQIVGGSNLRILGVVKSDEGFYQCVAENEAG 407
cd00931   1 PTFTQKPPPDTVVAGGEDVTLECRASGNPPPTITWLKNGKPLSLLDgrYTVLDNNGTLTISNVTKEDAGTYTCVATNSAG 80


           ....*....
1       408 NAQTSAQLI 416
cd00931  81 GASASARLT 89
```

Title	PssmId	Multi-Dom	E-value
cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	8e-13
cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	1e-12
cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	9e-12
cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	3e-11
cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	3e-10
cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	6e-09
cd00063, FN3, Fibronectin type 3 domain; One of three types of internal repeats found ...	28945	No	8e-07
cd00931, IGcam, Immunoglobulin domain cell adhesion molecule (cam) subfamily; members ...	28983	No	1e-06
pfam06583, Neogenin_C, Neogenin C-terminus. This family represents the C-terminus of e...	87114	No	3e-96
pfam07686, V-set, Immunoglobulin V-set domain. This domain is found in antibodies as w...	87333	Yes	2e-04



CDART: Conserved Domain Architecture Retrieval Tool

About CDART

Query

Similar domain architectures

55 Sequences
Coelomata
neogenin homolog 1

6 Sequences
cellular organisms
neural cell adhesi

2 Sequences
Drosophila melanog
CG33519-PC, isofor

XP_315475
Anopheles gambiae
AGAP005471-PA

2 Sequences
Eutheria
PREDICTED: similar

2 Sequences
Laurasiatheria
tyrosine kinase wi

2 Sequences
Homo sapiens
Obscurin (Obscurin

3 Sequences
Mus musculus
tyrosine kinase re

2 Sequences
Eutheria
leucine rich repea

Result page: Previous 1 2 3 4 5 6 7 8 9 10 11 Next

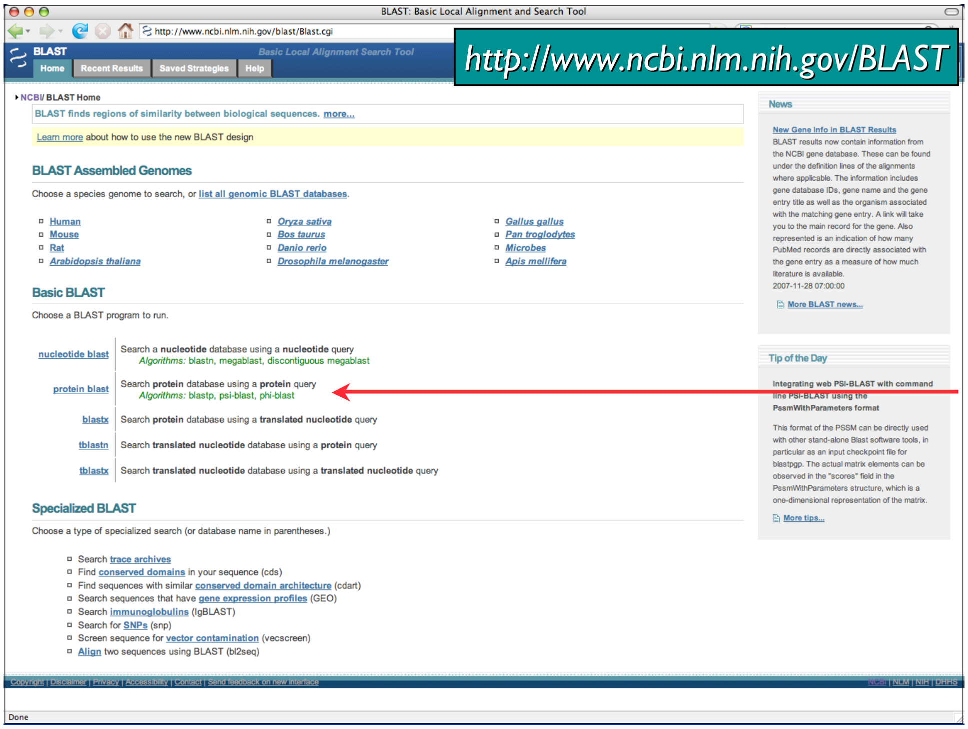
Subset by Taxonomy

Subset by selected domains:

□ 🟦	cd00063	Fibronectin type 3 domain; One of three types of ...
	includes:	smart00060 pfam00041
□ 🟩	cd00160	Guanine nucleotide exchange factor for Rho/Rac/Cd...
	includes:	smart00325 pfam00621
□ 🟦	cd00180	Serine/Threonine protein kinases, catalytic domai... cd00174 COG0510 COG2187 COG2334 COG3001 COG3173 COG3178 COG3231 COG3570 COG3642 COG4857 smart00090 smart00219 smart00326 smart00587 smart00750 pfam03109 pfam03881 pfam04655 pfam07914 PRK09902 PRK10271 PRK12396 pfam06293 PRK01723 PRK04750 PRK09550 PRK11768 pfam00018 pfam00069 pfam01163 pfam01633 pfam01636 pfam02958 pfam07653 pfam07714 cd05119 cd05144 cd05145 cd05146 cd05147 cd00192 cd05032 cd05033 cd05034 cd05035 cd05036 cd05037 cd05038 cd05039 cd05040 cd05041 cd05042

PSI-BLAST

- Position-Specific Iterated BLAST search
- Easy-to-use version of a profile-based search
  - Perform BLAST search against protein database
  - Use results to calculate a position-specific scoring matrix
  - PSSM replaces query for next round of searches
  - May be iterated until no new significant alignments are found
    - Convergence – all related sequences deemed found
    - Divergence – query is too broad, make cutoffs more stringent


BLAST: Basic Local Alignment and Search Tool
http://www.ncbi.nlm.nih.gov/blast/Blast.cgi
BLAST
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help

http://www.ncbi.nlm.nih.gov/BLAST

NCBI/ BLAST Home

BLAST finds regions of similarity between biological sequences. more...

Learn more about how to use the new BLAST design

BLAST Assembled Genomes

Choose a species genome to search, or list all genomic BLAST databases.

- Human
- Mouse
- Rat
- *Arabidopsis thaliana*
- *Oryza sativa*
- *Bos taurus*
- *Danio rerio*
- *Drosophila melanogaster*
- *Gallus gallus*
- *Pan troglodytes*
- *Microbes*
- *Apis mellifera*

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

Specialized BLAST

Choose a type of specialized search (or database name in parentheses.)

- Search trace archives
- Find conserved domains in your sequence (cds)
- Find sequences with similar conserved domain architecture (cdart)
- Search sequences that have gene expression profiles (GEO)
- Search immunoglobulins (IgBLAST)
- Search for SNPs (snp)
- Screen sequence for vector contamination (vecscreen)
- Align two sequences using BLAST (bl2seq)

News

New Gene Info in BLAST Results
BLAST results now contain information from the NCBI gene database. These can be found under the definition lines of the alignments where applicable. The information includes gene database IDs, gene name and the gene entry title as well as the organism associated with the matching gene entry. A link will take you to the main record for the gene. Also represented is an indication of how many PubMed records are directly associated with the gene entry as a measure of how much literature is available.
2007-11-28 07:00:00

More BLAST news...

Tip of the Day

Integrating web PSI-BLAST with command line PSI-BLAST using the PsssmWithParameters format

This format of the PSSM can be directly used with other stand-alone Blast software tools, in particular as an input checkpoint file for blastpgp. The actual matrix elements can be observed in the "scores" field in the PssmWithParameters structure, which is a one-dimensional representation of the matrix.

More tips...

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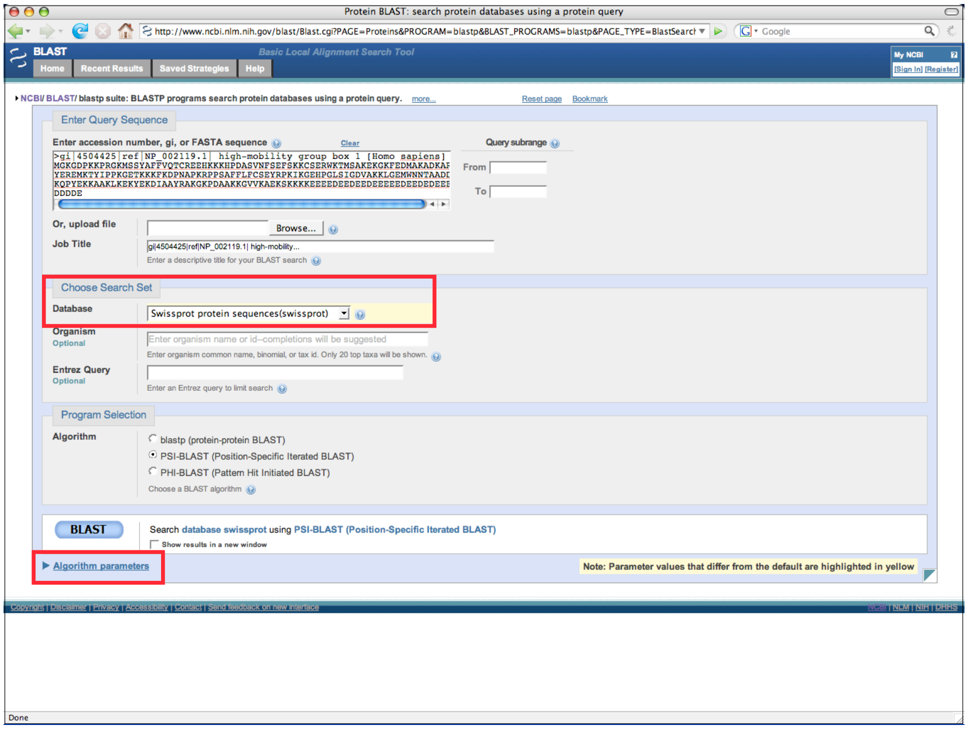
NCBI | NLM | NIH | DHHS
Done



Protein BLAST: search protein databases using a protein query

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch

NCBI/ BLAST/ blastp suite: BLASTP programs search protein databases using a protein query.

Enter Query Sequence

Enter accession number, gi, or FASTA sequence

```
>gi|4504425|ref|NP_002119.1| high-mobility group box 1 [Homo sapiens]
MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFEDMAKADKAF
YEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLFCSEYRPKIKGEHPGLSIGDVAKKLGEMWNNTAADI
KQPYEKKAAKLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKKKKEEEEDEEDEEDEEEEEDEEDEDEEE
DDDDE
```

Query subrange: From ___ To ___

Or, upload file: ___ Browse...

Job Title: gi|4504425|ref|NP_002119.1| high-mobility...

Enter a descriptive title for your BLAST search

Choose Search Set

Database: Swissprot protein sequences(swissprot)

Organism (Optional): Enter organism name or id--completions will be suggested

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Entrez Query (Optional): ___

Enter an Entrez query to limit search

Program Selection

Algorithm:
○ blastp (protein-protein BLAST)
● PSI-BLAST (Position-Specific Iterated BLAST)
○ PHI-BLAST (Pattern Hit Initiated BLAST)

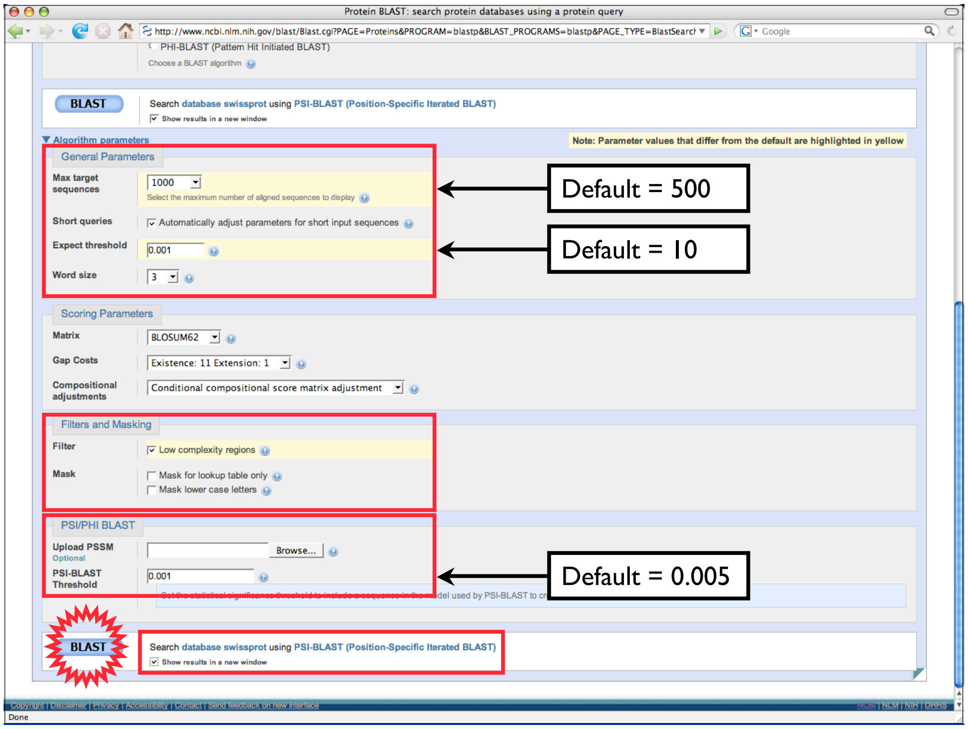
Choose a BLAST algorithm

BLAST — Search database swissprot using PSI-BLAST (Position-Specific Iterated BLAST)

□ Show results in a new window

▸ Algorithm parameters

Note: Parameter values that differ from the default are highlighted in yellow



Algorithm parameters

Note: Parameter values that differ from the default are highlighted in yellow

General Parameters

Max target sequences: 1000 — Default = 500

Select the maximum number of aligned sequences to display

Short queries: ☑ Automatically adjust parameters for short input sequences

Expect threshold: 0.001 — Default = 10

Word size: 3

Scoring Parameters

Matrix: BLOSUM62

Gap Costs: Existence: 11 Extension: 1

Compositional adjustments: Conditional compositional score matrix adjustment

Filters and Masking

Filter: ☑ Low complexity regions

Mask: □ Mask for lookup table only
□ Mask lower case letters

PSI/PHI BLAST

Upload PSSM (Optional): ___ Browse...

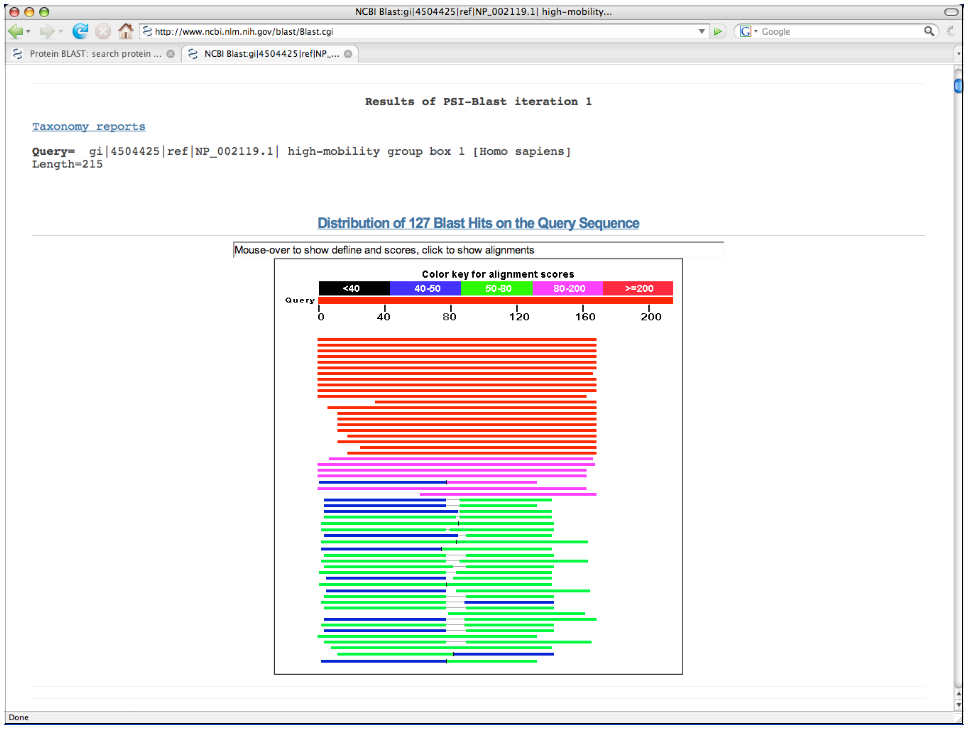
PSI-BLAST Threshold: 0.001 — Default = 0.005

Set the statistical significance threshold to include a sequence in the model used by PSI-BLAST to cr

BLAST — Search database swissprot using PSI-BLAST (Position-Specific Iterated BLAST)

☑ Show results in a new window

PHI-BLAST (Pattern Hit Initiated BLAST)
Choose a BLAST algorithm
Copyright | Disclaimer | Privacy | Accessibility | Contact | Send feedback on new interface
NCBI | NLM | NIH | DHHS
Done


NCBI Blast:gi|4504425|ref|NP_002119.1| high-mobility...
http://www.ncbi.nlm.nih.gov/blast/Blast.cgi
Google
Protein BLAST: search protein ...
NCBI Blast:gi|4504425|ref|NP_...
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
0
40
80
120
160
200
Done

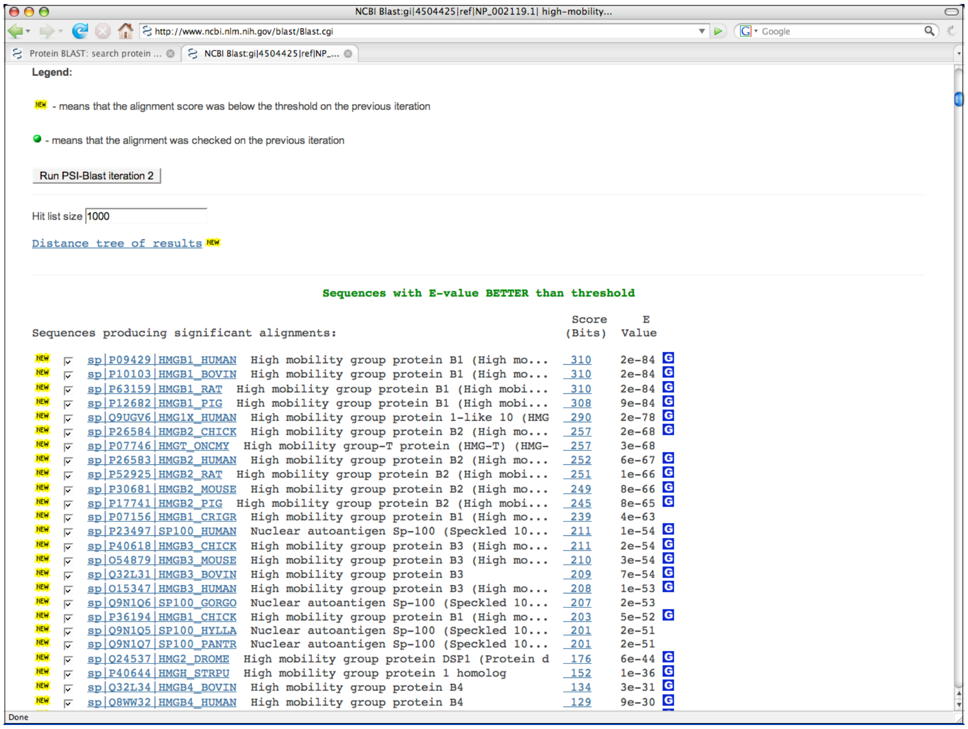
```
Results of PSI-Blast iteration 1

Taxonomy reports

Query=  gi|4504425|ref|NP_002119.1| high-mobility group box 1 [Homo sapiens]
Length=215
```

Distribution of 127 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments


NCBI Blast:gi|4504425|ref|NP_002119.1| high-mobility...
http://www.ncbi.nlm.nih.gov/blast/Blast.cgi
Google
Protein BLAST: search protein ...
NCBI Blast:gi|4504425|ref|NP_...
Run PSI-Blast iteration 2
Done

Legend:

NEW - means that the alignment score was below the threshold on the previous iteration

● - means that the alignment was checked on the previous iteration

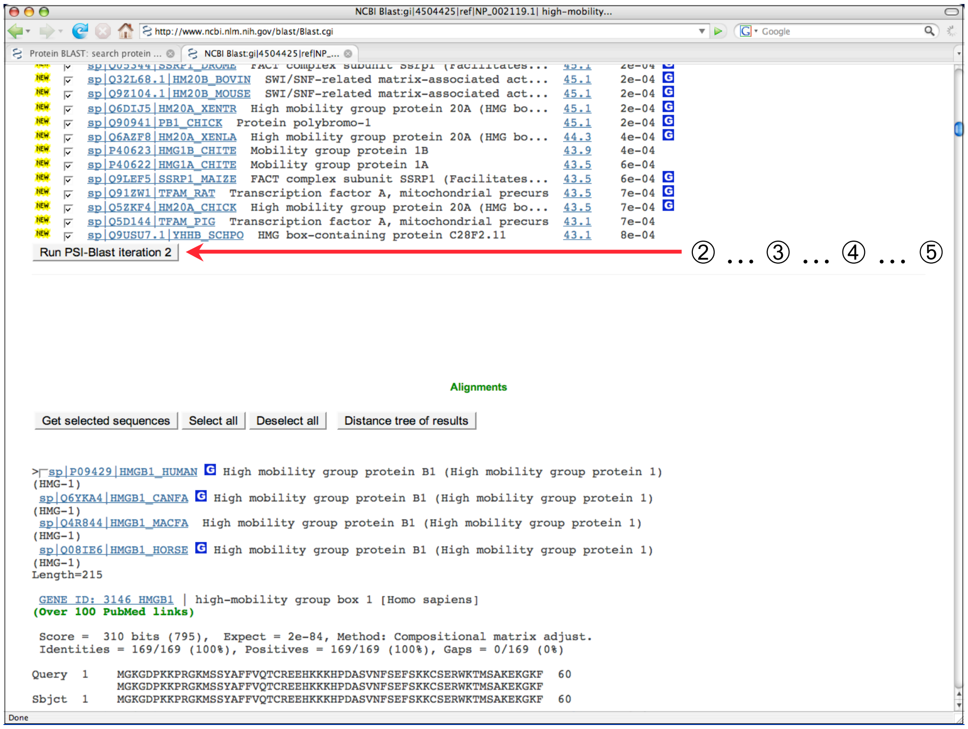
Hit list size 1000

Distance tree of results NEW

```
                         Sequences with E-value BETTER than threshold

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

NEW ☑ sp|P09429|HMGB1_HUMAN  High mobility group protein B1 (High mo...   310    2e-84 G
NEW ☑ sp|P10103|HMGB1_BOVIN  High mobility group protein B1 (High mo...   310    2e-84 G
NEW ☑ sp|P63159|HMGB1_RAT  High mobility group protein B1 (High mobi...   310    2e-84 G
NEW ☑ sp|P12682|HMGB1_PIG  High mobility group protein B1 (High mobi...   308    9e-84 G
NEW ☑ sp|Q9UGV6|HMG1X_HUMAN  High mobility group protein 1-like 10 (HMG   290    2e-78 G
NEW ☑ sp|P26584|HMGB2_CHICK  High mobility group protein B2 (High mo...   257    2e-68 G
NEW ☑ sp|P07746|HMGT_ONCMY  High mobility group-T protein (HMG-T) (HMG-   257    3e-68
NEW ☑ sp|P26583|HMGB2_HUMAN  High mobility group protein B2 (High mo...   252    6e-67 G
NEW ☑ sp|P52925|HMGB2_RAT  High mobility group protein B2 (High mobi...   251    1e-66 G
NEW ☑ sp|P30681|HMGB2_MOUSE  High mobility group protein B2 (High mo...   249    8e-66 G
NEW ☑ sp|P17741|HMGB2_PIG  High mobility group protein B2 (High mobi...   245    8e-65 G
NEW ☑ sp|P07156|HMGB1_CRIGR  High mobility group protein B1 (High mo...   239    4e-63
NEW ☑ sp|P23497|SP100_HUMAN  Nuclear autoantigen Sp-100 (Speckled 10...   211    1e-54 G
NEW ☑ sp|P40618|HMGB3_CHICK  High mobility group protein B3 (High mo...   211    2e-54 G
NEW ☑ sp|O54879|HMGB3_MOUSE  High mobility group protein B3 (High mo...   210    3e-54 G
NEW ☑ sp|Q32L31|HMGB3_BOVIN  High mobility group protein B3               209    7e-54 G
NEW ☑ sp|O15347|HMGB3_HUMAN  High mobility group protein B3 (High mo...   208    1e-53 G
NEW ☑ sp|Q9N1Q6|SP100_GORGO  Nuclear autoantigen Sp-100 (Speckled 10...   207    2e-53
NEW ☑ sp|P36194|HMGB1_CHICK  High mobility group protein B1 (High mo...   203    5e-52 G
NEW ☑ sp|Q9N1Q5|SP100_HYLLA  Nuclear autoantigen Sp-100 (Speckled 10...   201    2e-51
NEW ☑ sp|Q9N1Q7|SP100_PANTR  Nuclear autoantigen Sp-100 (Speckled 10...   201    2e-51
NEW ☑ sp|Q24537|HMG2_DROME  High mobility group protein DSP1 (Protein d   176    6e-44 G
NEW ☑ sp|P40644|HMGH_STRPU  High mobility group protein 1 homolog         152    1e-36 G
NEW ☑ sp|Q32L34|HMGB4_BOVIN  High mobility group protein B4               134    3e-31 G
NEW ☑ sp|Q8WW32|HMGB4_HUMAN  High mobility group protein B4               129    9e-30 G
```



NCBI Blast:gi|4504425|ref|NP_002119.1| high-mobility...

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

Google
Protein BLAST: search protein ...
NCBI Blast:gi|4504425|ref|NP_...

```
NEW ☑ sp|Q05344|SSRP1_DROME  FACT complex subunit SSRP1 (Facilitates...  45.1   2e-04 G
NEW ☑ sp|Q32L68.1|HM20B_BOVIN  SWI/SNF-related matrix-associated act...  45.1   2e-04 G
NEW ☑ sp|Q9Z104.1|HM20B_MOUSE  SWI/SNF-related matrix-associated act...  45.1   2e-04 G
NEW ☑ sp|Q6DIJ5|HM20A_XENTR  High mobility group protein 20A (HMG bo...  45.1   2e-04 G
NEW ☑ sp|Q90941|PB1_CHICK  Protein polybromo-1                           45.1   2e-04 G
NEW ☑ sp|Q6AZF8|HM20A_XENLA  High mobility group protein 20A (HMG bo...  44.3   4e-04 G
NEW ☑ sp|P40623|HMG1B_CHITE  Mobility group protein 1B                   43.9   4e-04
NEW ☑ sp|P40622|HMG1A_CHITE  Mobility group protein 1A                   43.5   6e-04
NEW ☑ sp|Q9LEF5|SSRP1_MAIZE  FACT complex subunit SSRP1 (Facilitates...  43.5   6e-04 G
NEW ☑ sp|Q91ZW1|TFAM_RAT  Transcription factor A, mitochondrial precurs  43.5   7e-04 G
NEW ☑ sp|Q5ZKF4|HM20A_CHICK  High mobility group protein 20A (HMG bo...  43.5   7e-04 G
NEW ☑ sp|Q5D144|TFAM_PIG  Transcription factor A, mitochondrial precurs  43.1   7e-04
NEW ☑ sp|Q9USU7.1|YHHB_SCHPO  HMG box-containing protein C28F2.11        43.1   8e-04
```

② … ③ … ④ … ⑤

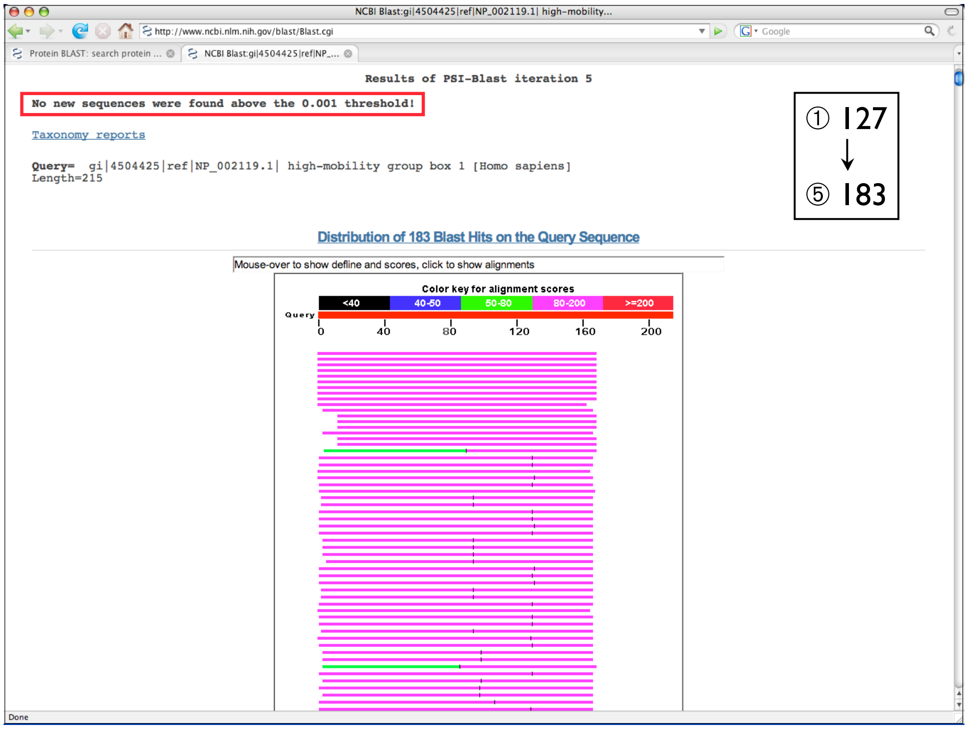
Alignments

```
>sp|P09429|HMGB1_HUMAN G High mobility group protein B1 (High mobility group protein 1)
(HMG-1)
 sp|Q6YKA4|HMGB1_CANFA G High mobility group protein B1 (High mobility group protein 1)
(HMG-1)
 sp|Q4R844|HMGB1_MACFA  High mobility group protein B1 (High mobility group protein 1)
(HMG-1)
 sp|Q08IE6|HMGB1_HORSE G High mobility group protein B1 (High mobility group protein 1)
(HMG-1)
Length=215

 GENE ID: 3146 HMGB1 | high-mobility group box 1 [Homo sapiens]
(Over 100 PubMed links)

 Score =  310 bits (795),  Expect = 2e-84, Method: Compositional matrix adjust.
 Identities = 169/169 (100%), Positives = 169/169 (100%), Gaps = 0/169 (0%)

Query  1    MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKF  60
            MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKF
Sbjct  1    MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKF  60
```



NCBI Blast:gi|4504425|ref|NP_002119.1| high-mobility...

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

Google
Protein BLAST: search protein ...
NCBI Blast:gi|4504425|ref|NP_...

Results of PSI-Blast iteration 5

No new sequences were found above the 0.001 threshold!

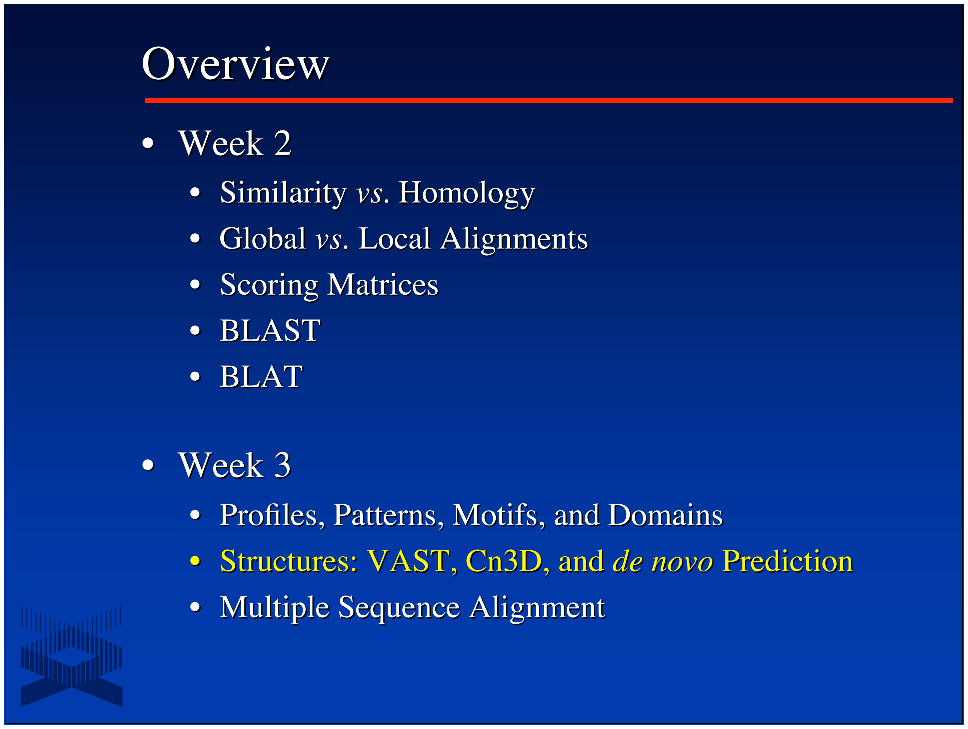
Taxonomy reports

```
Query=  gi|4504425|ref|NP_002119.1| high-mobility group box 1 [Homo sapiens]
Length=215
```

① 127 ↓ ⑤ 183

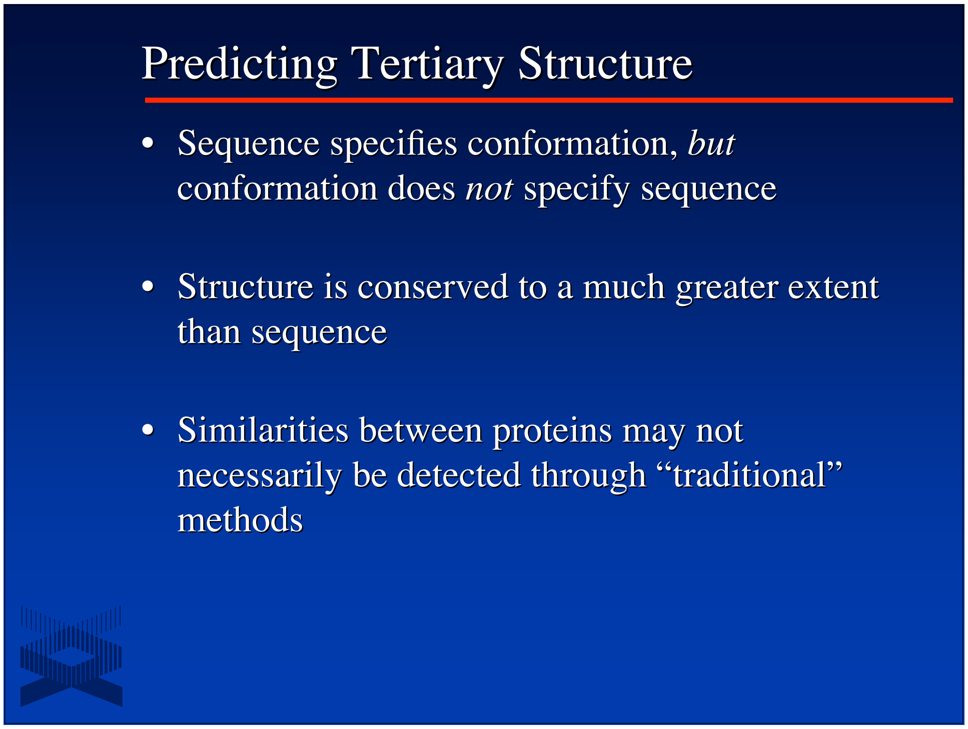
Distribution of 183 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments



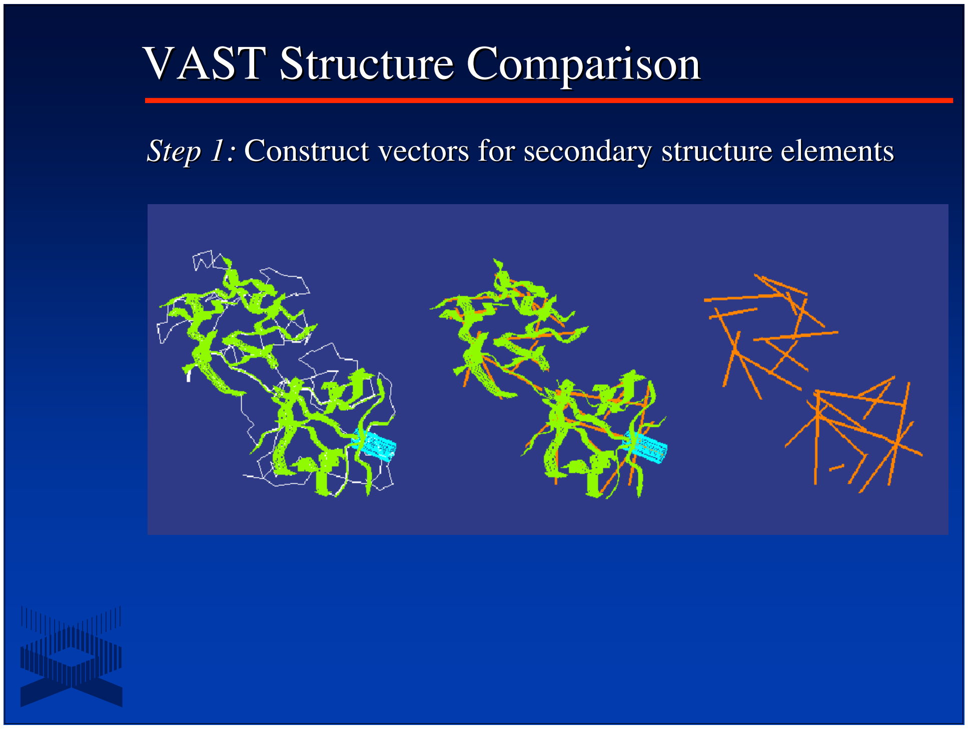
Overview

- Week 2
  - Similarity *vs.* Homology
  - Global *vs.* Local Alignments
  - Scoring Matrices
  - BLAST
  - BLAT
- Week 3
  - Profiles, Patterns, Motifs, and Domains
  - Structures: VAST, Cn3D, and *de novo* Prediction
  - Multiple Sequence Alignment



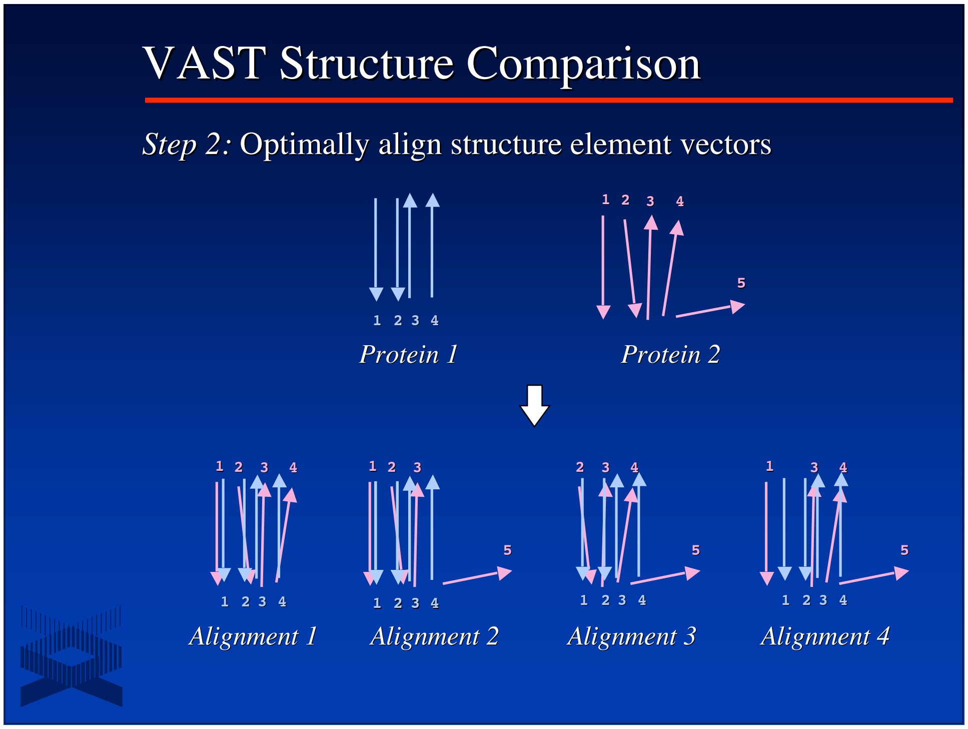
Predicting Tertiary Structure

- Sequence specifies conformation, *but* conformation does *not* specify sequence
- Structure is conserved to a much greater extent than sequence
- Similarities between proteins may not necessarily be detected through “traditional” methods



VAST Structure Comparison

Step 1: Construct vectors for secondary structure elements



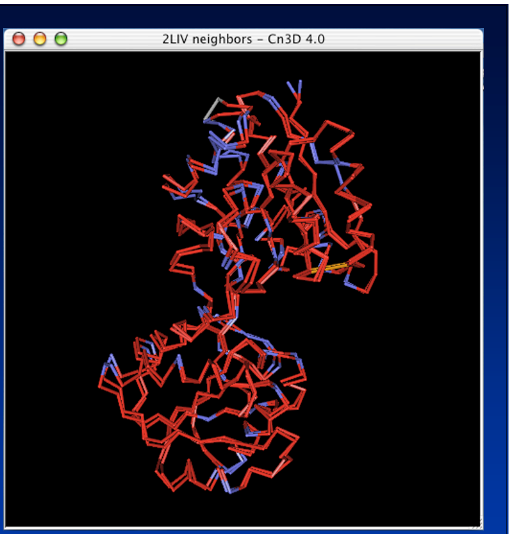
VAST Structure Comparison

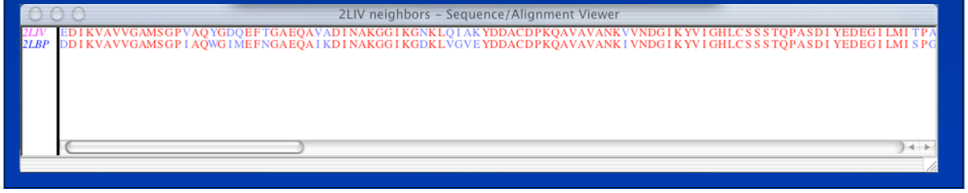
Step 2: Optimally align structure element vectors

Cn3D Viewer

Rendering: Tubes

Coloring: Identity
Red – matches
Blue – mismatches

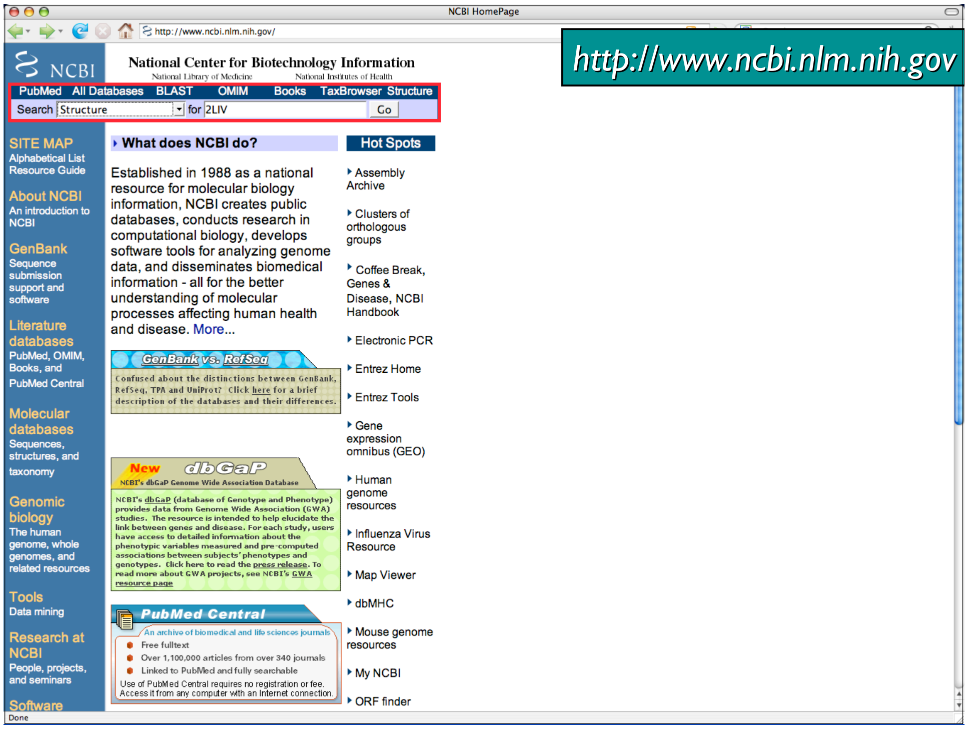




```
2LIV  EDIKVAVVGAMSGPVAQYGDQEFTGAEQAVADINAKGGIKGNKLQIAKYDDACDPKQAVAVANKVVNDGIKYVIGHLCSSSTQPASDIYEDEGILMITPA
2LBP  DDIKVAVVGAMSGPIAQWGIMEFNGAEQAIKDINAKGGIKGDKLVGVEYDDACDPKQAVAVANKIVNDGIKYVIGHLCSSSTQPASDIYEDEGILMISPG
```

VAST Shortcomings

- Not the best method for determining structural similarities
- Reducing a structure to a series of vectors necessarily results in a loss of information (less confidence in prediction)
- Regardless of the “simplicity” of the method, provides a simple and fast first answer to the question of structural similarity



http://www.ncbi.nlm.nih.gov

NCBI HomePage

http://www.ncbi.nlm.nih.gov/

National Center for Biotechnology Information
National Library of Medicine National Institutes of Health

PubMed All Databases BLAST OMIM Books TaxBrowser Structure

Search Structure for 2LIV Go

SITE MAP
Alphabetical List
Resource Guide

About NCBI
An introduction to NCBI

GenBank
Sequence submission support and software

Literature databases
PubMed, OMIM, Books, and PubMed Central

Molecular databases
Sequences, structures, and taxonomy

Genomic biology
The human genome, whole genomes, and related resources

Tools
Data mining

Research at NCBI
People, projects, and seminars

Software

What does NCBI do?

Established in 1988 as a national resource for molecular biology information, NCBI creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information - all for the better understanding of molecular processes affecting human health and disease. More...

GenBank vs. RefSeq

Confused about the distinctions between GenBank, RefSeq, TPA and UniProt? Click here for a brief description of the databases and their differences.

New dbGaP
NCBI's dbGaP Genome Wide Association Database

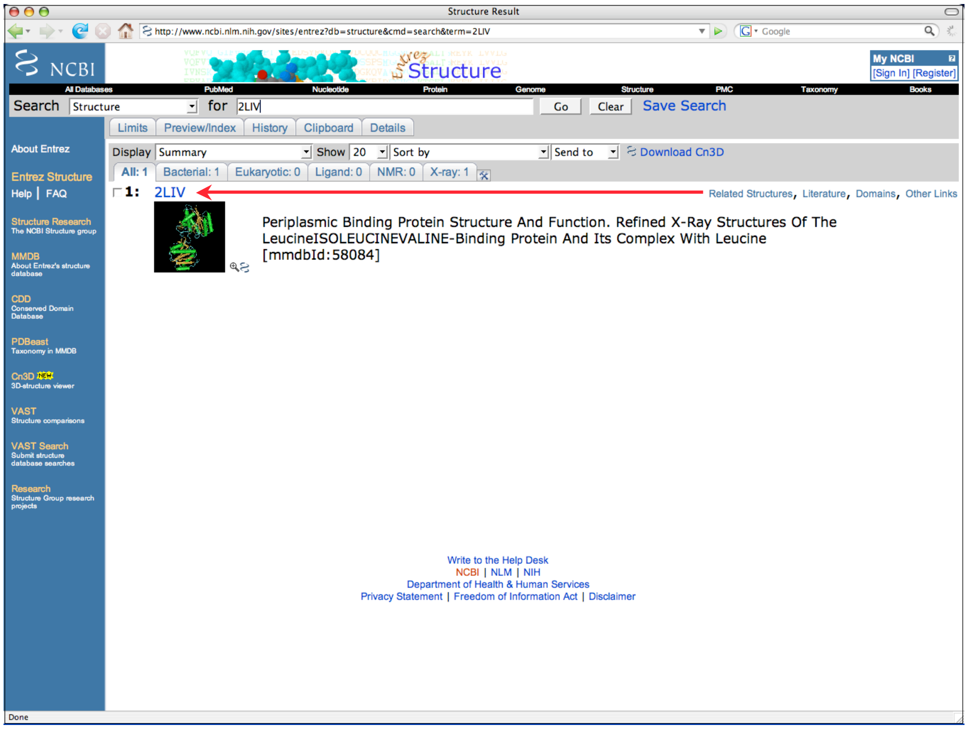
NCBI's dbGaP (database of Genotype and Phenotype) provides data from Genome Wide Association (GWA) studies. The resource is intended to help elucidate the link between genes and disease. For each study, users have access to detailed information about the phenotypic variables measured and pre-computed associations between subjects' phenotypes and genotypes. Click here to read the press release. To read more about GWA projects, see NCBI's GWA resource page

PubMed Central
An archive of biomedical and life sciences journals
- Free fulltext
- Over 1,100,000 articles from over 340 journals
- Linked to PubMed and fully searchable

Use of PubMed Central requires no registration or fee. Access it from any computer with an Internet connection.

Hot Spots
- Assembly Archive
- Clusters of orthologous groups
- Coffee Break, Genes & Disease, NCBI Handbook
- Electronic PCR
- Entrez Home
- Entrez Tools
- Gene expression omnibus (GEO)
- Human genome resources
- Influenza Virus Resource
- Map Viewer
- dbMHC
- Mouse genome resources
- My NCBI
- ORF finder

Done



Structure Result

http://www.ncbi.nlm.nih.gov/sites/entrez?db=structure&cmd=search&term=2LIV

Google

NCBI Entrez Structure

My NCBI [Sign In] [Register]

All Databases PubMed Nucleotide Protein Genome Structure PMC Taxonomy Books

Search Structure for 2LIV Go Clear Save Search

Limits Preview/Index History Clipboard Details

Display Summary Show 20 Sort by Send to Download Cn3D

All: 1 Bacterial: 1 Eukaryotic: 0 Ligand: 0 NMR: 0 X-ray: 1

1: 2LIV Related Structures, Literature, Domains, Other Links

Periplasmic Binding Protein Structure And Function. Refined X-Ray Structures Of The LeucineISOLEUCINEVALINE-Binding Protein And Its Complex With Leucine [mmdbId:58084]

About Entrez

Entrez Structure
Help | FAQ

Structure Research
The NCBI Structure group

MMDB
About Entrez's structure database

CDD
Conserved Domain Database

PDBeast
Taxonomy in MMDB

Cn3D NEW
3D-structure viewer

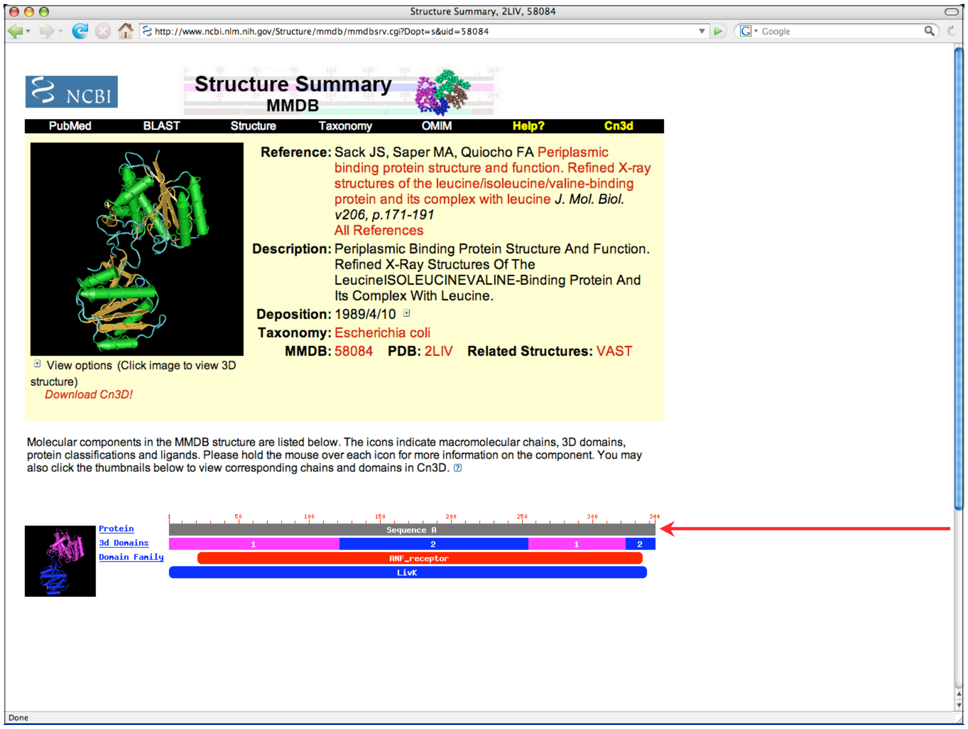
VAST
Structure comparisons

VAST Search
Submit structure database searches

Research
Structure Group research projects

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Privacy Statement | Freedom of Information Act | Disclaimer

Done



Structure Summary, 2LIV, 58084
http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?Dopt=s&uid=58084
NCBI
Structure Summary
MMDB
PubMed
BLAST
Structure
Taxonomy
OMIM
Help?
Cn3d

Reference: Sack JS, Saper MA, Quiocho FA Periplasmic binding protein structure and function. Refined X-ray structures of the leucine/isoleucine/valine-binding protein and its complex with leucine *J. Mol. Biol. v206, p.171-191*
All References

Description: Periplasmic Binding Protein Structure And Function. Refined X-Ray Structures Of The LeucineISOLEUCINEVALINE-Binding Protein And Its Complex With Leucine.

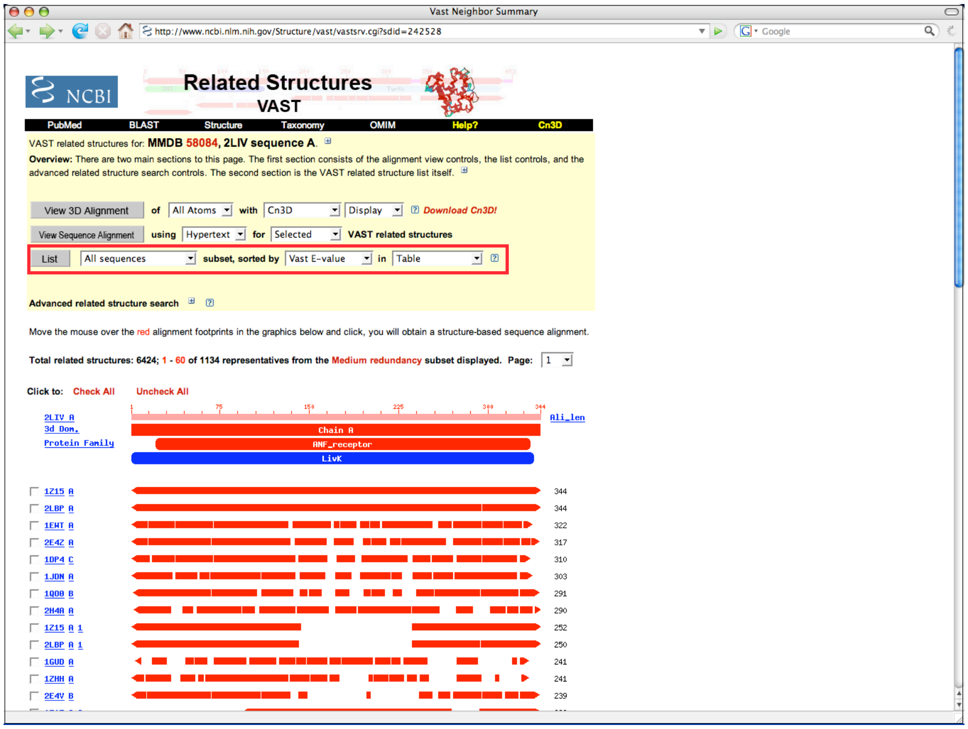
Deposition: 1989/4/10 ⊞

Taxonomy: Escherichia coli

MMDB: 58084 **PDB:** 2LIV **Related Structures:** VAST

⊞ View options (Click image to view 3D structure)
Download Cn3D!

Molecular components in the MMDB structure are listed below. The icons indicate macromolecular chains, 3D domains, protein classifications and ligands. Please hold the mouse over each icon for more information on the component. You may also click the thumbnails below to view corresponding chains and domains in Cn3D. ?



VAST related structures for: **MMDB 58084, 2LIV sequence A.** ⊞

Overview: There are two main sections to this page. The first section consists of the alignment view controls, the list controls, and the advanced related structure search controls. The second section is the VAST related structure list itself. ⊞

View 3D Alignment **of** All Atoms **with** Cn3D Display ? *Download Cn3D!*

View Sequence Alignment **using** Hypertext **for** Selected **VAST related structures**

List All sequences **subset, sorted by** Vast E-value **in** Table ?

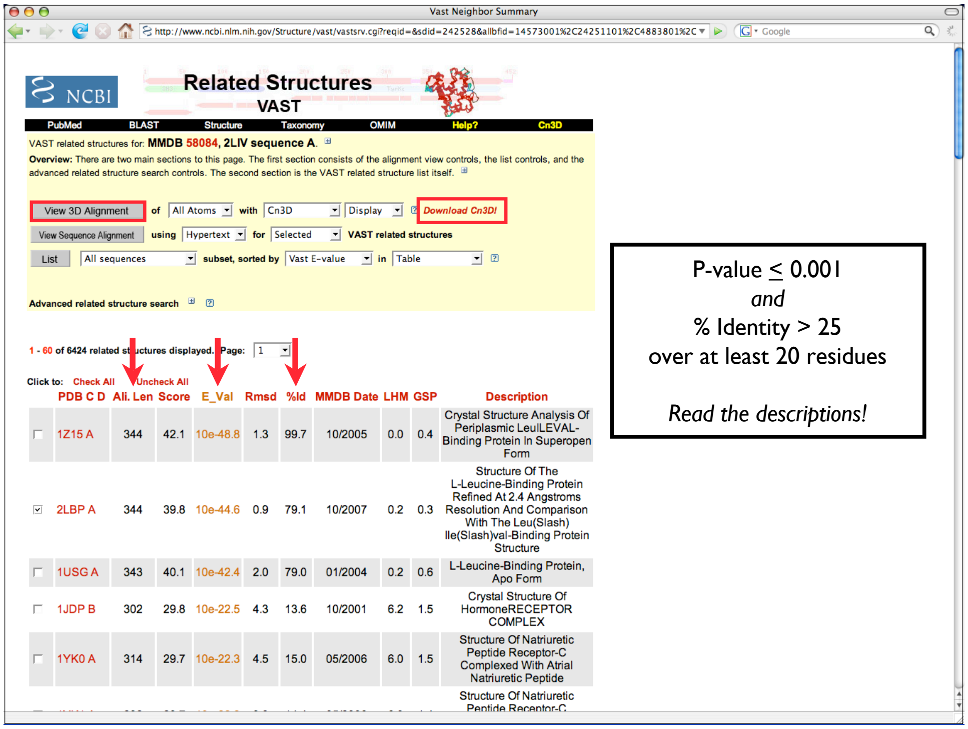
Advanced related structure search ⊞ ?

Move the mouse over the red alignment footprints in the graphics below and click, you will obtain a structure-based sequence alignment.

Total related structures: 6424; 1 - 60 of 1134 representatives from the Medium redundancy subset displayed. Page: 1

Click to: Check All Uncheck All

□ 1Z15 A 344
□ 2LBP A 344
□ 1EWT A 322
□ 2E4Z A 317
□ 1DP4 C 310
□ 1JDN A 303
□ 1QO8 B 291
□ 2H4A A 290
□ 1Z15 A 1 252
□ 2LBP A 1 250
□ 1GUD A 241
□ 1ZHH A 241
□ 2E4V B 239


Vast Neighbor Summary
http://www.ncbi.nlm.nih.gov/Structure/vast/vastsrv.cgi?reqid=&sdid=242528&allbfid=14573001%2C24251101%2C4883801%2C
Google
NCBI
Related Structures
VAST
PubMed
BLAST
Structure
Taxonomy
OMIM
Help?
Cn3D

VAST related structures for: **MMDB 58084, 2LIV sequence A.** ⊞

Overview: There are two main sections to this page. The first section consists of the alignment view controls, the list controls, and the advanced related structure search controls. The second section is the VAST related structure list itself. ⊞

View 3D Alignment of All Atoms with Cn3D Display ? Download Cn3D!

View Sequence Alignment using Hypertext for Selected VAST related structures

List All sequences subset, sorted by Vast E-value in Table ?

Advanced related structure search ⊞ ?

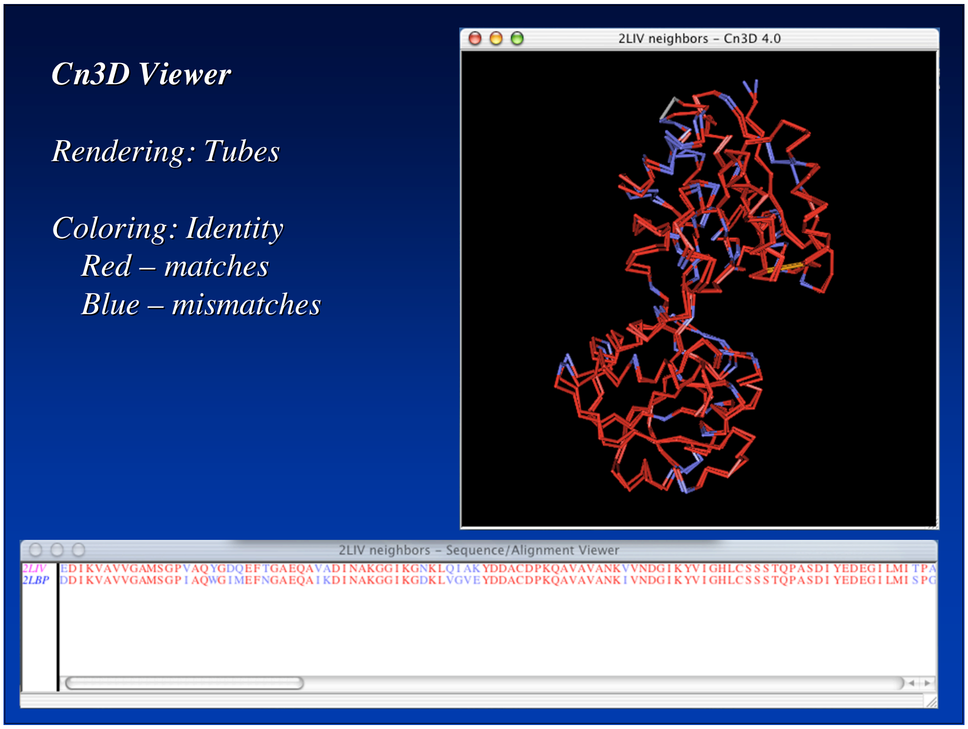
1 - 60 of 6424 related structures displayed. Page: 1

Click to: Check All Uncheck All

	PDB C D	Ali. Len	Score	E_Val	Rmsd	%Id	MMDB Date	LHM	GSP	Description
□	1Z15 A	344	42.1	10e-48.8	1.3	99.7	10/2005	0.0	0.4	Crystal Structure Analysis Of Periplasmic LeuILEVAL-Binding Protein In Superopen Form
☑	2LBP A	344	39.8	10e-44.6	0.9	79.1	10/2007	0.2	0.3	Structure Of The L-Leucine-Binding Protein Refined At 2.4 Angstroms Resolution And Comparison With The Leu(Slash) Ile(Slash)val-Binding Protein Structure
□	1USG A	343	40.1	10e-42.4	2.0	79.0	01/2004	0.2	0.6	L-Leucine-Binding Protein, Apo Form
□	1JDP B	302	29.8	10e-22.5	4.3	13.6	10/2001	6.2	1.5	Crystal Structure Of HormoneRECEPTOR COMPLEX
□	1YK0 A	314	29.7	10e-22.3	4.5	15.0	05/2006	6.0	1.5	Structure Of Natriuretic Peptide Receptor-C Complexed With Atrial Natriuretic Peptide
										Structure Of Natriuretic Peptide Receptor-C

P-value ≤ 0.001
and
% Identity > 25
over at least 20 residues

Read the descriptions!

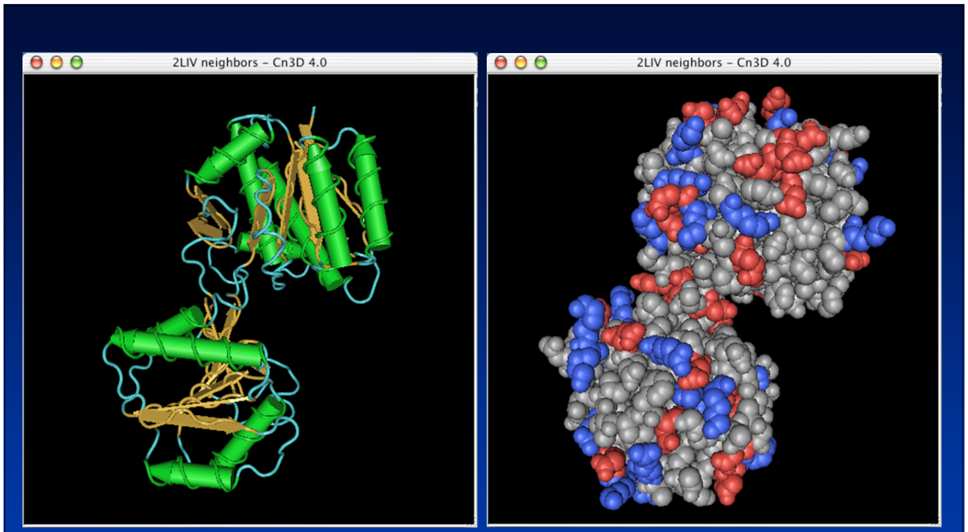

2LIV neighbors – Cn3D 4.0
2LIV neighbors – Sequence/Alignment Viewer

Cn3D Viewer

Rendering: Tubes

Coloring: Identity
Red – matches
Blue – mismatches

```
2LIV EDIKVAVVGAMSGPVAQYGDQEFTGAEQAVADINAKGGIKGNKLQIAKYDDACDPKQAVAVANKVVNDGIKYVIGHLCSSSTQPASDIYEDEGILMITPA
2LBP DDIKVAVVGAMSGPIAQWGIMEFNGAEQAIKDINAKGGIKGDKLVGVEYDDACDPKQAVAVANKIVNDGIKYVIGHLCSSSTQPASDIYEDEGILMISPG
```



Worms	**Rendering**	Spacefill
Secondary Structure	**Coloring**	Charge

Current Protocols in Bioinformatics

CPBI Unit 1.3
Entrez and Cn3D

Searching the NCBI Databases Using Entrez

UNIT 1.3

One of the most widely used interfaces for the retrieval of information from biological databases is the NCBI Entrez system. Entrez capitalizes on the fact that there are pre-existing, logical relationships between the individual entries found in numerous public databases. For example, a paper in MEDLINE (or, more properly, PubMed) may describe the sequencing of a gene whose sequence appears in GenBank. The nucleotide sequence, in turn, may code for a protein product whose sequence is stored in the protein databases. The three-dimensional structure of that protein may be known, and the coordinates for that structure may appear in the structure database. Finally, the gene may have been mapped to a specific region of a given chromosome, with that information being stored in a mapping database. The existence of such natural connections, mostly biological in nature, argued for the development of a method through which all the information about a particular biological entity could be found without having to sequentially visit and query disparate databases.

Basic Protocols 1 and 2 describe simple, text-based searches, illustrating the types of information that can be retrieved through the Entrez system; Basic Protocol 2 also illustrates the use of Cn3D, a viewer that is used to visualize three-dimensional structures. The Alternate Protocol builds upon Basic Protocol 1, using additional, built-in features of the Entrez system, as well as alternative ways of issuing the initial query. The Support Protocol reviews how to save frequently issued queries.

QUERYING ENTREZ

BASIC PROTOCOL 1

The Entrez Web interface is located at *http://www.ncbi.nlm.nih.gov/Entrez*. Most of the Web pages at the NCBI Web site provide a direct link to Entrez, either in a blue bar running across the top of the page or in the left-hand sidebar. Entrez queries can also be issued from the NCBI home page (*http://www.ncbi.nlm.nih.gov*). The best way to illustrate the integrated nature of the Entrez system and to drive home the power of neighboring (see Commentary) is by considering three biological examples, described in Basic Protocols 1 and 2 and the Alternate Protocol.

Necessary Resources

Software

An up-to-date Web browser, such as Netscape Communicator, MS Internet Explorer, Apple Safari, or Mozilla Firefox

Select and search an Entrez database

1. Begin at the Entrez home page (*http://www.ncbi.nlm.nih.gov/Entrez*).
2. In the "Search across databases" text box, enter the following:

```
DCC AND "Vogelstein B"
```

Using Boolean operators such as AND, OR, and NOT is the simplest way to query the Entrez system. Note that all Boolean operators must be capitalized for the query to return the expected results.

Contributed by Andreas D. Baxevanis
Current Protocols in Bioinformatics (2006) 1.3.1-1.3.24
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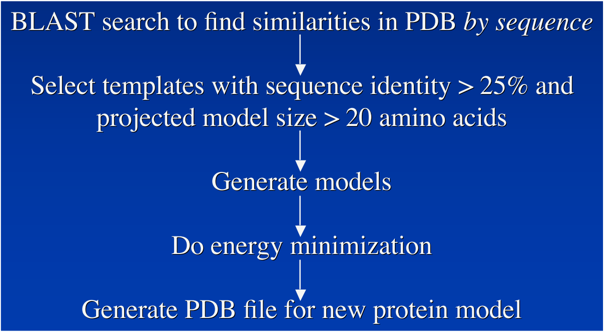
Using Biological Databases
1.3.1
Supplement 13

http://nihlibrary.nih.gov
Search "Online Journals" for "Current Protocols in Bioinformatics"

SWISS-MODEL

- Automated comparative protein modelling server
- Web front-end at *http://www.expasy.org/swissmod*
- Results returned by E-mail



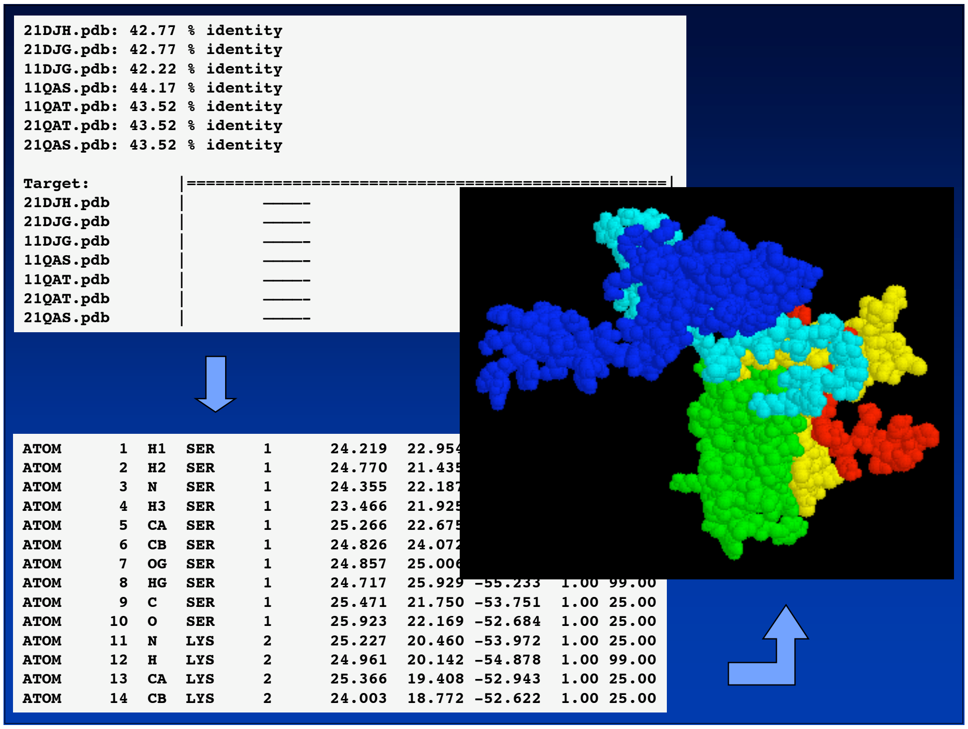
BLAST search to find similarities in PDB *by sequence*

Select templates with sequence identity > 25% and projected model size > 20 amino acids

Generate models

Do energy minimization

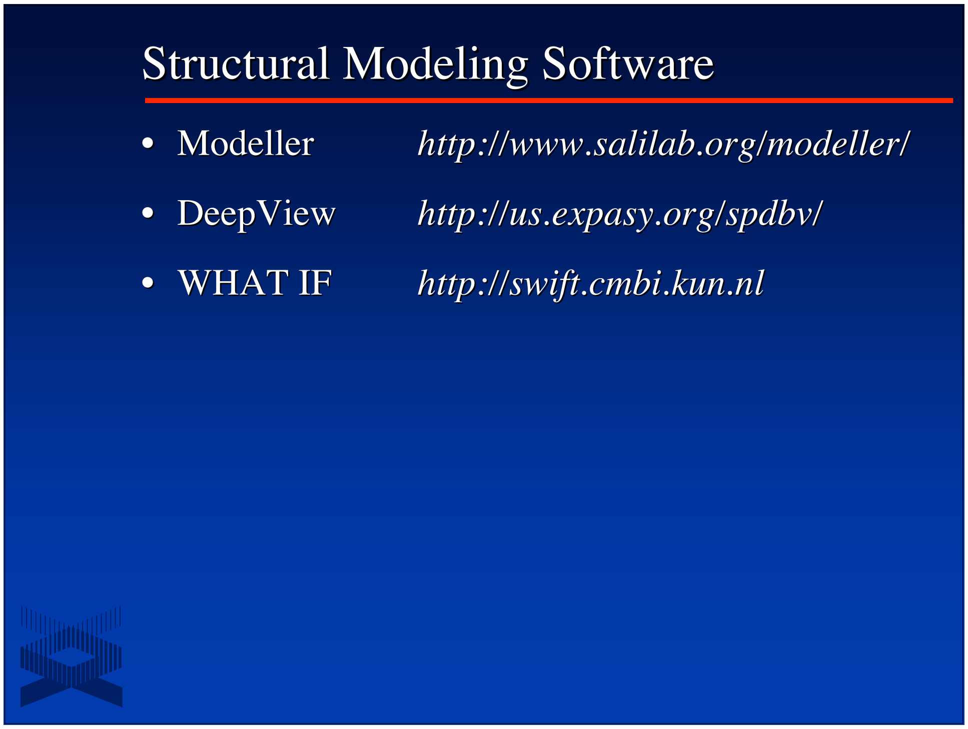
Generate PDB file for new protein model



```
21DJH.pdb: 42.77 % identity
21DJG.pdb: 42.77 % identity
11DJG.pdb: 42.22 % identity
11QAS.pdb: 44.17 % identity
11QAT.pdb: 43.52 % identity
21QAT.pdb: 43.52 % identity
21QAS.pdb: 43.52 % identity

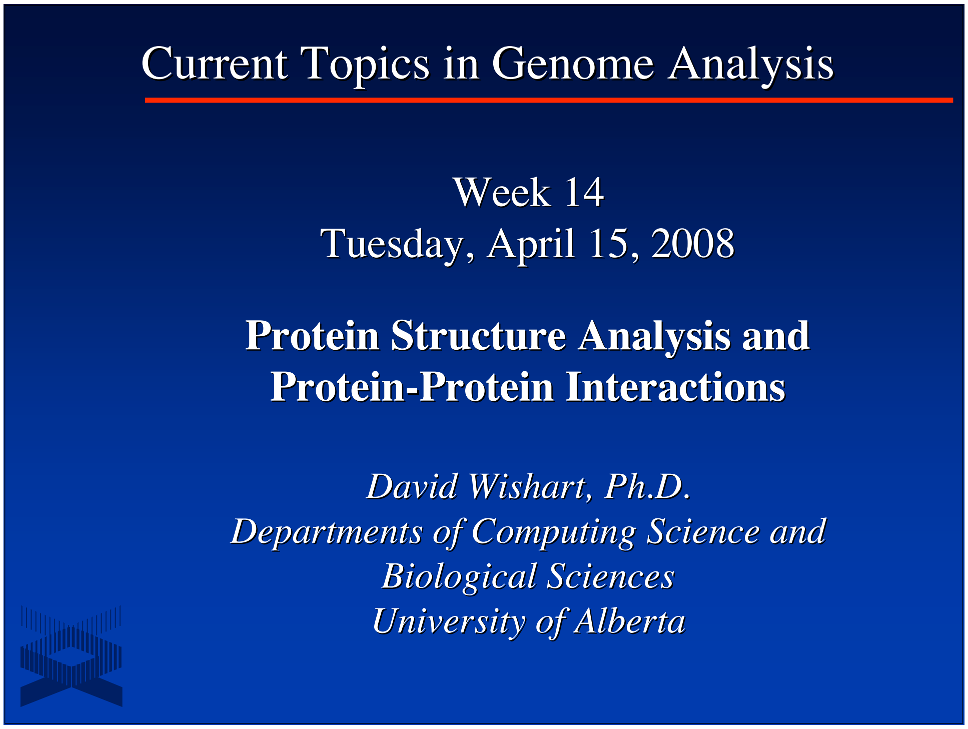
Target:          |==================================================|
21DJH.pdb        |        -----
21DJG.pdb        |        -----
11DJG.pdb        |        -----
11QAS.pdb        |        -----
11QAT.pdb        |        -----
21QAT.pdb        |        -----
21QAS.pdb        |        -----
```

```
ATOM      1  H1  SER     1      24.219  22.954
ATOM      2  H2  SER     1      24.770  21.435
ATOM      3  N   SER     1      24.355  22.187
ATOM      4  H3  SER     1      23.466  21.925
ATOM      5  CA  SER     1      25.266  22.675
ATOM      6  CB  SER     1      24.826  24.072
ATOM      7  OG  SER     1      24.857  25.006
ATOM      8  HG  SER     1      24.717  25.929 -55.233  1.00 99.00
ATOM      9  C   SER     1      25.471  21.750 -53.751  1.00 25.00
ATOM     10  O   SER     1      25.923  22.169 -52.684  1.00 25.00
ATOM     11  N   LYS     2      25.227  20.460 -53.972  1.00 25.00
ATOM     12  H   LYS     2      24.961  20.142 -54.878  1.00 99.00
ATOM     13  CA  LYS     2      25.366  19.408 -52.943  1.00 25.00
ATOM     14  CB  LYS     2      24.003  18.772 -52.622  1.00 25.00
```



Structural Modeling Software

- Modeller *http://www.salilab.org/modeller/*
- DeepView *http://us.expasy.org/spdbv/*
- WHAT IF *http://swift.cmbi.kun.nl*

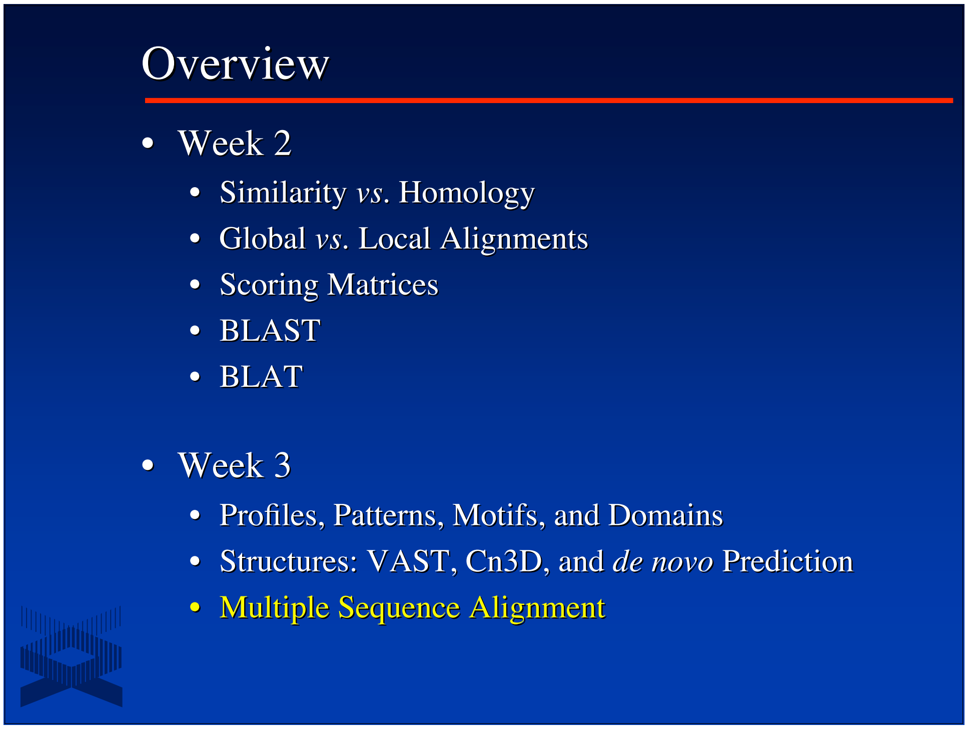


Current Topics in Genome Analysis

Week 14
Tuesday, April 15, 2008

Protein Structure Analysis and Protein-Protein Interactions

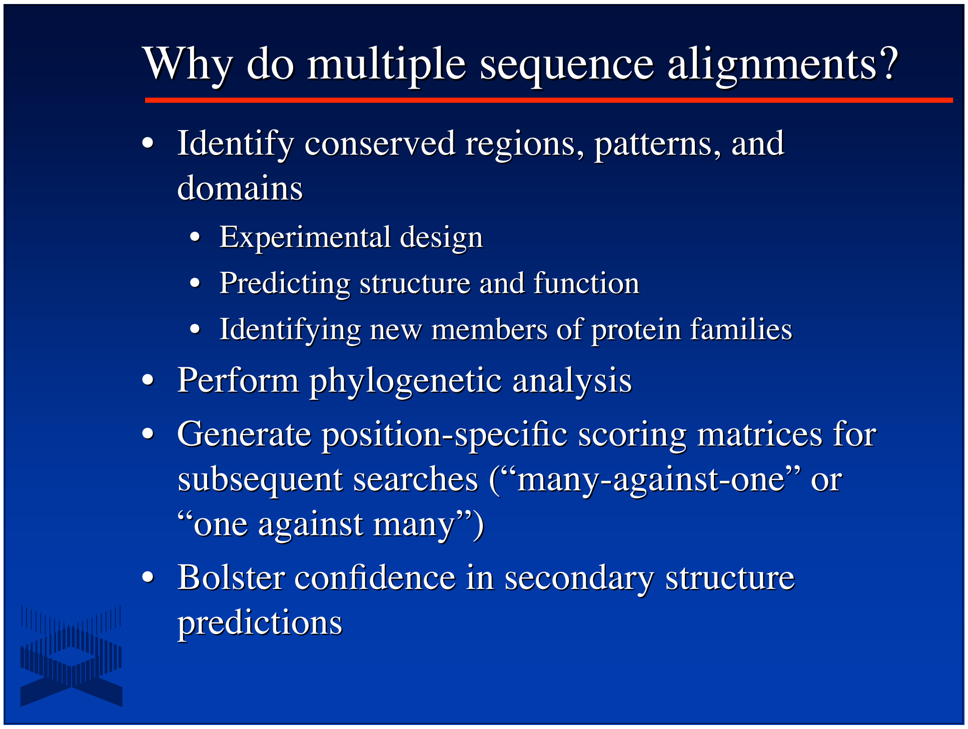
David Wishart, Ph.D.
Departments of Computing Science and Biological Sciences
University of Alberta



Overview

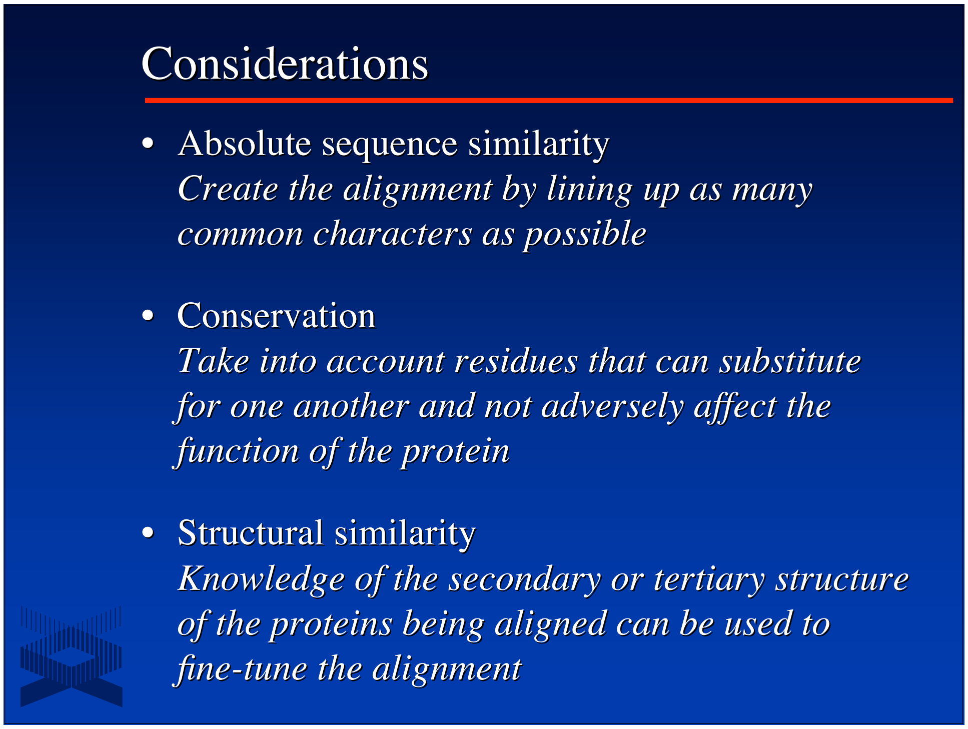
- Week 2
  - Similarity *vs*. Homology
  - Global *vs*. Local Alignments
  - Scoring Matrices
  - BLAST
  - BLAT

- Week 3
  - Profiles, Patterns, Motifs, and Domains
  - Structures: VAST, Cn3D, and *de novo* Prediction
  - Multiple Sequence Alignment



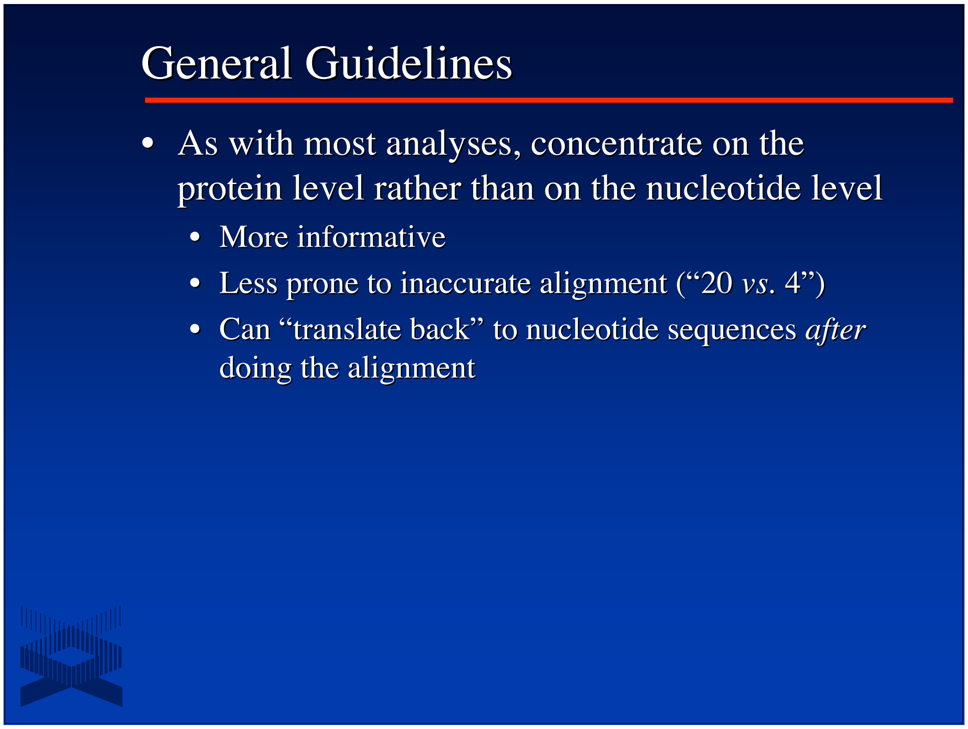
Why do multiple sequence alignments?

- Identify conserved regions, patterns, and domains
  - Experimental design
  - Predicting structure and function
  - Identifying new members of protein families
- Perform phylogenetic analysis
- Generate position-specific scoring matrices for subsequent searches (“many-against-one” or “one against many”)
- Bolster confidence in secondary structure predictions



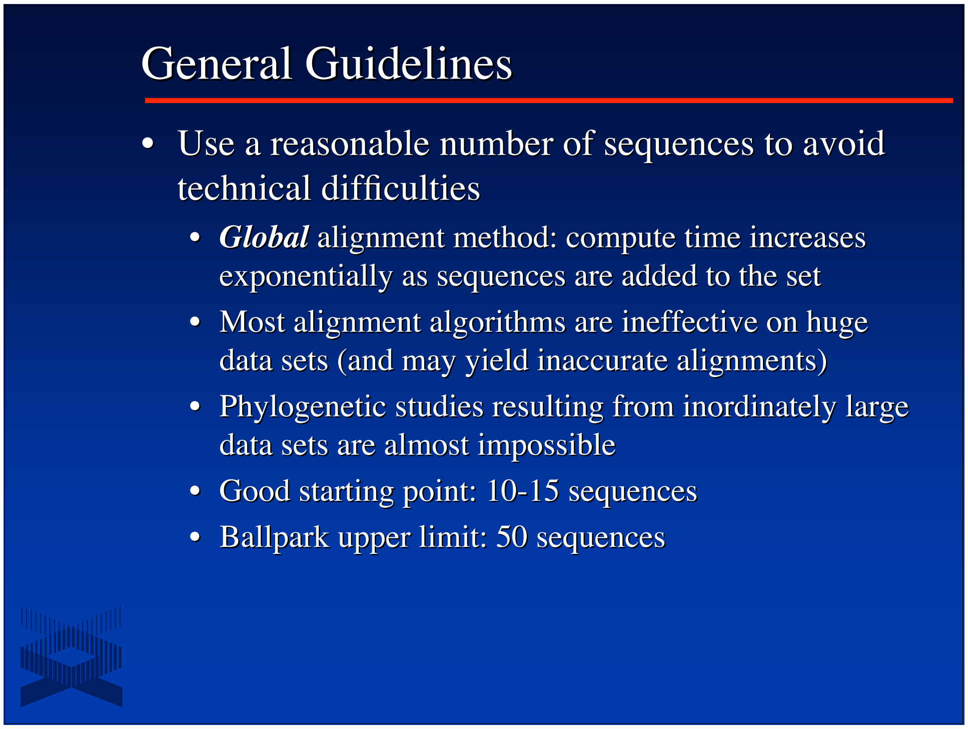
Considerations

- Absolute sequence similarity
  Create the alignment by lining up as many common characters as possible
- Conservation
  Take into account residues that can substitute for one another and not adversely affect the function of the protein
- Structural similarity
  Knowledge of the secondary or tertiary structure of the proteins being aligned can be used to fine-tune the alignment



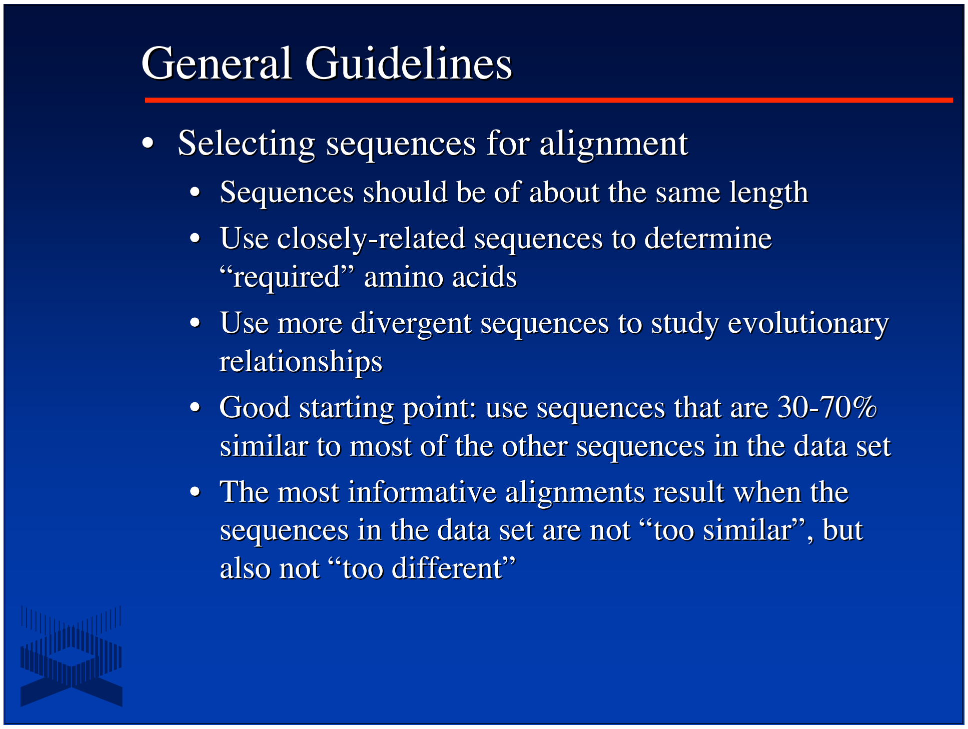
General Guidelines

- As with most analyses, concentrate on the protein level rather than on the nucleotide level
  - More informative
  - Less prone to inaccurate alignment ("20 *vs.* 4")
  - Can "translate back" to nucleotide sequences *after* doing the alignment



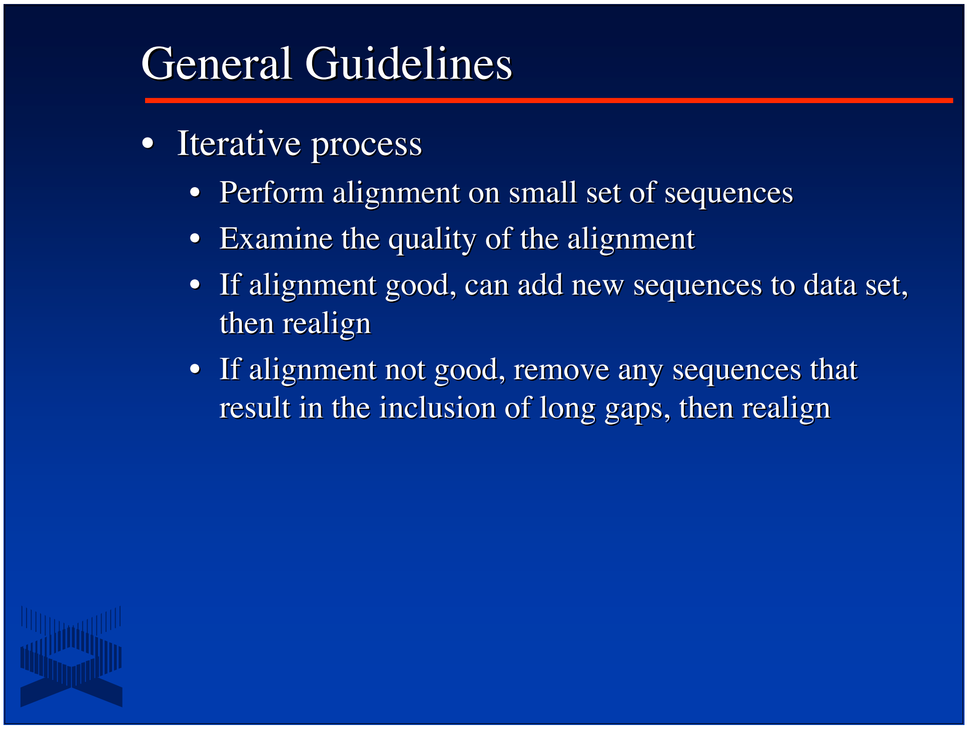
General Guidelines

- Use a reasonable number of sequences to avoid technical difficulties
  - ***Global*** alignment method: compute time increases exponentially as sequences are added to the set
  - Most alignment algorithms are ineffective on huge data sets (and may yield inaccurate alignments)
  - Phylogenetic studies resulting from inordinately large data sets are almost impossible
  - Good starting point: 10-15 sequences
  - Ballpark upper limit: 50 sequences



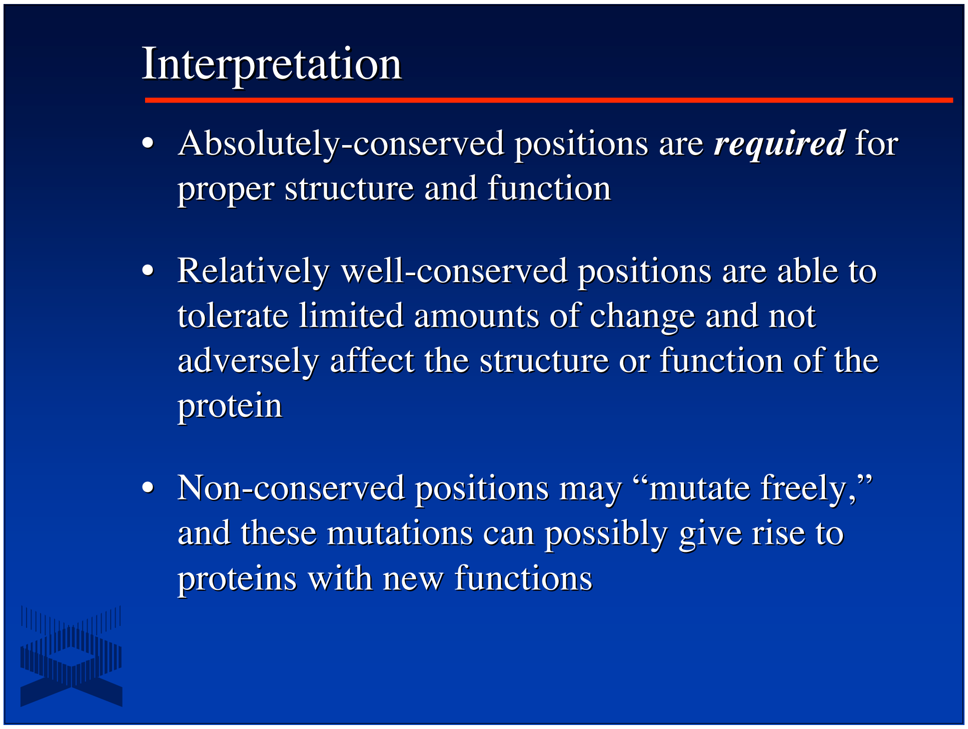
General Guidelines

- Selecting sequences for alignment
  - Sequences should be of about the same length
  - Use closely-related sequences to determine “required” amino acids
  - Use more divergent sequences to study evolutionary relationships
  - Good starting point: use sequences that are 30-70% similar to most of the other sequences in the data set
  - The most informative alignments result when the sequences in the data set are not “too similar”, but also not “too different”



General Guidelines

- Iterative process
  - Perform alignment on small set of sequences
  - Examine the quality of the alignment
  - If alignment good, can add new sequences to data set, then realign
  - If alignment not good, remove any sequences that result in the inclusion of long gaps, then realign

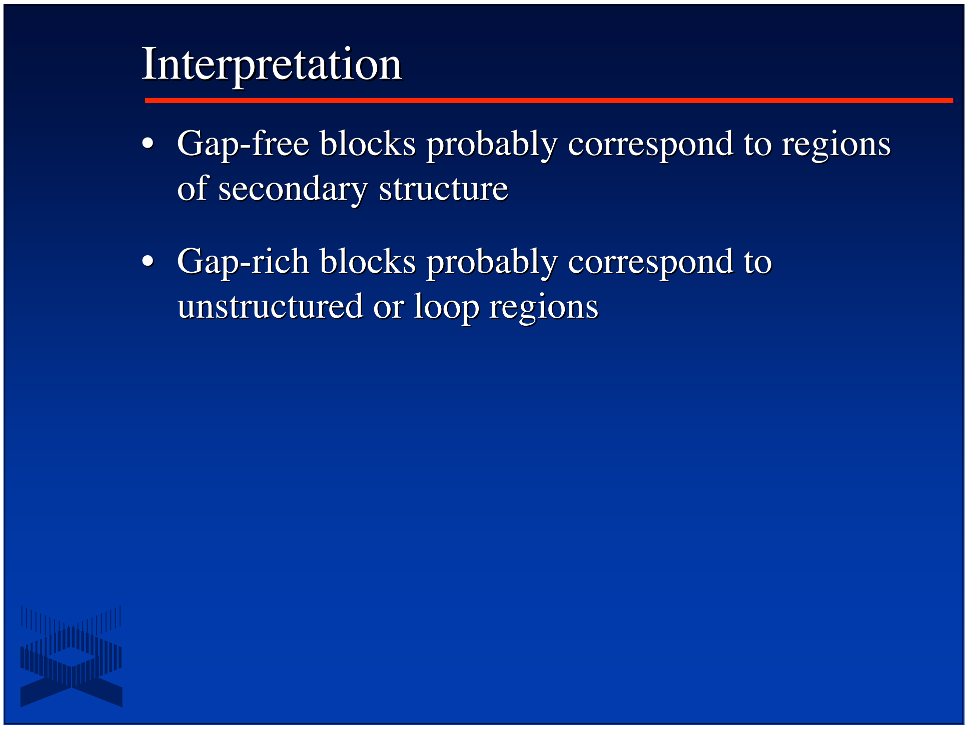


Interpretation

- Absolutely-conserved positions are ***required*** for proper structure and function

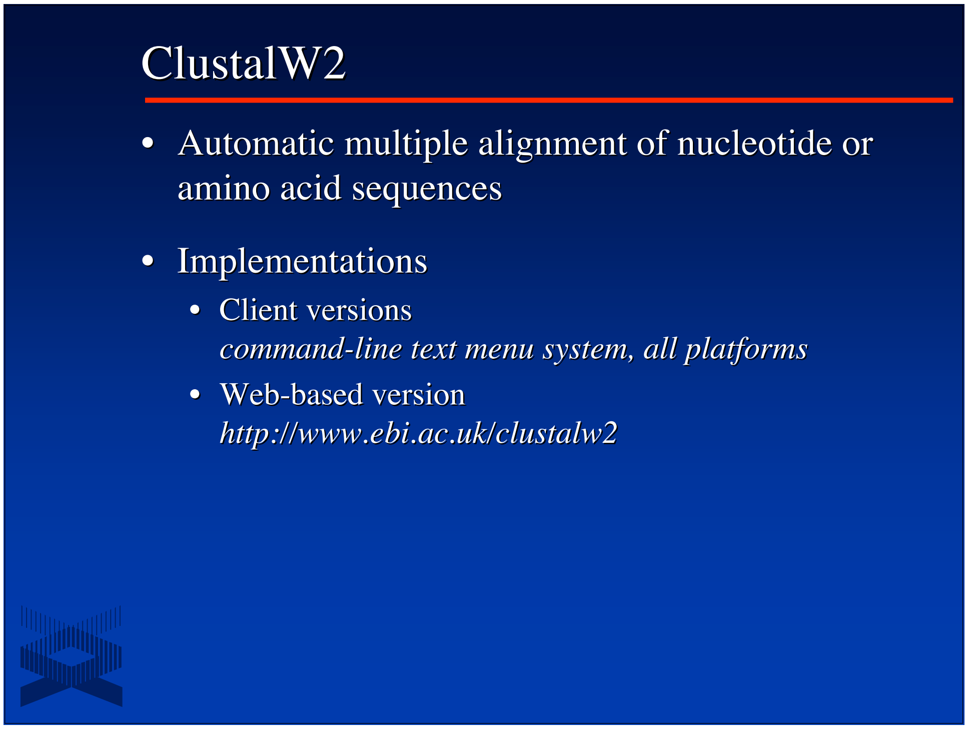
- Relatively well-conserved positions are able to tolerate limited amounts of change and not adversely affect the structure or function of the protein

- Non-conserved positions may “mutate freely,” and these mutations can possibly give rise to proteins with new functions



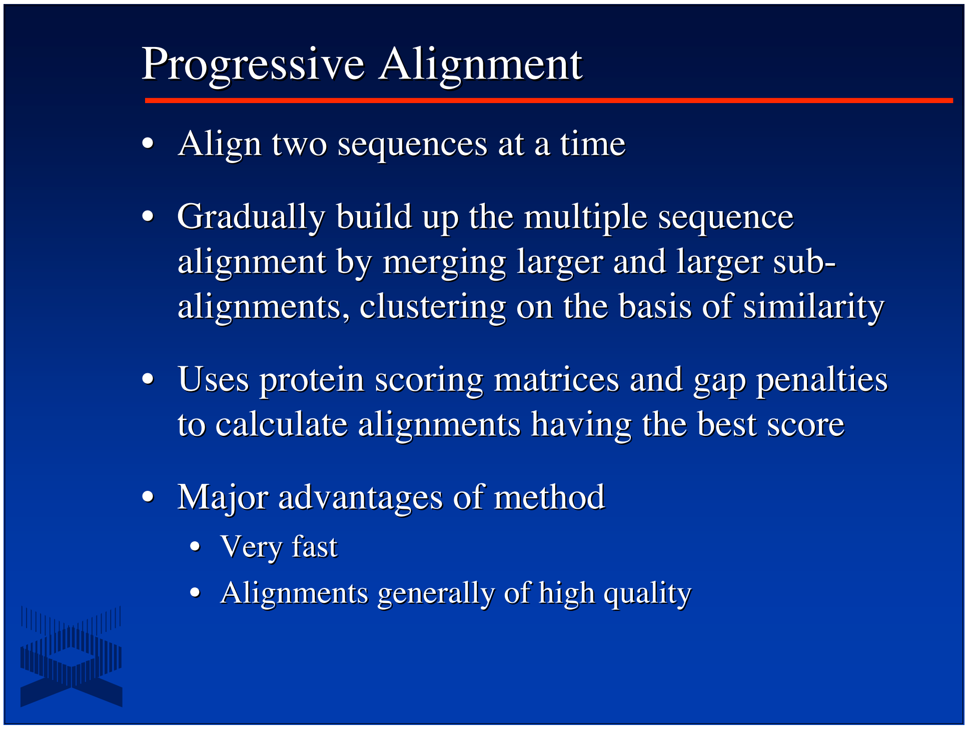
Interpretation

- Gap-free blocks probably correspond to regions of secondary structure
- Gap-rich blocks probably correspond to unstructured or loop regions



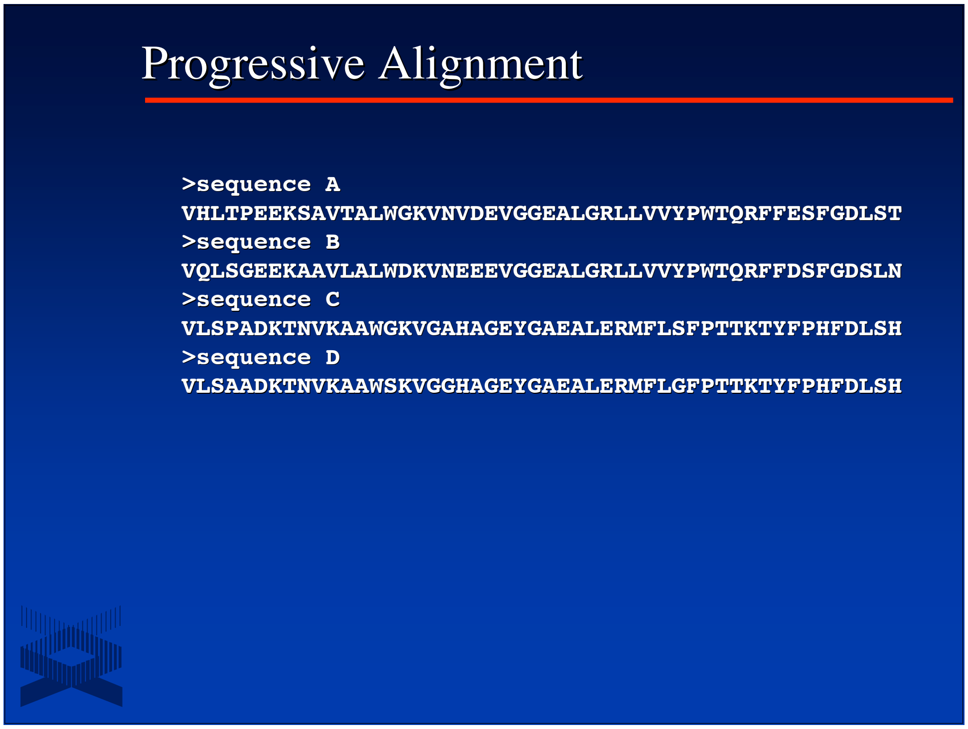
ClustalW2

- Automatic multiple alignment of nucleotide or amino acid sequences
- Implementations
  - Client versions
    command-line text menu system, all platforms
  - Web-based version
    http://www.ebi.ac.uk/clustalw2



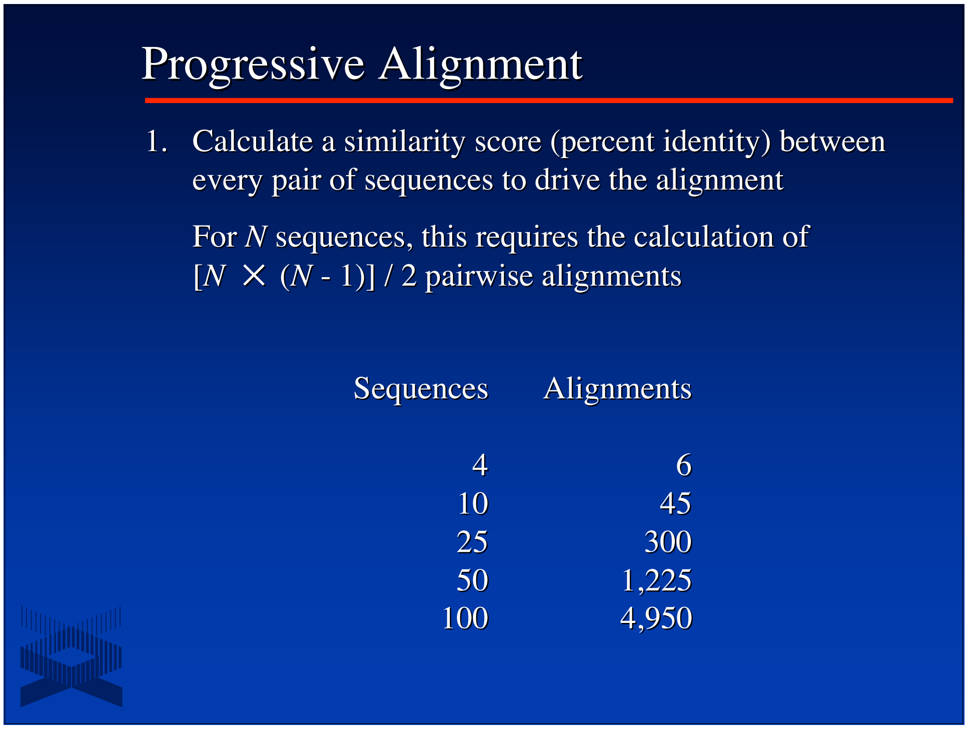
Progressive Alignment

- Align two sequences at a time
- Gradually build up the multiple sequence alignment by merging larger and larger sub-alignments, clustering on the basis of similarity
- Uses protein scoring matrices and gap penalties to calculate alignments having the best score
- Major advantages of method
  - Very fast
  - Alignments generally of high quality



Progressive Alignment

```
>sequence A
VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLST
>sequence B
VQLSGEEKAAVLALWDKVNEEEVGGEALGRLLVVYPWTQRFFDSFGDSLN
>sequence C
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSH
>sequence D
VLSAADKTNVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSH
```

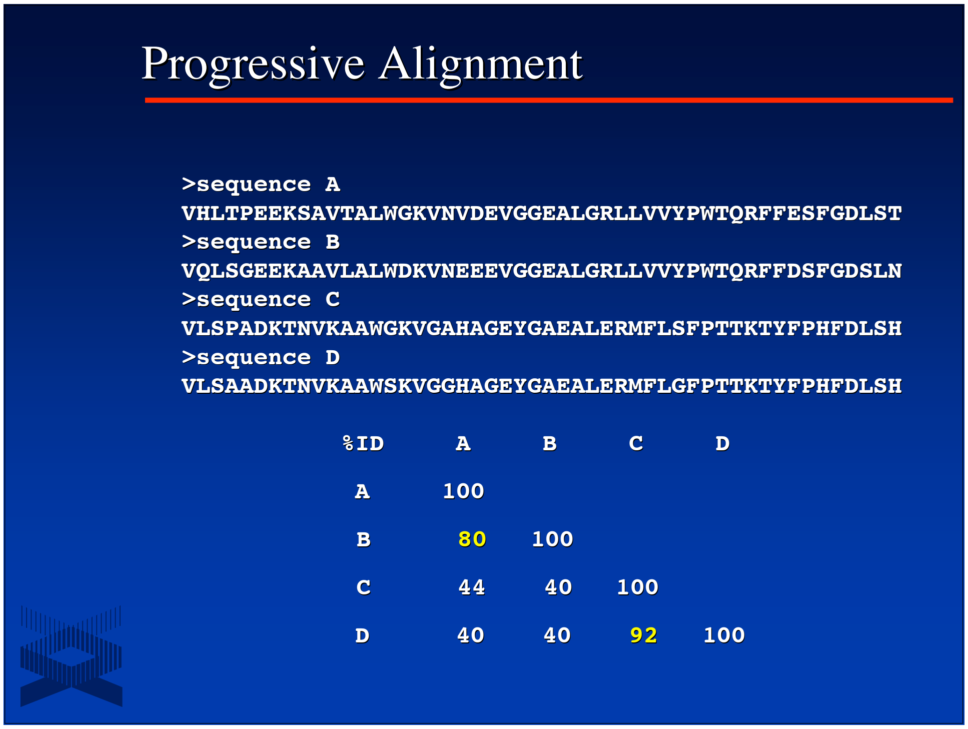


Progressive Alignment

1. Calculate a similarity score (percent identity) between every pair of sequences to drive the alignment

   For *N* sequences, this requires the calculation of [*N* × (*N* - 1)] / 2 pairwise alignments

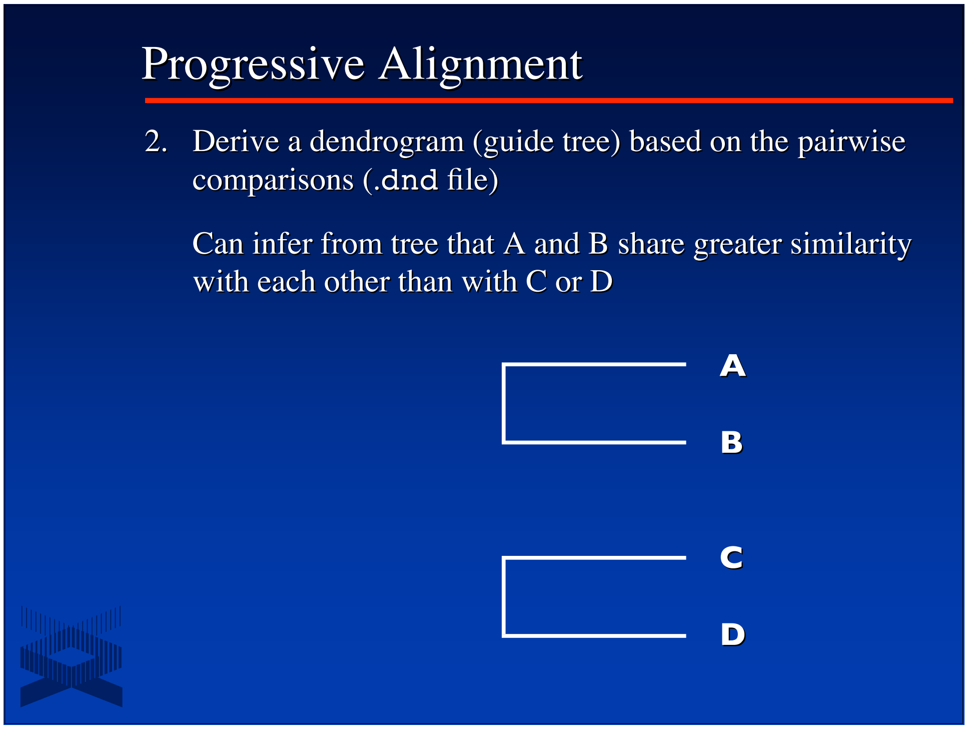
Sequences	Alignments
4	6
10	45
25	300
50	1,225
100	4,950



Progressive Alignment

```
>sequence A
VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLST
>sequence B
VQLSGEEKAAVLALWDKVNEEEVGGEALGRLLVVYPWTQRFFDSFGDSLN
>sequence C
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSH
>sequence D
VLSAADKTNVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSH
```

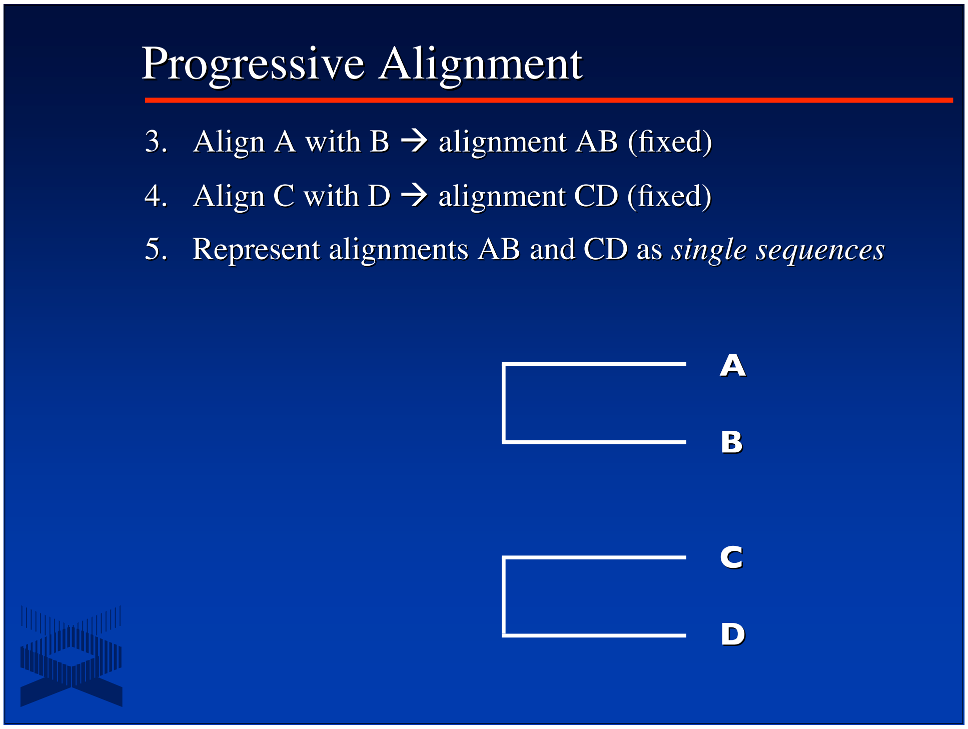
%ID	A	B	C	D
A	100			
B	80	100		
C	44	40	100	
D	40	40	92	100



Progressive Alignment

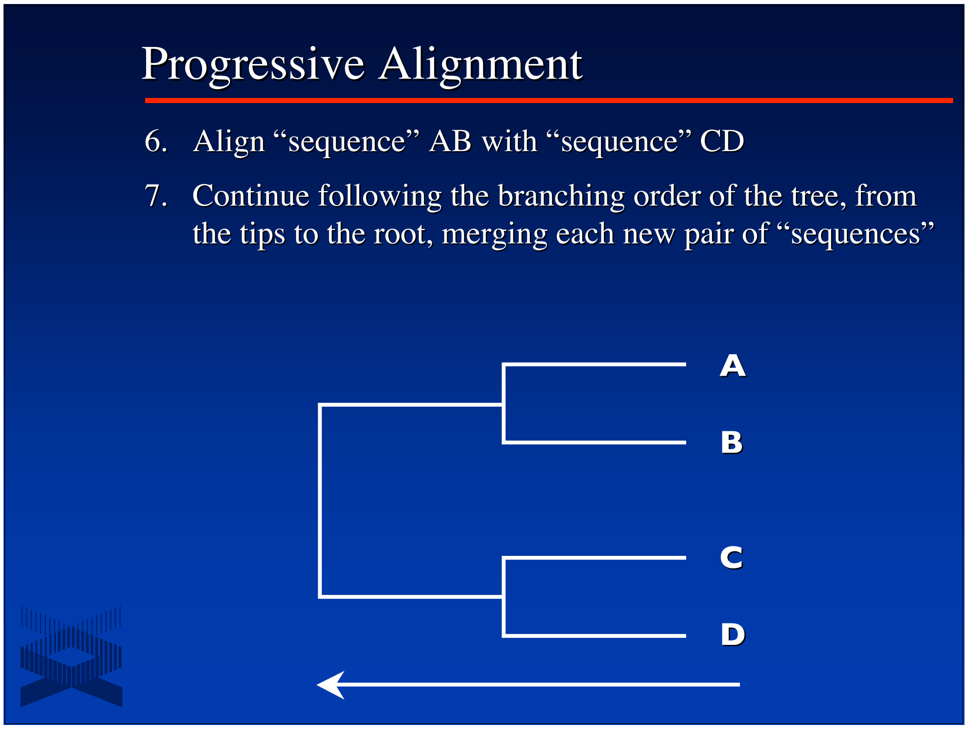
2. Derive a dendrogram (guide tree) based on the pairwise comparisons (.dnd file)

   Can infer from tree that A and B share greater similarity with each other than with C or D



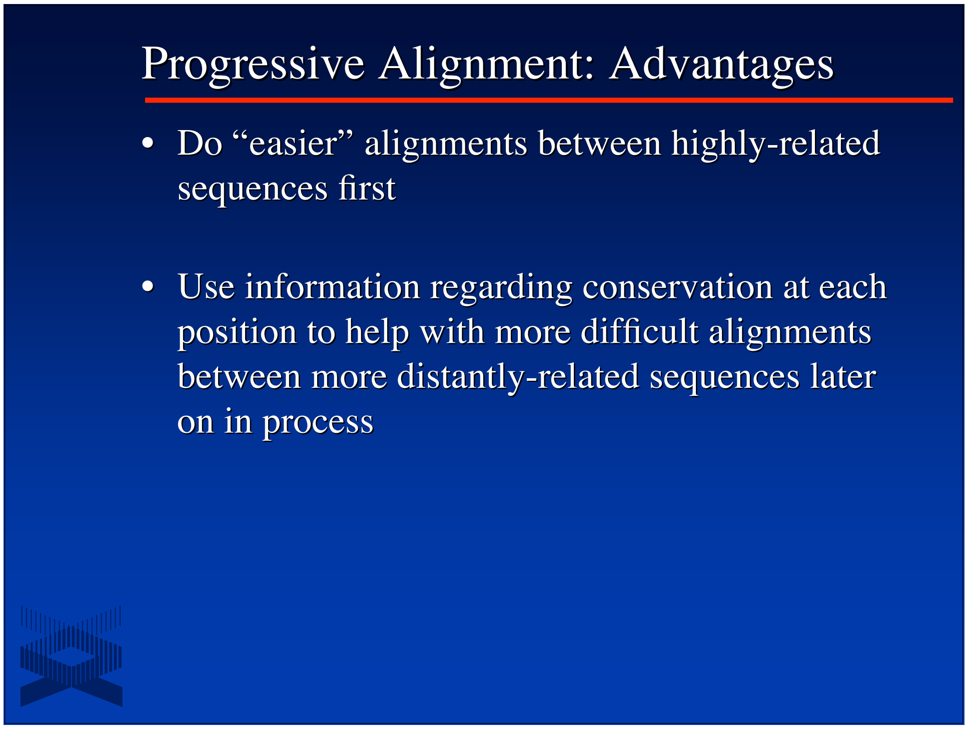
Progressive Alignment

3. Align A with B → alignment AB (fixed)
4. Align C with D → alignment CD (fixed)
5. Represent alignments AB and CD as *single sequences*



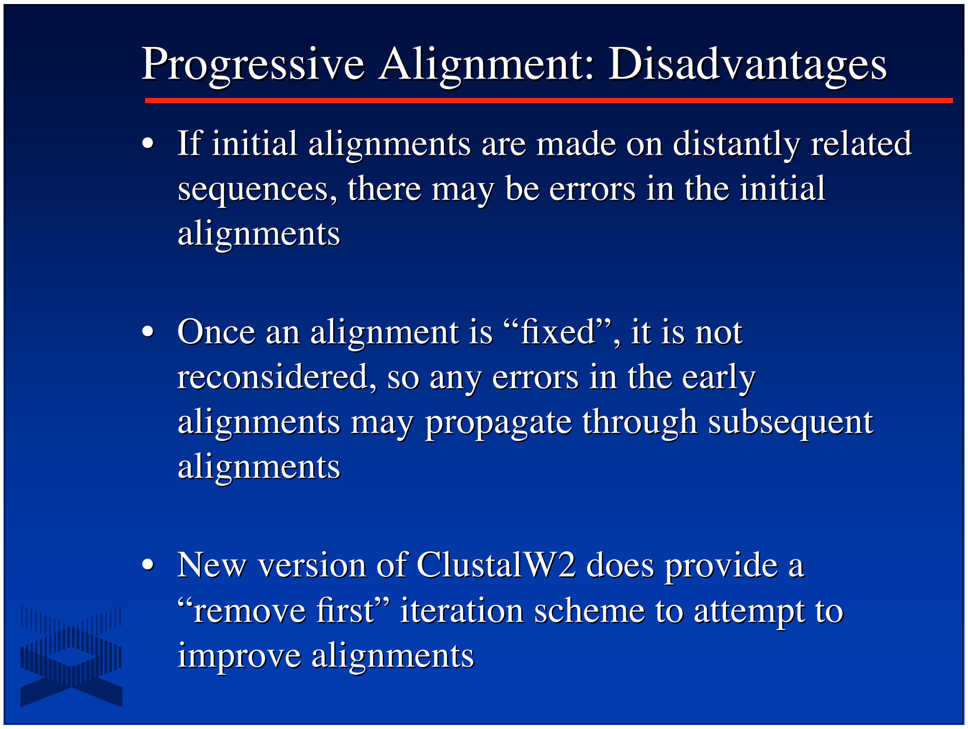
Progressive Alignment

6. Align “sequence” AB with “sequence” CD
7. Continue following the branching order of the tree, from the tips to the root, merging each new pair of “sequences”



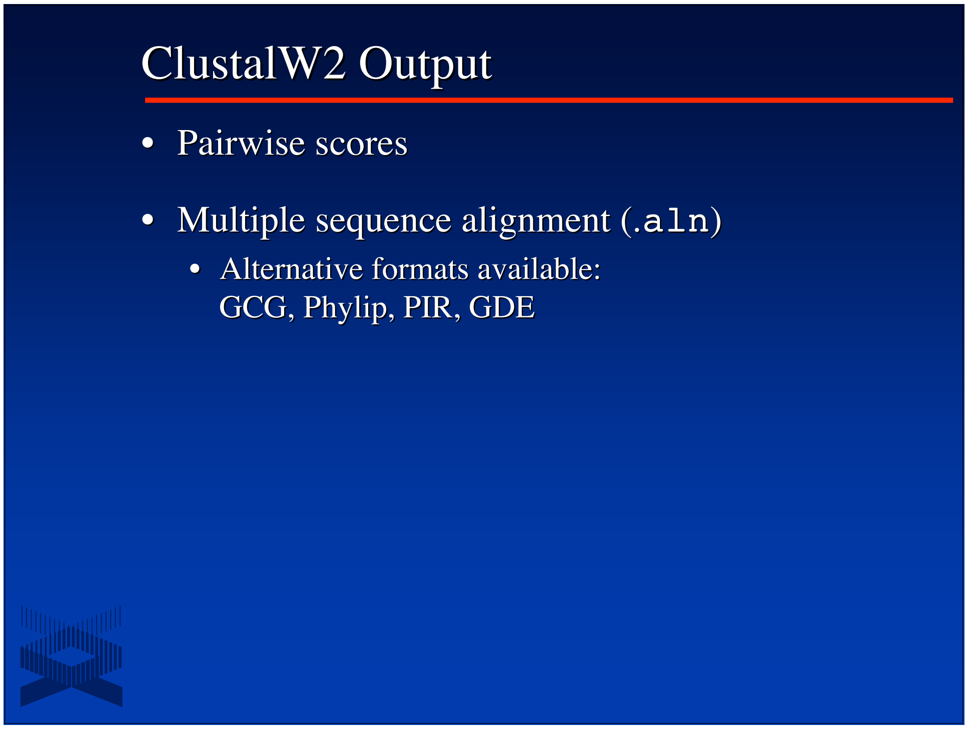
Progressive Alignment: Advantages

- Do “easier” alignments between highly-related sequences first
- Use information regarding conservation at each position to help with more difficult alignments between more distantly-related sequences later on in process



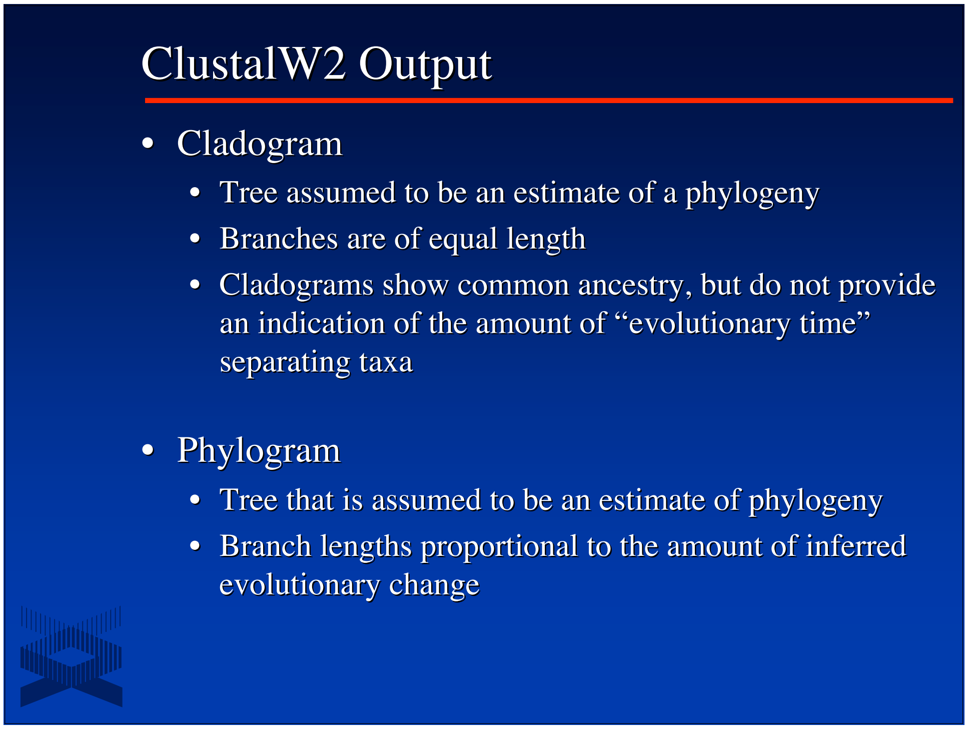
Progressive Alignment: Disadvantages

- If initial alignments are made on distantly related sequences, there may be errors in the initial alignments
- Once an alignment is “fixed”, it is not reconsidered, so any errors in the early alignments may propagate through subsequent alignments
- New version of ClustalW2 does provide a “remove first” iteration scheme to attempt to improve alignments



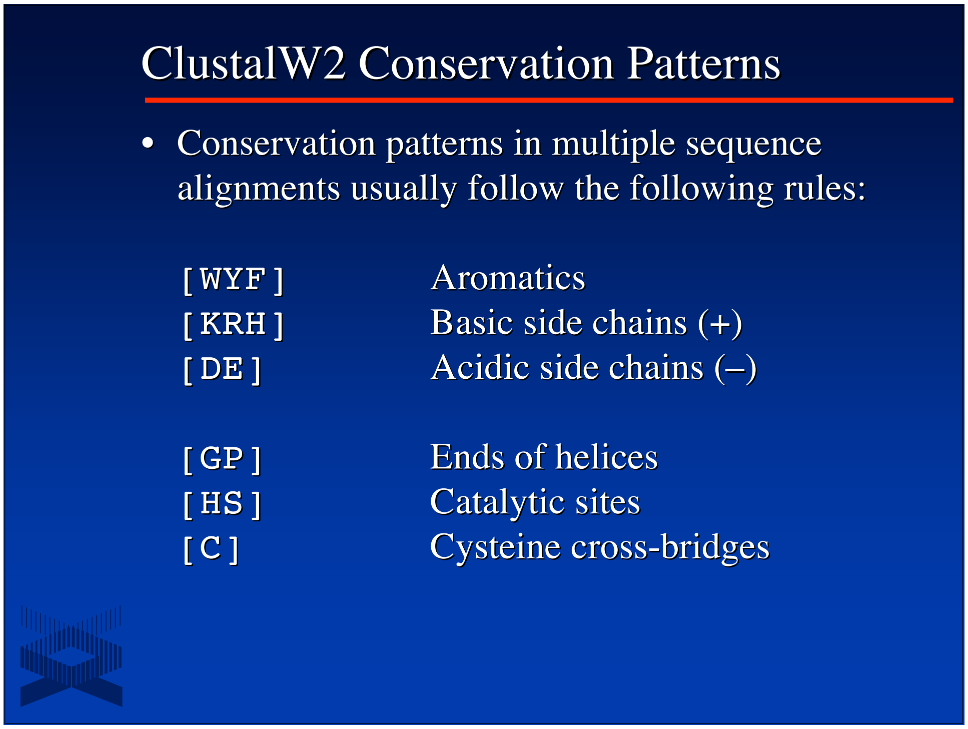
ClustalW2 Output

- Pairwise scores
- Multiple sequence alignment (`.aln`)
  - Alternative formats available: GCG, Phylip, PIR, GDE



ClustalW2 Output

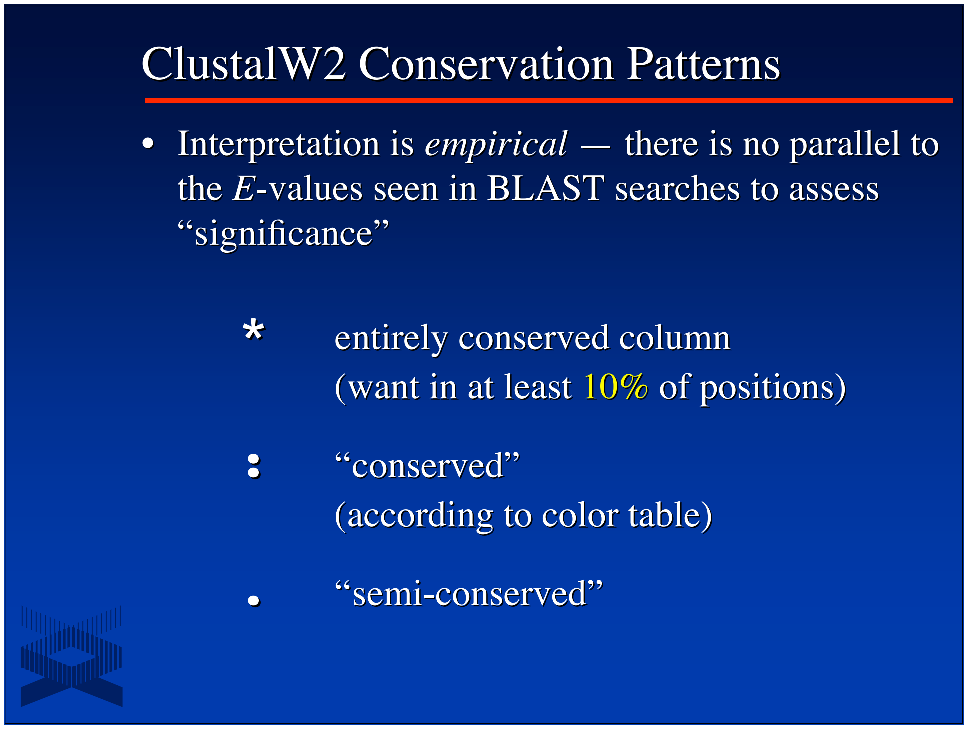
- Cladogram
  - Tree assumed to be an estimate of a phylogeny
  - Branches are of equal length
  - Cladograms show common ancestry, but do not provide an indication of the amount of “evolutionary time” separating taxa
- Phylogram
  - Tree that is assumed to be an estimate of phylogeny
  - Branch lengths proportional to the amount of inferred evolutionary change



ClustalW2 Conservation Patterns

- Conservation patterns in multiple sequence alignments usually follow the following rules:

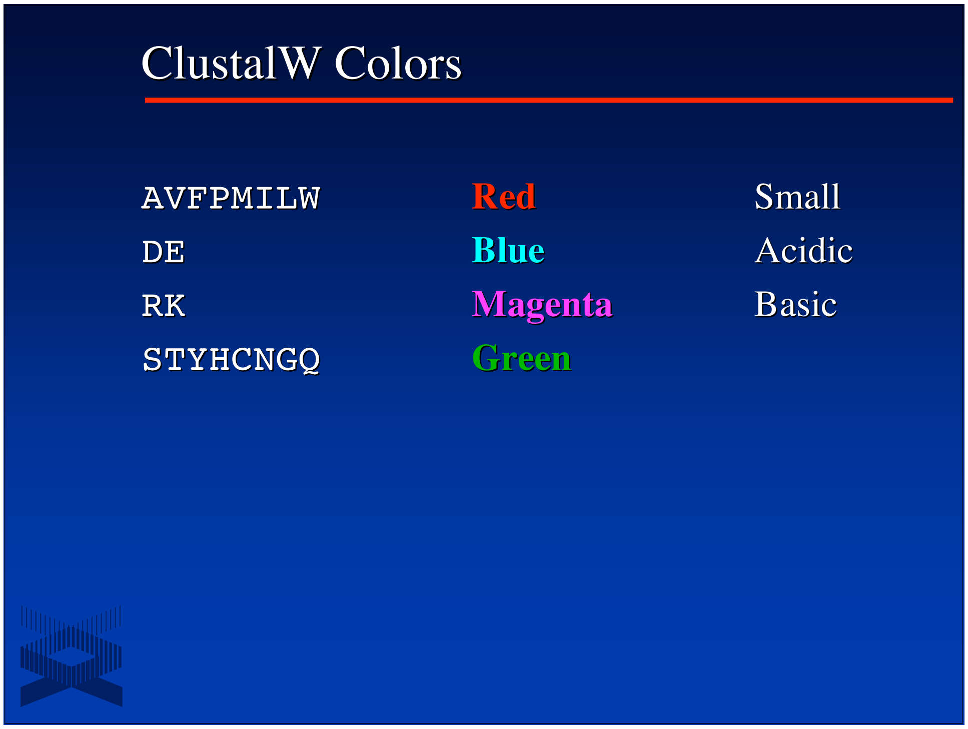
`[WYF]`	Aromatics
`[KRH]`	Basic side chains (+)
`[DE]`	Acidic side chains (–)
`[GP]`	Ends of helices
`[HS]`	Catalytic sites
`[C]`	Cysteine cross-bridges



ClustalW2 Conservation Patterns

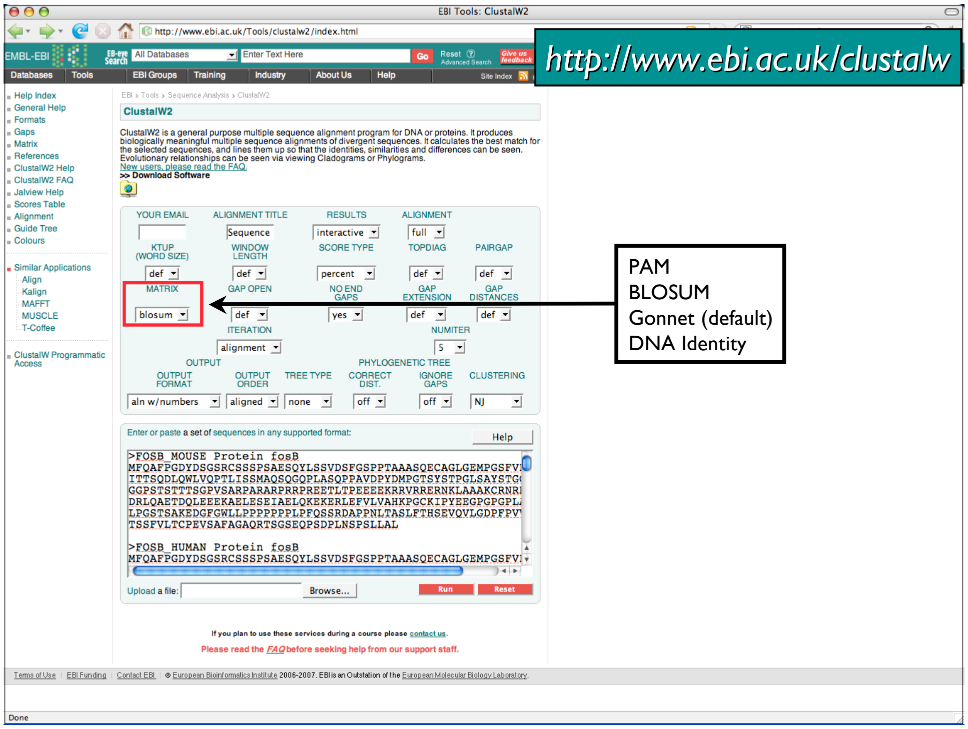
- Interpretation is *empirical* — there is no parallel to the *E*-values seen in BLAST searches to assess "significance"

*	entirely conserved column (want in at least 10% of positions)
:	"conserved" (according to color table)
.	"semi-conserved"



ClustalW Colors

AVFPMILW	Red	Small
DE	Blue	Acidic
RK	Magenta	Basic
STYHCNGQ	Green	



http://www.ebi.ac.uk/clustalw

EBI Tools: ClustalW2

http://www.ebi.ac.uk/Tools/clustalw2/index.html

ClustalW2

ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms.
New users, please read the FAQ.
>> Download Software

YOUR EMAIL:
ALIGNMENT TITLE: Sequence
RESULTS: interactive
ALIGNMENT: full
KTUP (WORD SIZE): def
WINDOW LENGTH: def
SCORE TYPE: percent
TOPDIAG: def
PAIRGAP: def
MATRIX: blosum
GAP OPEN: def
NO END GAPS: yes
GAP EXTENSION: def
GAP DISTANCES: def
ITERATION: alignment
NUMITER: 5

OUTPUT
OUTPUT FORMAT: aln w/numbers
OUTPUT ORDER: aligned

PHYLOGENETIC TREE
TREE TYPE: none
CORRECT DIST.: off
IGNORE GAPS: off
CLUSTERING: NJ

Enter or paste a set of sequences in any supported format:

```
>FOSB_MOUSE Protein fosB
MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVI
ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTG(
GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRI
DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPL
LPGSTSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQVLGDPFPV
TSSFVLTCPEVSAFAGAQRTSGSEQPSDPLNSPSLLAL

>FOSB_HUMAN Protein fosB
MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVI
```

Upload a file:

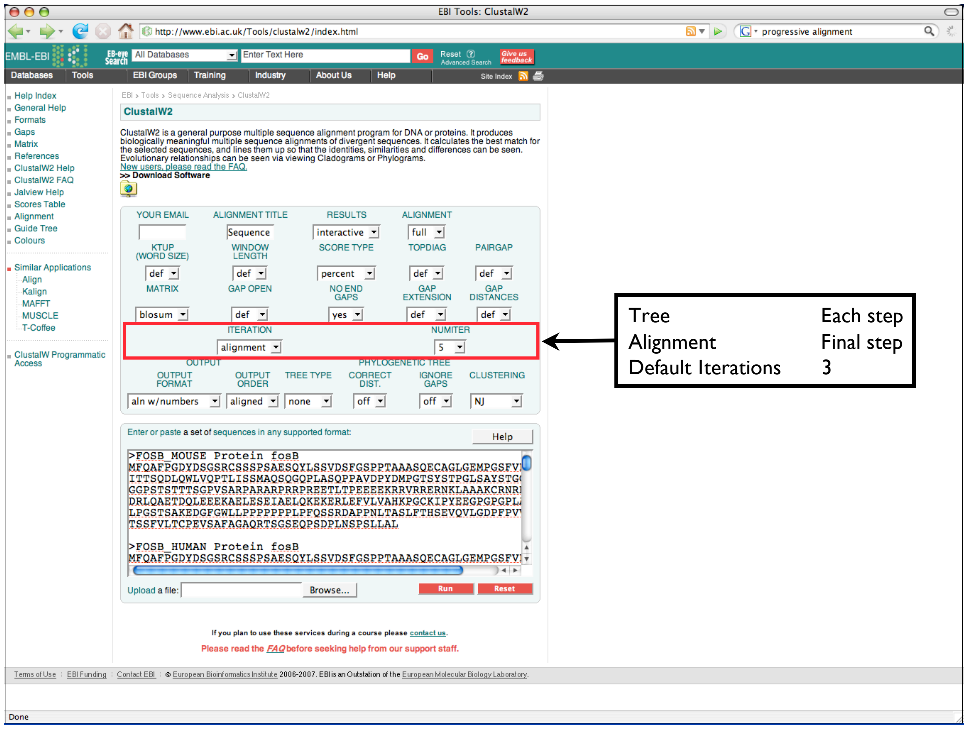
If you plan to use these services during a course please contact us.

Please read the *FAQ* before seeking help from our support staff.

Terms of Use | EBI Funding | Contact EBI | © European Bioinformatics Institute 2006-2007. EBI is an Outstation of the European Molecular Biology Laboratory.

Done

PAM
BLOSUM
Gonnet (default)
DNA Identity



progressive alignment

EBI Tools: ClustalW2

http://www.ebi.ac.uk/Tools/clustalw2/index.html

ClustalW2

ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms.
New users, please read the FAQ.
>> Download Software

YOUR EMAIL:
ALIGNMENT TITLE: Sequence
RESULTS: interactive
ALIGNMENT: full
KTUP (WORD SIZE): def
WINDOW LENGTH: def
SCORE TYPE: percent
TOPDIAG: def
PAIRGAP: def
MATRIX: blosum
GAP OPEN: def
NO END GAPS: yes
GAP EXTENSION: def
GAP DISTANCES: def
ITERATION: alignment
NUMITER: 5

OUTPUT
OUTPUT FORMAT: aln w/numbers
OUTPUT ORDER: aligned

PHYLOGENETIC TREE
TREE TYPE: none
CORRECT DIST.: off
IGNORE GAPS: off
CLUSTERING: NJ

Enter or paste a set of sequences in any supported format:

>FOSB_MOUSE Protein fosB
MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVI
ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTG(
GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRI
DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPL
LPGSTSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQVLGDPFPV
TSSFVLTCPEVSAFAGAQRTSGSEQPSDPLNSPSLLAL

>FOSB_HUMAN Protein fosB
MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVI

Upload a file:

If you plan to use these services during a course please contact us.

Please read the *FAQ* before seeking help from our support staff.

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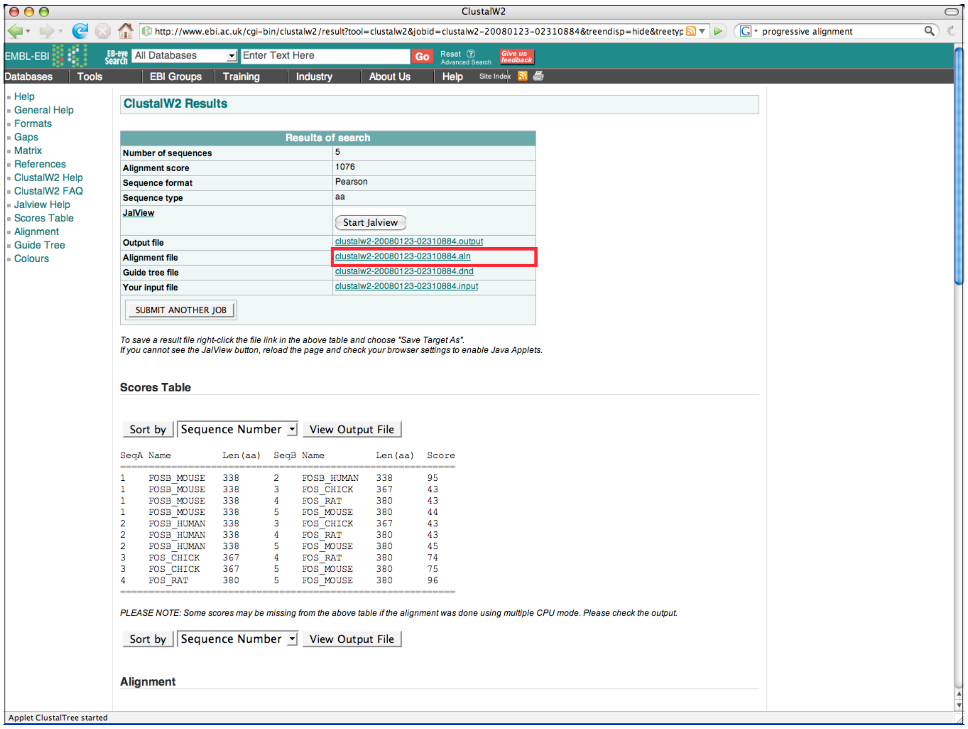
Done

Tree	Each step
Alignment	Final step
Default Iterations	3



ClustalW2 Results

Results of search	
Number of sequences	5
Alignment score	1076
Sequence format	Pearson
Sequence type	aa
JalView	Start Jalview
Output file	clustalw2-20080123-02310884.output
Alignment file	clustalw2-20080123-02310884.aln
Guide tree file	clustalw2-20080123-02310884.dnd
Your input file	clustalw2-20080123-02310884.input

SUBMIT ANOTHER JOB

To save a result file right-click the file link in the above table and choose "Save Target As".
If you cannot see the JalView button, reload the page and check your browser settings to enable Java Applets.

Scores Table

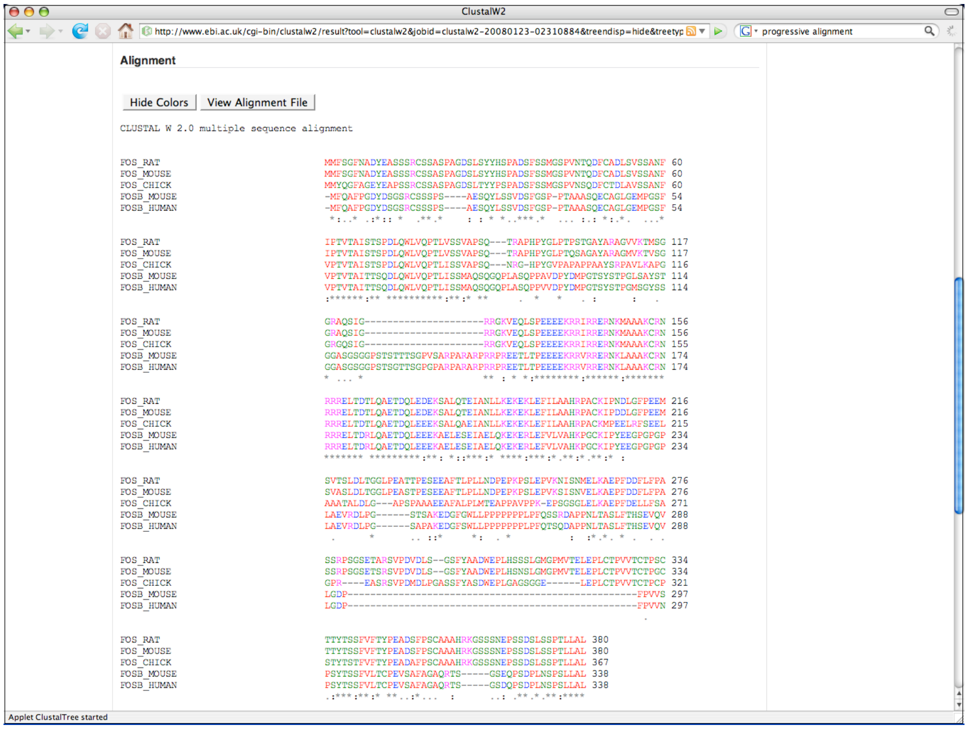
Sort by | Sequence Number | View Output File

```
SeqA Name         Len(aa)  SeqB Name         Len(aa)  Score
=============================================================
1    FOSB_MOUSE   338      2    FOSB_HUMAN   338      95
1    FOSB_MOUSE   338      3    FOS_CHICK    367      43
1    FOSB_MOUSE   338      4    FOS_RAT      380      43
1    FOSB_MOUSE   338      5    FOS_MOUSE    380      44
2    FOSB_HUMAN   338      3    FOS_CHICK    367      43
2    FOSB_HUMAN   338      4    FOS_RAT      380      43
2    FOSB_HUMAN   338      5    FOS_MOUSE    380      45
3    FOS_CHICK    367      4    FOS_RAT      380      74
3    FOS_CHICK    367      5    FOS_MOUSE    380      75
4    FOS_RAT      380      5    FOS_MOUSE    380      96
=============================================================
```

PLEASE NOTE: Some scores may be missing from the above table if the alignment was done using multiple CPU mode. Please check the output.

Sort by | Sequence Number | View Output File

Alignment


ClustalW2
http://www.ebi.ac.uk/cgi-bin/clustalw2/result?tool=clustalw2&jobid=clustalw2-20080123-02310884&treendisp=hide&treetyp
progressive alignment
Applet ClustalTree started

Alignment

Hide Colors | View Alignment File

```
CLUSTAL W 2.0 multiple sequence alignment


FOS_RAT                     MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANF 60
FOS_MOUSE                   MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANF 60
FOS_CHICK                   MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANF 60
FOSB_MOUSE                  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSF 54
FOSB_HUMAN                  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSF 54
                             *:..*  .:*:: *   .**.*     : : * *..***.*   ... :.: *:.*.   ...*

FOS_RAT                     IPTVTAISTSPDLQWLVQPTLVSSVAPSQ---TRAPHPYGLPTPSTGAYARAGVVKTMSG 117
FOS_MOUSE                   IPTVTAISTSPDLQWLVQPTLVSSVAPSQ---TRAPHPYGLPTQSAGAYARAGMVKTVSG 117
FOS_CHICK                   VPTVTAISTSPDLQWLVQPTLISSVAPSQ---NRG-HPYGVPAPAPPAAYSRPAVLKAPG 116
FOSB_MOUSE                  VPTVTAITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYST 114
FOSB_HUMAN                  VPTVTAITTSQDLQWLVQPTLISSMAQSQGQPLASQPPVVDPYDMPGTSYSTPGMSGYSS 114
                            :******:** **********:**:* **      .  *   *   . :       :   .

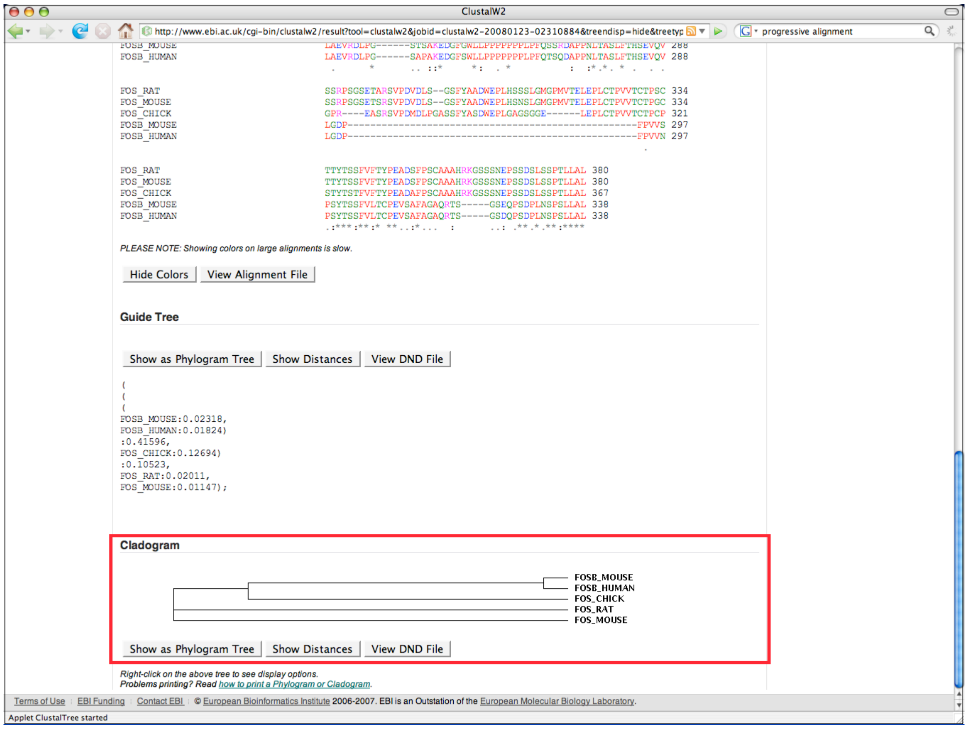
FOS_RAT                     GRAQSIG---------------------RRGKVEQLSPEEEEKRRIRRERNKMAAAKCRN 156
FOS_MOUSE                   GRAQSIG---------------------RRGKVEQLSPEEEEKRRIRRERNKMAAAKCRN 156
FOS_CHICK                   GRGQSIG---------------------RRGKVEQLSPEEEEKRRIRRERNKMAAAKCRN 155
FOSB_MOUSE                  GGASGSGGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRN 174
FOSB_HUMAN                  GGASGSGGPSTSGTTSGPGPARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRN 174
                            *  ... *                    ** : * *:********:******:*******

FOS_RAT                     RRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPACKIPNDLGFPEEM 216
FOS_MOUSE                   RRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPACKIPDDLGFPEEM 216
FOS_CHICK                   RRRELTDTLQAETDQLEEEKSALQAEIANLLKEKEKLEFILAAHRPACKMPEELRFSEEL 215
FOSB_MOUSE                  RRRELTDRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGP 234
FOSB_HUMAN                  RRRELTDRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGP 234
                            ******* *********:**: *::***:* ****:***:*.**:*.**:*  :

FOS_RAT                     SVTSLDLTGGLPEATTPESEEAFTLPLLNDPEPKPSLEPVKNISNMELKAEPFDDFLFPA 276
FOS_MOUSE                   SVASLDLTGGLPEASTPESEEAFTLPLLNDPEPKPSLEPVKSISNVELKAEPFDDFLFPA 276
FOS_CHICK                   AAATALDLG---APSPAAAEEAFALPLMTEAPPAVPPK-EPSGSGLELKAEPFDELLFSA 271
FOSB_MOUSE                  LAEVRDLPG------STSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQV 288
FOSB_HUMAN                  LAEVRDLPG------SAPAKEDGFSWLLPPPPPPPLPFQTSQDAPPNLTASLFTHSEVQV 288
                             .      *       .. ::*     *:  . *           :  :*.*. *  .  . .

FOS_RAT                     SSRPSGSETARSVPDVDLS--GSFYAADWEPLHSSSLGMGPMVTELEPLCTPVVTCTPSC 334
FOS_MOUSE                   SSRPSGSETSRSVPDVDLS--GSFYAADWEPLHSNSLGMGPMVTELEPLCTPVVTCTPGC 334
FOS_CHICK                   GPR----EASRSVPDMDLPGASSFYASDWEPLGAGSGGE------LEPLCTPVVTCTPCP 321
FOSB_MOUSE                  LGDP--------------------------------------------------FPVVS 297
FOSB_HUMAN                  LGDP--------------------------------------------------FPVVN 297
                                                                                  .

FOS_RAT                     TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_MOUSE                   TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_CHICK                   STYTSTFVFTYPEADAFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 367
FOSB_MOUSE                  PSYTSSFVLTCPEVSAFAGAQRTS-----GSEQPSDPLNSPSLLAL 338
FOSB_HUMAN                  PSYTSSFVLTCPEVSAFAGAQRTS-----GSDQPSDPLNSPSLLAL 338
                            .:***:**:* **..:*...   :       ..: .**.*.**:****
```



ClustalW2
http://www.ebi.ac.uk/cgi-bin/clustalw2/result?tool=clustalw2&jobid=clustalw2-20080123-02310884&treendisp=hide&treetyp
progressive alignment

```
FOSB_MOUSE                    LAEVRDLPG------STSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQV 288
FOSB_HUMAN                    LAEVRDLPG------SAPAKEDGFSWLLPPPPPPPLPFQTSQDAPPNLTASLFTHSEVQV 288
                               .      *      .. ::*     *:  . *           :  :*.*. *  .  . .

FOS_RAT                       SSRPSGSETARSVPDVDLS--GSFYAADWEPLHSSSLGMGPMVTELEPLCTPVVTCTPSC 334
FOS_MOUSE                     SSRPSGSETSRSVPDVDLS--GSFYAADWEPLHSNSLGMGPMVTELEPLCTPVVTCTPGC 334
FOS_CHICK                     GPR----EASRSVPDMDLPGASSFYASDWEPLGAGSGGE------LEPLCTPVVTCTPCP 321
FOSB_MOUSE                    LGDP---------------------------------------------------FPVVS 297
FOSB_HUMAN                    LGDP---------------------------------------------------FPVVN 297
                                                                                     .

FOS_RAT                       TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_MOUSE                     TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_CHICK                     STYTSTFVFTYPEADAFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 367
FOSB_MOUSE                    PSYTSSFVLTCPEVSAFAGAQRTS-----GSEQPSDPLNSPSLLAL 338
FOSB_HUMAN                    PSYTSSFVLTCPEVSAFAGAQRTS-----GSDQPSDPLNSPSLLAL 338
                              .:*** :** :* **..:*...  :      ..: .**.*.**:****
```

PLEASE NOTE: Showing colors on large alignments is slow.

Hide Colors | View Alignment File

Guide Tree

Show as Phylogram Tree | Show Distances | View DND File

```
(
(
(
FOSB_MOUSE:0.02318,
FOSB_HUMAN:0.01824)
:0.41596,
FOS_CHICK:0.12694)
:0.10523,
FOS_RAT:0.02011,
FOS_MOUSE:0.01147);
```

Cladogram

Show as Phylogram Tree | Show Distances | View DND File

Right-click on the above tree to see display options.
Problems printing? Read how to print a Phylogram or Cladogram.

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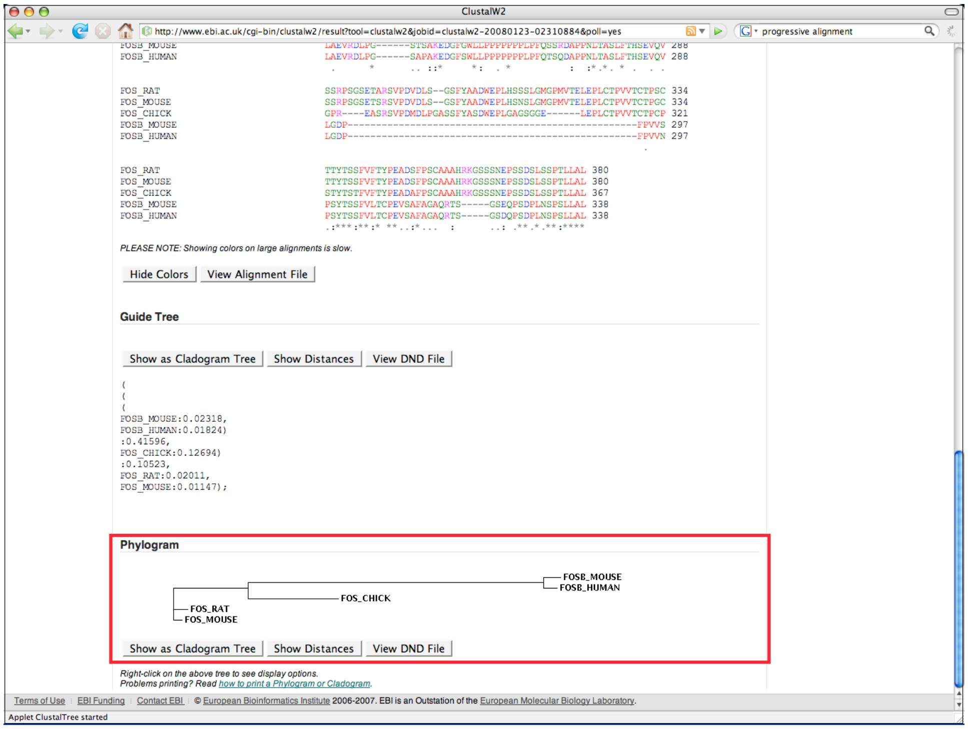
Applet ClustalTree started



ClustalW2
http://www.ebi.ac.uk/cgi-bin/clustalw2/result?tool=clustalw2&jobid=clustalw2-20080123-02310884&poll=yes
progressive alignment

```
FOSB_MOUSE                    LAEVRDLPG------STSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQV 288
FOSB_HUMAN                    LAEVRDLPG------SAPAKEDGFSWLLPPPPPPPLPFQTSQDAPPNLTASLFTHSEVQV 288
                               .      *      .. ::*     *:  . *           :  :*.*. *  .  . .

FOS_RAT                       SSRPSGSETARSVPDVDLS--GSFYAADWEPLHSSSLGMGPMVTELEPLCTPVVTCTPSC 334
FOS_MOUSE                     SSRPSGSETSRSVPDVDLS--GSFYAADWEPLHSNSLGMGPMVTELEPLCTPVVTCTPGC 334
FOS_CHICK                     GPR----EASRSVPDMDLPGASSFYASDWEPLGAGSGGE------LEPLCTPVVTCTPCP 321
FOSB_MOUSE                    LGDP---------------------------------------------------FPVVS 297
FOSB_HUMAN                    LGDP---------------------------------------------------FPVVN 297
                                                                                     .

FOS_RAT                       TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_MOUSE                     TTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 380
FOS_CHICK                     STYTSTFVFTYPEADAFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL 367
FOSB_MOUSE                    PSYTSSFVLTCPEVSAFAGAQRTS-----GSEQPSDPLNSPSLLAL 338
FOSB_HUMAN                    PSYTSSFVLTCPEVSAFAGAQRTS-----GSDQPSDPLNSPSLLAL 338
                              .:*** :** :* **..:*...  :      ..: .**.*.**:****
```

PLEASE NOTE: Showing colors on large alignments is slow.

Hide Colors | View Alignment File

Guide Tree

Show as Cladogram Tree | Show Distances | View DND File

```
(
(
(
FOSB_MOUSE:0.02318,
FOSB_HUMAN:0.01824)
:0.41596,
FOS_CHICK:0.12694)
:0.10523,
FOS_RAT:0.02011,
FOS_MOUSE:0.01147);
```

Phylogram

Show as Cladogram Tree | Show Distances | View DND File

Right-click on the above tree to see display options.
Problems printing? Read how to print a Phylogram or Cladogram.

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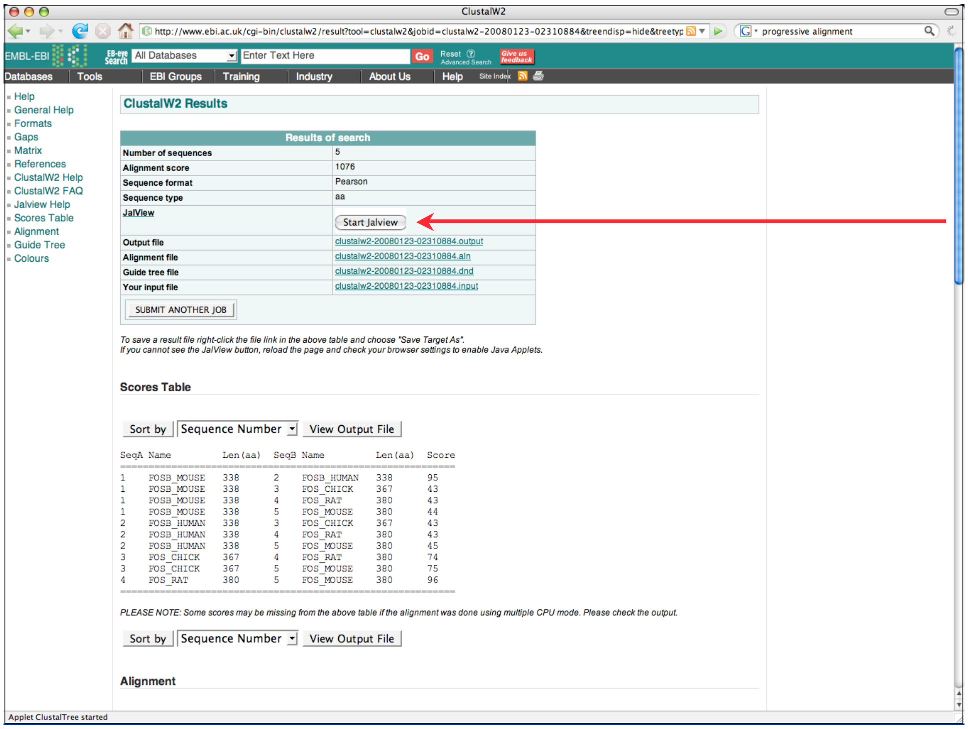
Applet ClustalTree started

Jalview

- Java applet available within ClustalW2 results
- Used to manually edit ClustalW2 alignments
- Color residues based on various properties
- Pairwise alignment of selected sequences
- Consensus sequence calculations
- Removal of redundant sequences
- Calculation of phylogenetic trees
- Color PostScript output



ClustalW2 Results

Results of search	
Number of sequences	5
Alignment score	1076
Sequence format	Pearson
Sequence type	aa
JalView	Start Jalview
Output file	clustalw2-20080123-02310884.output
Alignment file	clustalw2-20080123-02310884.aln
Guide tree file	clustalw2-20080123-02310884.dnd
Your input file	clustalw2-20080123-02310884.input

SUBMIT ANOTHER JOB

To save a result file right-click the file link in the above table and choose "Save Target As".
If you cannot see the JalView button, reload the page and check your browser settings to enable Java Applets.

Scores Table

Sort by | Sequence Number | View Output File

```
SeqA Name         Len(aa)  SeqB Name         Len(aa)  Score
============================================================
1    FOSB_MOUSE   338      2    FOSB_HUMAN   338      95
1    FOSB_MOUSE   338      3    FOS_CHICK    367      43
1    FOSB_MOUSE   338      4    FOS_RAT      380      43
1    FOSB_MOUSE   338      5    FOS_MOUSE    380      44
2    FOSB_HUMAN   338      3    FOS_CHICK    367      43
2    FOSB_HUMAN   338      4    FOS_RAT      380      43
2    FOSB_HUMAN   338      5    FOS_MOUSE    380      45
3    FOS_CHICK    367      4    FOS_RAT      380      74
3    FOS_CHICK    367      5    FOS_MOUSE    380      75
4    FOS_RAT      380      5    FOS_MOUSE    380      96
============================================================
```

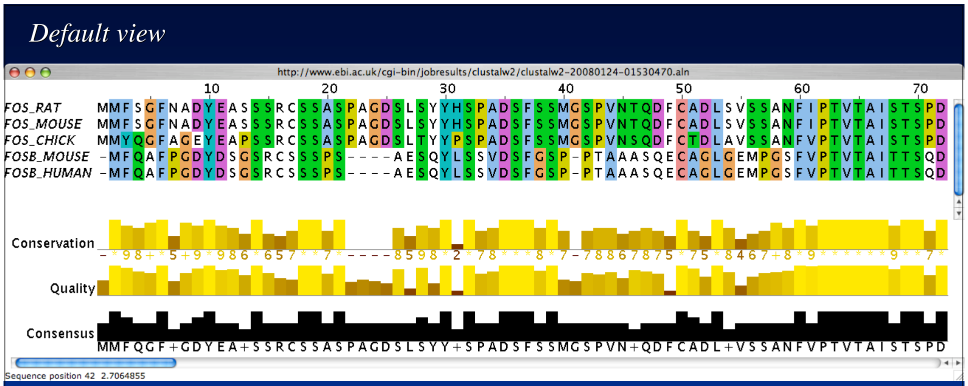
PLEASE NOTE: Some scores may be missing from the above table if the alignment was done using multiple CPU mode. Please check the output.

Sort by | Sequence Number | View Output File

Alignment

Applet ClustalTree started

Default view



http://www.ebi.ac.uk/cgi-bin/jobresults/clustalw2/clustalw2-20080124-01530470.aln

```
                      10        20        30        40        50        60        70
FOS_RAT      MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_MOUSE    MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_CHICK    MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANFVPTVTAISTSPD
FOSB_MOUSE   -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD
FOSB_HUMAN   -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD

Conservation -*98+*5+9*986*657**7*----8598*2*78***8*7-78867875*75*8467+8*9******9**7*
Quality
Consensus    MMFQGF+GDYEA+SSRCSSASPAGDSLSYY+SPADSFSSMGSPVN+QDFCADL+VSSANFVPTVTAISTSPD
```

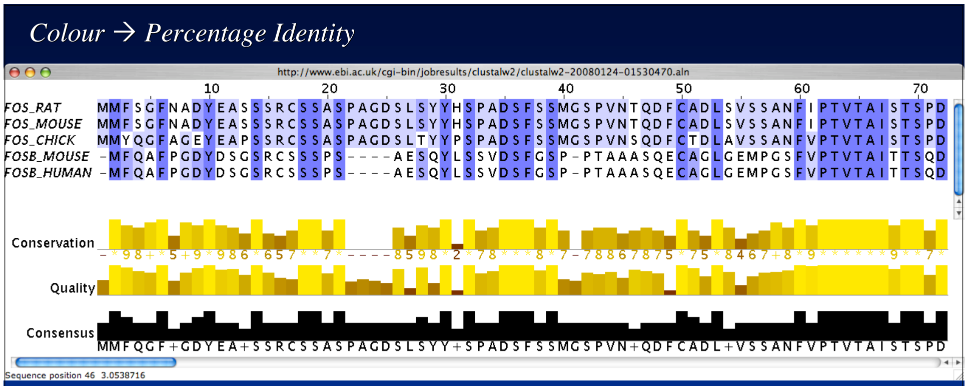
Sequence position 42 2.7064855

Conservation *Conservation of total alignment (indication of percent identity)*

Quality *Alignment quality, based on BLOSUM62 scores*

Consensus *Based on percent identity*

Colour → Percentage Identity



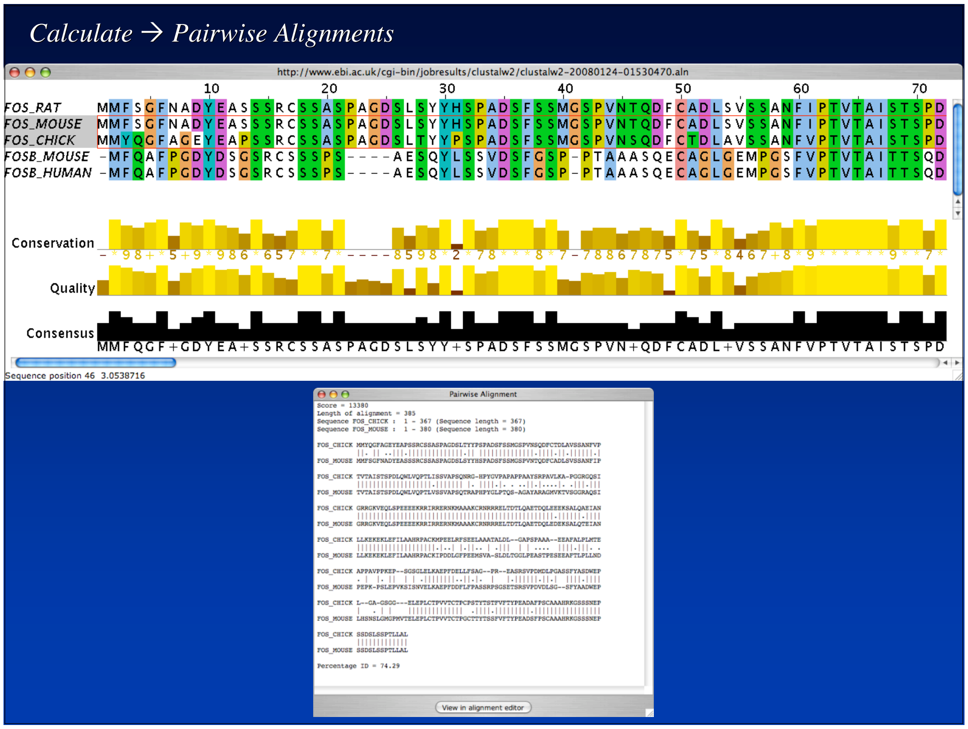
http://www.ebi.ac.uk/cgi-bin/jobresults/clustalw2/clustalw2-20080124-01530470.aln

```
                      10        20        30        40        50        60        70
FOS_RAT      MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_MOUSE    MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_CHICK    MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANFVPTVTAISTSPD
FOSB_MOUSE   -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD
FOSB_HUMAN   -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD

Conservation -*98+*5+9*986*657**7*----8598*2*78***8*7-78867875*75*8467+8*9******9**7*
Quality
Consensus    MMFQGF+GDYEA+SSRCSSASPAGDSLSYY+SPADSFSSMGSPVN+QDFCADL+VSSANFVPTVTAISTSPD
```

Sequence position 46 3.0538716

Agreement	***Background Color***
81 - 100%	Dark blue
61 - 80%	Medium blue
41 - 60%	Light blue
< 40%	White



Calculate → Pairwise Alignments

http://www.ebi.ac.uk/cgi-bin/jobresults/clustalw2/clustalw2-20080124-01530470.aln

```
                     10        20        30        40        50        60        70
FOS_RAT     MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_MOUSE   MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_CHICK   MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANFVPTVTAISTSPD
FOSB_MOUSE  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD
FOSB_HUMAN  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD

Conservation -*98+*5+9*986*657**7*----8598*2*78***8*7-78867875*75*8467+8*9*******9**7*
     Quality
   Consensus
            MMFQGF+GDYEA+SSRCSSASPAGDSLSYY+SPADSFSSMGSPVN+QDFCADL+VSSANFVPTVTAISTSPD
Sequence position 46  3.0538716
```

Pairwise Alignment

```
Score = 13380
Length of alignment = 385
Sequence FOS_CHICK :  1 - 367 (Sequence length = 367)
Sequence FOS_MOUSE :  1 - 380 (Sequence length = 380)

FOS_CHICK MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANFVP
          ||. || ..|||.|||||||||||||.|| |||||||||||||.||||.||.||||||.|
FOS_MOUSE MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIP

FOS_CHICK TVTAISTSPDLQWLVQPTLISSVAPSQNRG-HPYGVPAPAPPAAYSRPAVLKA-PGGRGQSI
          |||||||||||||||||||.||||||| |. ||||.|. . ..||.|....|. .|||.|||
FOS_MOUSE TVTAISTSPDLQWLVQPTLVSSVAPSQTRAPHPYGLPTQS-AGAYARAGMVKTVSGGRAQSI

FOS_CHICK GRRGKVEQLSPEEEEKRRIRRERNKMAAAKCRNRRRELTDTLQAETDQLEEEKSALQAEIAN
          ||||||||||||||||||||||||||||||||||||||||||||||||||.||||||.||||
FOS_MOUSE GRRGKVEQLSPEEEEKRRIRRERNKMAAAKCRNRRRELTDTLQAETDQLEDEKSALQTEIAN

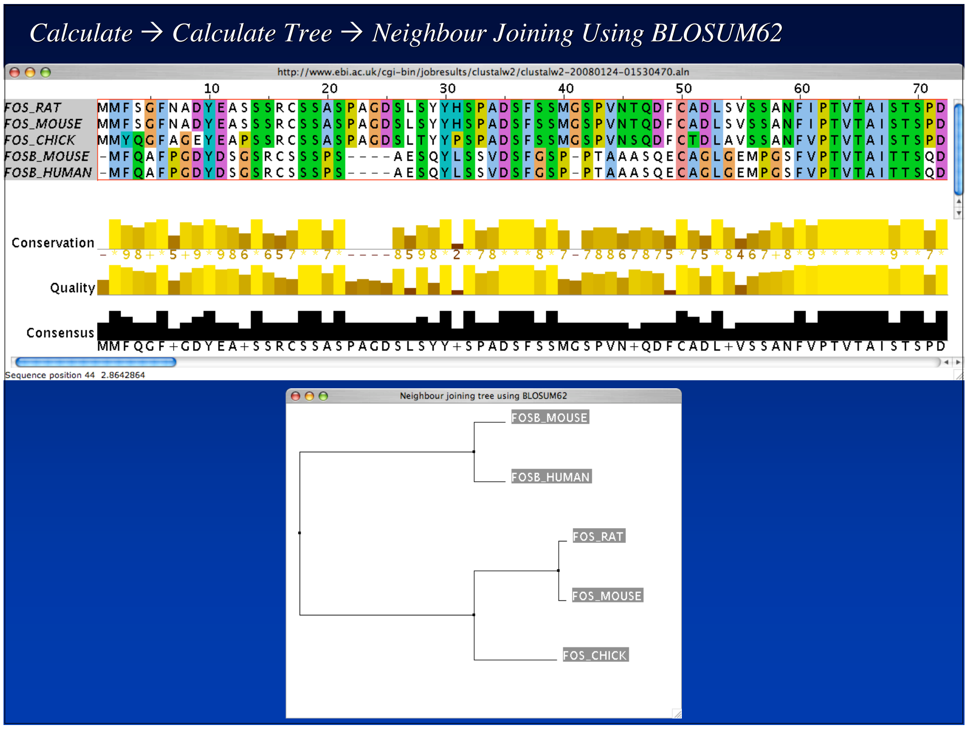
FOS_CHICK LLKEKEKLEFILAAHRPACKMPEELRFSEELAAATALDL--GAPSPAAA--EEAFALPLMTE
          ||||||||||||||||||||.|..| |.||.. | .|||  | | ....  ||||.|||. .
FOS_MOUSE LLKEKEKLEFILAAHRPACKIPDDLGFPEEMSVA-SLDLTGGLPEASTPESEEAFTLPLLND

FOS_CHICK APPAVPPKEP--SGSGLELKAEPFDELLFSAG--PR--EASRSVPDMDLPGASSFYASDWEP
          . |  |. ||  | | .||||||||..||.|.  |   |.||||||.||.|  ||||.||||
FOS_MOUSE PEPK-PSLEPVKSISNVELKAEPFDDFLFPASSRPSGSETSRSVPDVDLSG--SFYAADWEP

FOS_CHICK L--GA-GSGG---ELEPLCTPVVTCTPCPSTYTSTFVFTYPEADAFPSCAAAHRKGSSSNEP
          |   . | |    ||||||||||||||  .||||.|||||||||.||||||||||||||||||
FOS_MOUSE LHSNSLGMGPMVTELEPLCTPVVTCTPGCTTYTSSFVFTYPEADSFPSCAAAHRKGSSSNEP

FOS_CHICK SSDSLSSPTLLAL
          |||||||||||||
FOS_MOUSE SSDSLSSPTLLAL

Percentage ID = 74.29
```



Calculate → Calculate Tree → Neighbour Joining Using BLOSUM62

http://www.ebi.ac.uk/cgi-bin/jobresults/clustalw2/clustalw2-20080124-01530470.aln

```
                     10        20        30        40        50        60        70
FOS_RAT     MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_MOUSE   MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNTQDFCADLSVSSANFIPTVTAISTSPD
FOS_CHICK   MMYQGFAGEYEAPSSRCSSASPAGDSLTYYPSPADSFSSMGSPVNSQDFCTDLAVSSANFVPTVTAISTSPD
FOSB_MOUSE  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD
FOSB_HUMAN  -MFQAFPGDYDSGSRCSSSPS----AESQYLSSVDSFGSP-PTAAASQECAGLGEMPGSFVPTVTAITTSQD

Conservation -*98+*5+9*986*657**7*----8598*2*78***8*7-78867875*75*8467+8*9*******9**7*
     Quality
   Consensus
            MMFQGF+GDYEA+SSRCSSASPAGDSLSYY+SPADSFSSMGSPVN+QDFCADL+VSSANFVPTVTAISTSPD
```

```
Sequence position 44  2.8642864
```

Neighbour joining tree using BLOSUM62

Current Protocols in Bioinformatics

CPBI Unit 2.3
ClustalW

Multiple Sequence Alignment Using ClustalW and ClustalX

UNIT 2.3

The Clustal programs are widely used for carrying out automatic multiple alignment of sets of nucleotide or amino acid sequences. The most familiar version is ClustalW (Thompson et al., 1994), which uses a simple text menu system that is portable to more or less all computer systems. ClustalX (Thompson et al., 1997) features a graphical user interface and some powerful graphical utilities for aiding the interpretation of alignments, and is the preferred version for interactive usage. ClustalW and ClustalX are developed in parallel, and the same version-numbering system is used for both in order to synchronize changes (e.g., bug fixes, improvements, and additions). In January 2002, the latest version for both programs was 1.81. The programs can both be run interactively, but the protocols below give instructions on how to do this using ClustalX. Alternatively, ClustalW supports a full command-line interface which allows it to be used automatically as part of larger analyses (e.g., it can be run from scripts). In the simplest usage (see Basic Protocol), the programs are employed to take a set of homologous sequences (all DNA/RNA or all protein) and to produce a single multiple alignment. This covers the vast majority of Clustal usage and will be sufficient for most cases. Nonetheless, Clustal also has extensive facilities for adding sequences to existing alignments, merging existing alignments (so-called profile alignment as described in the Alternate Protocol), realignment of sections of alignment, detecting and fixing alignment errors, and basic phylogenetic analysis. Users may run Clustal remotely from several sites using the Web, or the programs may be downloaded to be run locally on PCs, Macintosh, or Unix computers (Support Protocol).

USING CLUSTALW AND CLUSTALX TO DO MULTIPLE ALIGNMENTS

BASIC PROTOCOL

The programs ClustalW and ClustalX provide alternative user interfaces to the Clustal multiple alignment software. The alignments produced by the two programs are exactly the same; the only difference between ClustalW and ClustalX is the way in which the user interacts with the program. ClustalW is now mainly used as a command-line program by Web servers and automatic batch systems, although the program does provide text menus which can be used to input sequences and perform multiple alignments. Most users who run Clustal interactively now use the graphical interface provided by ClustalX. This protocol therefore uses ClustalX (here on a Silicon Graphics Unix workstation) to illustrate the basic multiple alignment procedure. Although the example given here uses protein sequences, the same protocol can be performed with nucleic acid sequences.

Necessary Resources

Hardware

Unix (including Linux) workstation (e.g., Sun, Alpha, Silicon Graphics, PC), PC with MS Windows, or Power Macintosh

Software

ClustalW or ClustalX program (see Support Protocol)

Files

Sequences can be input to both ClustalW and ClustalX in one of seven file formats. All sequences must be in the same file. The formats that are automatically recognized are: NBRF/PIR, EMBL/Swiss-Prot, Pearson (FASTA; APPENDIX 1B), Clustal, GCG/MSF, GCG9/RSF, and GDE flat file. The sequences

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Recognizing Functional Domains

2.3.1

CPBI Unit 3.8
T-Coffee

Computing Multiple Sequence/Structure Alignments with the T-Coffee Package

UNIT 3.8

This unit describes how to assemble a multiple sequence alignment using the T-Coffee multiple sequence alignment package (Notredame et al., 2000). Although T-Coffee is slower than its close relative ClustalW (UNIT 2.3; Thompson et al., 1994), it is better suited for aligning distantly related sequences and mixing sequences and structures. T-Coffee is also much more flexible than most methods because it makes it possible to combine many alternative alignments into a single one, based on an estimate of consistency between the alignments. The protocols below show how such a combination can be done and how alternative alignment methods can be added as separate modules to the T-Coffee process.

This unit assumes that the user wants to align a set of sequences that are more or less homologous over their entire length (see Basic Protocol 1). These sequences may have been gathered using any appropriate database search strategy. Given such a data set, the user can assemble a multiple alignment in order to carry out family modeling (profile; see Basic Protocol 2), structural modeling (see Basic Protocol 3), or phyologenetic modeling (tree computation). This multiple sequence alignment may also be used to analyze the potential effect of nsSNPs (Non-Synonymous Single Nucleotide Polymorphisms) or simply to check whether a specific sequence is a true member of the family.

Most of this unit assumes that the user is familiar with the Unix environment (without being a specialist or a programmer; see APPENDIX 1C & APPENDIX 1D). The last section (see the Appendix at the end of the unit) is a bit more demanding in terms of computer skills, but it should be relatively straightforward to anyone with a basic knowledge of the scripting language Perl.

T-Coffee is very appropriate for generating high-quality alignments, but it is more demanding in terms of resources than other, similar programs. Given a standard 2-GHz PC with 500 Mb of memory, one should not hope to align more than 100 sequences that are up to 2000 residues long when using the default mode. This figure is simply an indication, since the memory requirement depends on the relatedness of the sequences being considered (close sequences require less time and less memory). Given these limitations, it is often a good strategy to start with a rapid multiple sequence alignment method (such as ClustalW; UNIT 2.3) in order to quickly identify potential problems within the data set, before refining the results with T-Coffee. Nevertheless, the authors provide two alternative strategies that make it possible to bypass some of the limitations of T-Coffee regarding memory usage.

In this unit, protocols are presented on how to use T-Coffee in a Unix/Linux environment, taking advantage of the rich command-line options of this program. Yet, for those who prefer clickable Web interfaces, much of what is presented here can also be executed by using the Web tool Tcoffee@igs (Poirot et al., 2003), available at *http://igs-server.cnrs-mrs.fr/Tcoffee/*. This service is provided to the community by the CNRS and HP servers. Other online versions of this software exist, and an exhaustive list is maintained on the T-Coffee home page (accessible from the Tcoffee@igs server at the abovementioned URL).

NOTE: Investigators who are unfamiliar with the Unix environment are encouraged to read APPENDIX 1C & APPENDIX 1D.

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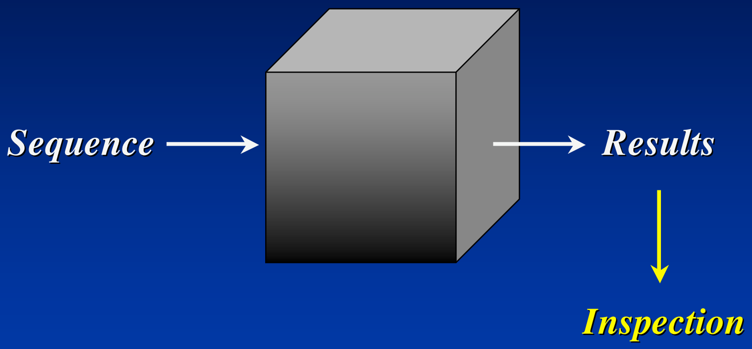
Finding Similarities and Inferring Homologies

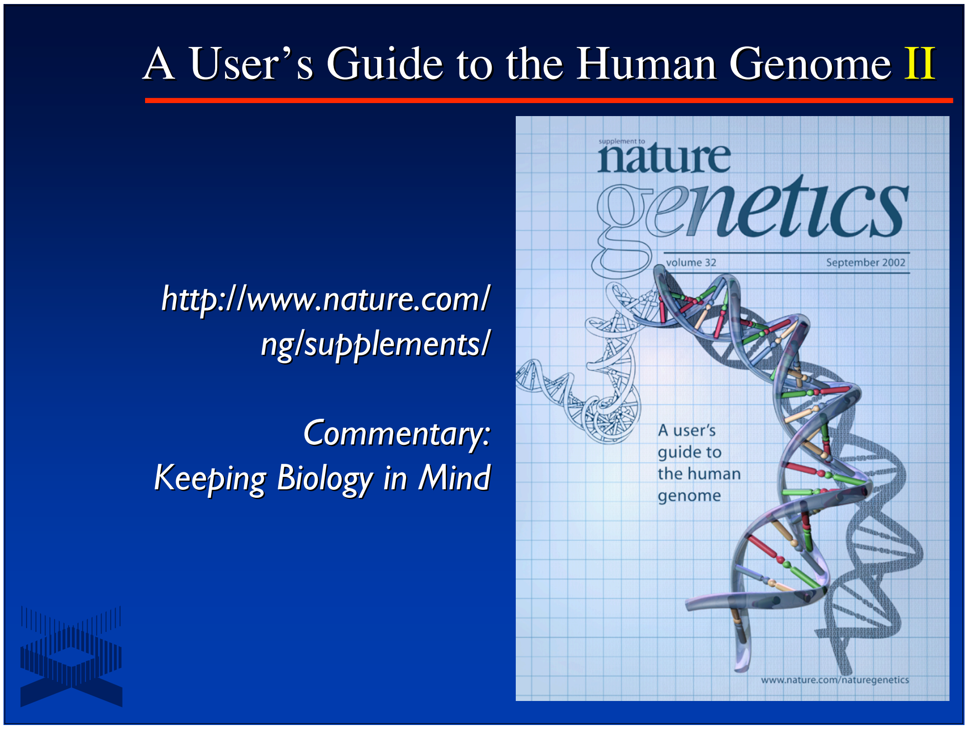
3.8.1

Supplement 4

http://nihlibrary.nih.gov
Search "Online Journals" for "Current Protocols in Bioinformatics"

Understanding Analyses





A User's Guide to the Human Genome II

http://www.nature.com/ ng/supplements/

Commentary: Keeping Biology in Mind

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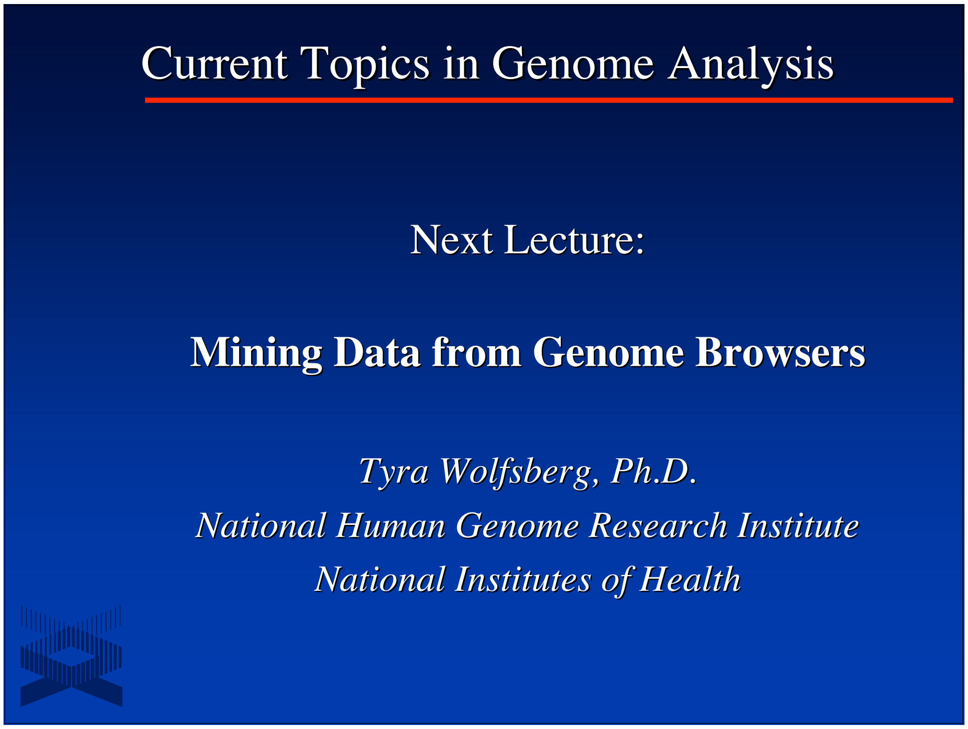

Current Topics in Genome Analysis

Next Lecture:

Mining Data from Genome Browsers

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