Workshop in Molecular Evolution
Protein Evolution / Similarity Searching

What BLAST Does / Why BLAST works

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Sequence Similarity - Conclusions

- *Homologous* sequences share a common ancestor, but most sequences are *non-homologous*
- Always compare Protein Sequences
- Sequence Homology can be reliably inferred from statistically significant similarity (non-homology cannot from non-similarity)
- Homologous proteins share common structures, but not necessarily common functions
- Sequence statistical significance estimates are accurate (verify this yourself)$10^{-6} < E() < 10^{-3}$ is statistically significant
- Scoring matrices set evolutionary look back horizons - not every discovery is distant
- PSI-BLAST can be more sensitive, but with lower statistical accuracy

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Establishing homology from statistically significant similarity
Why BLAST works

- For most proteins, homologs are easily found over long evolutionary distances (500 My – 2 By) using standard approaches (BLAST, FASTA)
- Difficult for distant relationships or very short domains
- Most default search parameters are optimized for distant relationships and work well

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This talk is not about:

- *Alignment*
  - Alignment quality may be more sensitive to parameter choice
  - Multiple sequences for biologically accurate alignments
- *Inferring Protein Function*
  - Homology (common ancestry) implies common structure (guaranteed), not necessarily common function
  - Homologs have different functions
  - Non-homologs have similar (or identical) functions
- *The best sequences for building trees*
  - Protein sequences are clearly best for establishing homology, but DNA sequences may be better for resolving recent divergence

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Protein Evolution and Sequence Similarity

- What is Homology and how do we recognize it?
- How do we measure sequence similarity – alignments and scoring matrices?
- DNA vs protein comparison
- Alignment Algorithms/Local sequence alignments
- Similarity scoring matrices
- When are we certain that an alignment is significant - similarity score statistics?
- When to trust similarity statistics?
- Improving sensitivity with PSI-BLAST

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MIT 7.91J / 7.36J / 20.490J
Foundations of Computational and Systems Biology

Lect. 1 Introduction/Sequence Comparison and Dynamic Programming

Definitions

- **Homologue:**

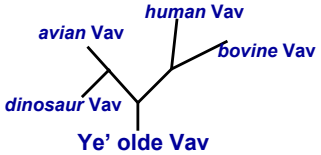
a relatively non-specific term (meaningless?) that conveys the idea that two sequences are somehow related

- **Orthologue:**

Ortho = (*greek*) straight….implies direct descent, 1 ancestor

Vav ***human***
↓
Vav ***bovine***
↓
Vav ***avian***
↓
Vav ***elegans***

-or-



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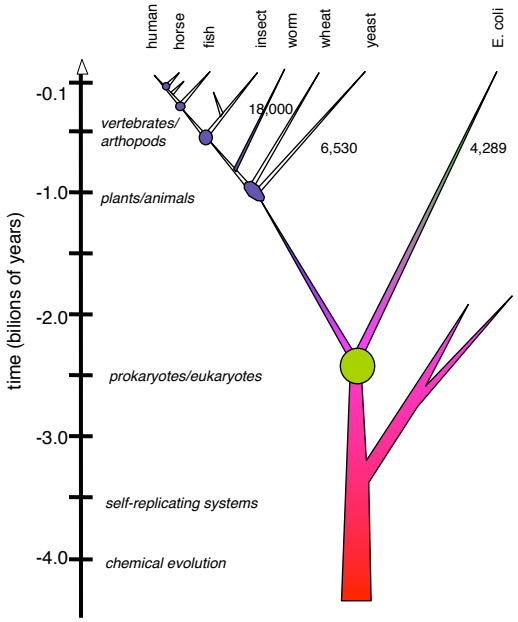
Homologues share a common ancestor



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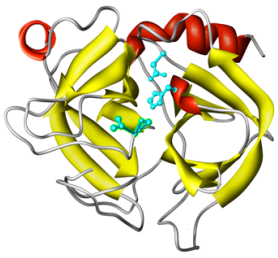
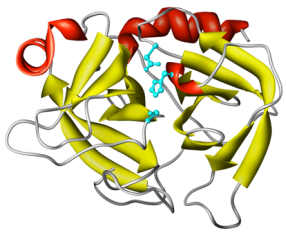
When do we infer homology?

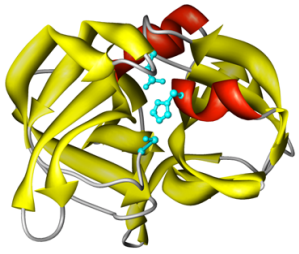
Homology <=> structural similarity
? sequence similarity



Bovine trypsin (5ptp)
Structure: E()< 10^{-23}; RMSD 0.0 A
Sequence: E()< 10^{-84} 100% 223/223



S. griseus trypsin (1sgt)
E()< 10^{-14} RMSD 1.6 A
E()< 10^{-19} 36%; 226/223



S. griseus protease A (2sga)
E()< 10^{-4}; RMSD 2.6 A
E()< 2.6 25%; 199/181

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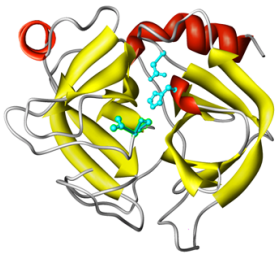
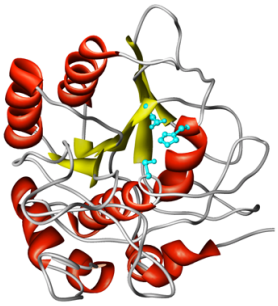
When can we infer non-homology?



Bovine trypsin (5ptp)
Structure: E()<10^{-23}
RMSD 0.0 A
Sequence: E()<10^{-84}
100% 223/223

Non-homologous proteins have different structures



Subtilisin (1sbt)
E() >100
E()<280; 25% 159/275

Cytochrome c4 (1etp)
E() > 100
E()<5.5; 23% 171/190

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Homology is confusing I: Homology defined Three(?) Ways

- Proteins/genes/DNA that share a common ancestor
- Specific positions/columns in a multiple sequence alignment that have a 1:1 relationship over evolutionary history
  - sequences are *50% homologous* ???
- Specific (morphological/functional) characters that share a recent divergence (clade)
  - bird/bat/butterfly wings are/are not homologous

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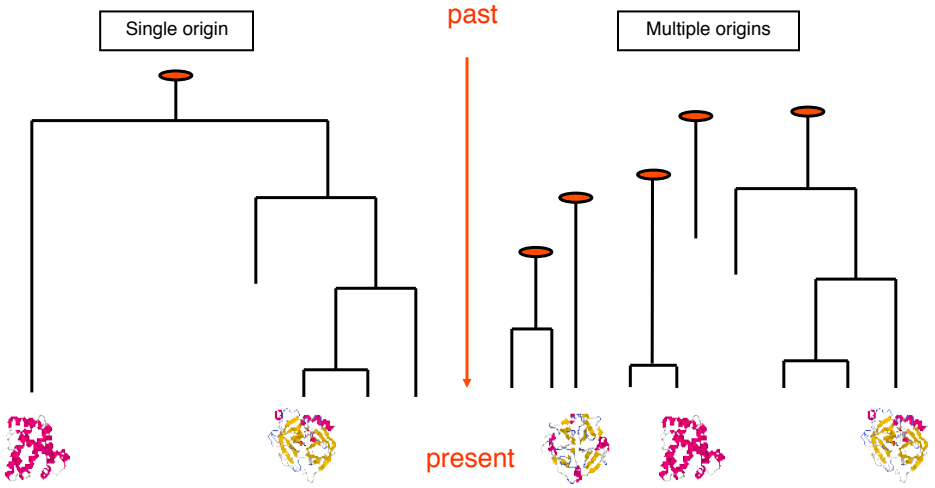
Homology is confusing II: Are All Sequences Homologous?

No Homology without excess similarity



Homology defined *My* Way

- Sequences are inferred to share a common ancestor based on statistically significant *excess* similarity. Any evidence of *excess* similarity can be used to infer homology
- Lack of evidence *cannot* be used to infer non-homology.
  - Proteins with different structures are non-homologous
- There are always two alternative hypotheses: homology (common ancestry), or convergence – one must weigh the evidence for each hypothesis (convergence is the *null* hypothesis).

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What BLAST does:

Similarity ? <=> Homology

Why BLAST works:

Statistical Significance ? <=> Biological Significance

Divergence ? Convergence

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Some important dates in history

Origin of the universe	–13.7[a]
Formation of the solar system	–4.6 ±0.4
First self-replicating system	–3.5 ±0.5
Prokaryotic-eukaryotic divergence	–2.5 ±0.3
Plant-animal divergence	-1.0
Invertebrate-vertebrate divergence	-0.5
Mammalian radiation beginning	-0.1

[a]Billions of years ago

Protein Family	PAMs[a]/100 res. /10^8 years	Protein Lookback time[b]	
Pseudogenes	400	45[c]	Primates,Rodents
Fibrinopeptides	90	200	Mammalian Radiation
Lactalbumins	27	670	Vertebrates
Ribonucleases	21	850	Animals
Hemoglobins	12	1.5[d]	Plants/Animals
Acid Proteases	8	2.3	Prokayrotic/Eukarotic
Triosphosphate isomerase	3	6	Archaen
Glutamate dehydrogenase	1	18	?

[a]PAMs, point accepted mutations. [b]Useful lookback time, 360 PAMs,15% identity.
[c]Millions of years. [d]Billions of years.

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E. coli proteins vs Human – Ancient Protein Domains

expect	%_id	alen	E coli descr	Human descr	sp_name
2.7e-206	53.8	944	glycine decarboxylase, P	Glycine dehydrogenase [de	GCSP_HUMAN
1.2e-176	59.5	706	methylmalonyl-CoA mutase	Methylmalonyl-CoA mutase,	MUTA_HUMAN
3.8e-176	50.6	803	glycogen phosphorylase [E	Glycogen phosphorylase, l	PHS1_HUMAN
9.9e-173	55.6	1222	B12-dependent homocystein	5-methyltetrahydrofolate-	METH_HUMAN
1.8e-165	41.8	1031	carbamoyl-phosphate synth	Carbamoyl-phosphate synth	CPSM_HUMAN
5.6e-159	65.7	542	glucosephosphate isomeras	Glucose-6-phosphate isome	G6PI_HUMAN
8.1e-143	53.7	855	aconitate hydrase 1 [Esch	Iron-responsive element b	IRE1_HUMAN
2.5e-134	73.0	459	membrane-bound ATP syntha	ATP synthase beta chain,	ATPB_HUMAN
3.3e-121	55.8	550	succinate dehydrogenase,	Succinate dehydrogenase [	DHSA_HUMAN
1.5e-113	60.6	401	putative aminotransferase	Cysteine desulfurase, mit	NFS1_HUMAN
4.4e-111	60.9	460	fumarase C= fumarate hydr	Fumarate hydratase, mitoc	FUMH_HUMAN
1.5e-109	56.1	474	succinate-semialdehyde de	Succinate semialdehyde de	SSDH_HUMAN
3.6e-106	44.7	789	maltodextrin phosphorylas	Glycogen phosphorylase, m	PHS2_HUMAN
1.4e-102	53.1	484	NAD+-dependent betaine al	Aldehyde dehydrogenase, E	DHAG_HUMAN
3.8e-98	53.0	449	pyridine nucleotide trans	NAD(P) transhydrogenase,	NNTM_HUMAN
5.8e-96	49.9	489	glycerol kinase [Escheric	Glycerol kinase, testis s	GKP2_HUMAN
2.1e-95	66.8	328	glyceraldehyde-3-phosphat	Glyceraldehyde 3-phosphat	G3P2_HUMAN
5.0e-91	62.5	368	alcohol dehydrogenase cla	Alcohol dehydrogenase cla	ADHX_HUMAN
6.7e-91	56.5	393	protein chain elongation	Elongation factor Tu, mit	EFTU_HUMAN
9.5e-91	56.6	392	protein chain elongation	Elongation factor Tu, mit	EFTU_HUMAN
2.2e-89	59.1	369	methionine adenosyltransf	S-adenosylmethionine synt	METK_HUMAN
6.5e-88	53.3	422	enolase [Escherichia coli	Alpha enolase (2-phospho-	ENOA_HUMAN
9.2e-88	43.3	536	NAD-linked malate dehydro	NADP-dependent malic enzy	MAOX_HUMAN
7.3e-86	55.5	389	2-amino-3-ketobutyrate Co	2-amino-3-ketobutyrate co	KBL_HUMAN
5.2e-83	44.4	543	degrades sigma32, integra	AFG3-like protein 2 (Para	AF32_HUMAN

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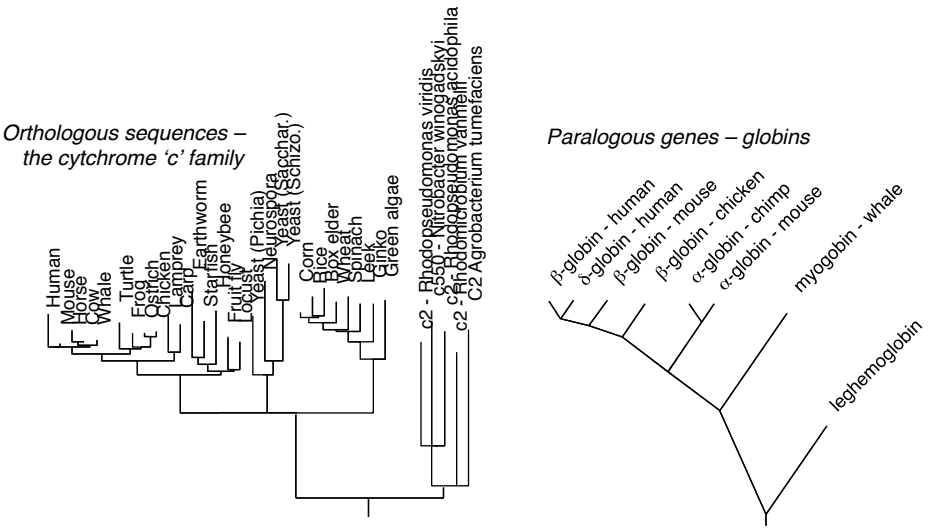
Orthologs and Paralogs – Inferring Function



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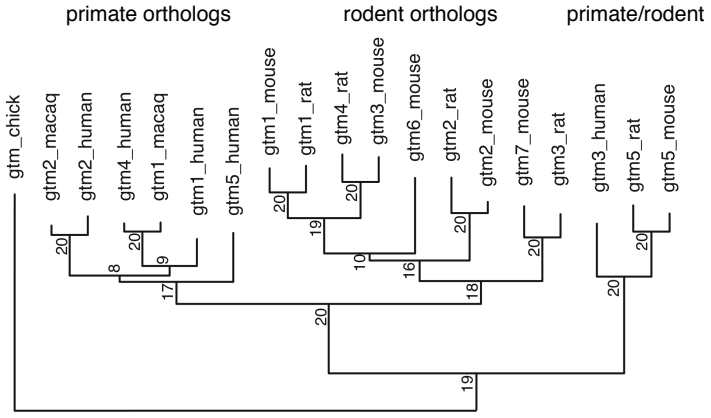
Orthology can be difficult to infer



Orthologs preserve function, but can be difficult to infer

- Over modest distances (human/mouse), post-speciation duplication is common
- Over large distances (human/fly, bacteria), duplication/loss/replacement may be common
- Homology inferences have false-negatives, but the false-positive rate can be reliably controlled
- Orthology inferences will have both false positives and false negatives
- Paralogous proteins often have similar functions

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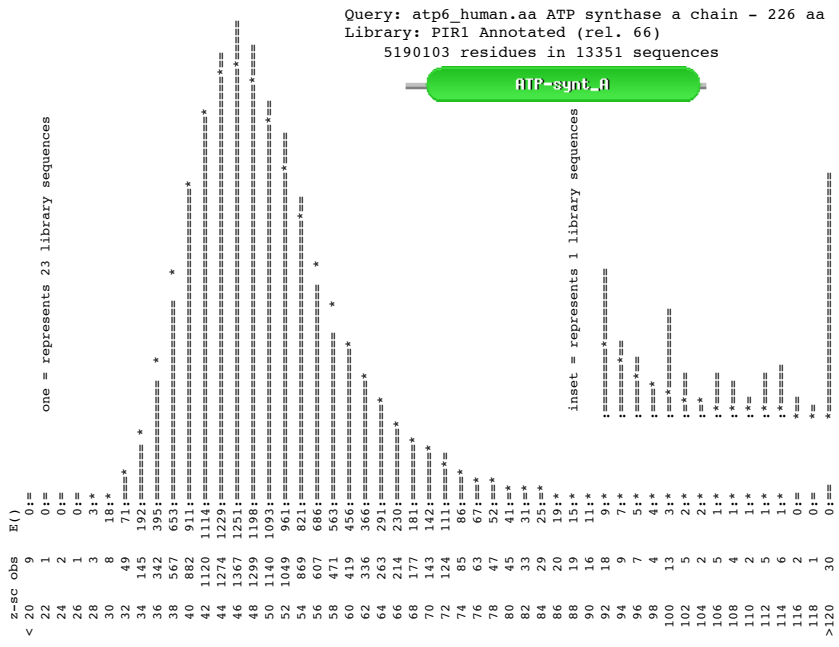

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Inferring Homology from Statistical Significance

- Real *UNRELATED* sequences have similarity scores that are indistinguishable from *RANDOM* sequences
- If a similarity is NOT *RANDOM*, then it must be NOT *UNRELATED*
- Therefore, NOT *RANDOM* (statistically significant) similarity must reflect *RELATED* sequences

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Query: atp6_human.aa ATP synthase a chain - 226 aa
Library: 5190103 residues in 13351 sequences

The best scores are:	(len)	s-w	bits	E(13351)	%_id	%_sim	alen
sp|P00846|ATP6_HUMAN ATP synthase a chain (AT	(226)	1400	325.8	5.8e-90	1.000	1.000	226
sp|P00847|ATP6_BOVIN ATP synthase a chain (AT	(226)	1157	270.5	2.5e-73	0.779	0.951	226
sp|P00848|ATP6_MOUSE ATP synthase a chain (AT	(226)	1118	261.7	1.2e-70	0.757	0.916	226
sp|P00849|ATP6_XENLA ATP synthase a chain (AT	(226)	745	176.8	4.0e-45	0.533	0.847	229
sp|P00851|ATP6_DROYA ATP synthase a chain (AT	(224)	473	115.0	1.7e-26	0.378	0.721	222
sp|P00854|ATP6_YEAST ATP synthase a chain pre	(259)	428	104.7	2.3e-23	0.353	0.694	232
sp|P00852|ATP6_EMENI ATP synthase a chain pre	(256)	365	90.4	4.8e-19	0.304	0.691	230
sp|P14862|ATP6_COCHE ATP synthase a chain (AT	(257)	353	87.7	3.2e-18	0.313	0.650	214
sp|P68526|ATP6_TRITI ATP synthase a chain (AT	(386)	309	77.6	5.1e-15	0.289	0.651	235
sp|P05499|ATP6_TOBAC ATP synthase a chain (AT	(395)	309	77.6	5.2e-15	0.283	0.635	233
sp|P07925|ATP6_MAIZE ATP synthase a chain (AT	(291)	283	71.7	2.3e-13	0.311	0.667	180
sp|P0AB98|ATP6_ECOLI ATP synthase a chain (AT	(271)	178	47.9	3.2e-06	0.233	0.585	236
sp|P0C2Y5|ATPI_ORYSA Chloroplast ATP synth (A	(247)	144	40.1	0.00062	0.242	0.580	231
sp|P06452|ATPI_PEA Chloroplast ATP synthase a	(247)	143	39.9	0.00072	0.250	0.586	232
sp|P27178|ATP6_SYNY3 ATP synthase a chain (AT	(276)	142	39.7	0.00095	0.265	0.571	170
sp|P06451|ATPI_SPIOL Chloroplast ATP synthase	(247)	138	38.8	0.0016	0.242	0.580	231
sp|P08444|ATP6_SYNP6 ATP synthase a chain (AT	(261)	127	36.3	0.0095	0.263	0.557	167
sp|P69371|ATPI_ATRBE Chloroplast ATP synthase	(247)	126	36.0	0.01	0.221	0.571	231
sp|P06289|ATPI_MARPO Chloroplast ATP synthase	(248)	126	36.0	0.011	0.240	0.575	167
sp|P30391|ATPI_EUGGR Chloroplast ATP synthase	(251)	123	35.4	0.017	0.257	0.579	214
sp|P19568|TLCA_RICPR ADP,ATP carrier protein	(498)	122	35.0	0.043	0.243	0.579	152
sp|P24966|CYB_TAYTA Cytochrome b	(379)	113	33.0	0.13	0.234	0.532	158
sp|P03892|NU2M_BOVIN NADH-ubiquinone oxidored	(347)	107	31.7	0.31	0.261	0.479	211
sp|P68092|CYB_STEAT Cytochrome b	(379)	104	31.0	0.54	0.277	0.547	137
sp|P03891|NU2M_HUMAN NADH-ubiquinone oxidored	(347)	103	30.8	0.58	0.201	0.537	149
sp|P00156|CYB_HUMAN Cytochrome b	(380)	102	30.5	0.74	0.268	0.585	205
sp|P15993|AROP_ECOLI Aromatic amino acid tr	(457)	103	30.7	0.78	0.234	0.622	111
sp|P24965|CYB_TRANA Cytochrome b	(379)	101	30.3	0.87	0.234	0.563	158
sp|P29631|CYB_POMTE Cytochrome b	(308)	99	29.9	0.95	0.274	0.584	113
sp|P24953|CYB_CAPHI Cytochrome b	(379)	99	29.8	1.2	0.236	0.564	140

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```
>>sp|P0AB98|ATP6_ECOLI ATP synthase a chain (ATPase protein 6) g  (271 aa)
 s-w opt: 178  Z-score: 218.2  bits: 47.9 E(): 3.2e-06
Smith-Waterman score: 178; 23.3% identity (58.5% similar) in 236 aa overlap (8-222:45-264)

                                          10        20        30        40
human                                   MNENLFASFIAPTILGLPAAVLIILFPPLLIPTSKYLINNRLITTQQ
                                          :..  ..:::   ....::  .   ...  . ... :. .
E coli NMTPQDYIGHHLNNLQLDLRTFSLVDPQNPPATFWTINIDSMFFSVVLGL---LFLVLFRSVAKKATSG-VPGKFQTAIE
          10        20        30        40        50          60        70         80

       50        60        70        80                90              100       110
human  WLIKLTSKQMMTMHNTKGRTWSLMLVSLIIFIATTNLLGLLP---------HSF-------TPTTQLSMNLAMAIPLWAG
        .: ... ..  :.. :..  . . .......   ::. :::         : .       .:.......:.::. ..
E coli LVIGFVNGSVKDMYHGKSKLIAPLALTIFVWVFLMNLMDLLPIDLLPYIAEHVLGLPALRVVPSADVNVTLSMALGVF--
              90       100       110       120       130       140       150

            120       130           140       150       160       170       180
human  TVIMGFRSKIKNALAHFLPQGTPTPL-----IPMLVIIETISLLIQPMALAVRLTANITAGHLLMHLIGSATLAMSTINL
        ... : :   .... :  . :  :.     ::. .:.: .::: .:..:..:: .:. ::.:.. ::..     :   :
E coli -ILILFYSIKMKGIGGFTKELTLQPFNHWAFIPVNLILEGVSLLSKPVSLGLRLFGNMYAGELIFILIAGLLPWWSQWIL
       160       170       180       190       200       210       220       230

        190       200       210       220
human  PSTLIIFTILILLTILEIAVALIQAYVFTLLVSLYLHDNT
            :: :::.          .::..: .:. .::
E coli NVPWAIFHILIIT---------LQAFIFMVLTIVYLSMASEEH
       240       250               260       270
```


The PAM250 matrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
Cys	12																			
Ser	0	2																		
Thr	-2	1	3																	
Pro	-1	1	0	6																
Ala	-2	1	1	1	2															
Gly	-3	1	0	-1	1	5														
Asn	-4	1	0	-1	0	0	2													
Asp	-5	0	0	-1	0	1	2	4												
Glu	-5	0	0	-1	0	0	1	3	4											
Gln	-5	-1	-1	0	0	-1	1	2	2	4										
His	-3	-1	-1	0	-1	-2	2	1	1	3	6									
Arg	-4	0	-1	0	-2	-3	0	-1	-1	1	2	6								
Lys	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5							
Met	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6						
Ile	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	5					
Leu	-6	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	6				
Val	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4			
Phe	-4	-3	-3	-5	-4	-5	-4	-6	-5	-5	-2	-4	-5	0	1	2	-1	9		
Tyr	0	-3	-3	-5	-3	-5	-2	-4	-4	-4	0	-4	-4	-2	-1	-1	-2	7	10	
Trp	-8	-2	-5	-6	-6	-7	-4	-7	-7	-5	-3	2	-3	-4	-5	-2	-6	0	0	17

Where do scoring matrices come from?

frequency of replacement in homologs

$$\lambda S = \log\left(\frac{q_{ij}}{p_i p_j}\right)$$

frequency of alignment by chance

- Scoring matrices can be designed for different evolutionary distances (less=shallow; more=deep)
- Deep matrices allow more substitution

Pam40

	A	R	N	D	E	I	L
A	8						
R	-9	12					
N	-4	-7	11				
D	-4	-13	3	11			
E	-3	-11	-2	4	11		
I	-6	-7	-7	-10	-7	12	
L	-8	-11	-9	-16	-12	-1	10

Pam250

	A	R	N	D	E	I	L
A	2						
R	-2	6					
N	0	0	2				
D	0	-1	2	4			
E	0	-1	1	3	4		
I	-1	-2	-2	-2	-2	5	
L	-2	-3	-3	-4	-3	2	6

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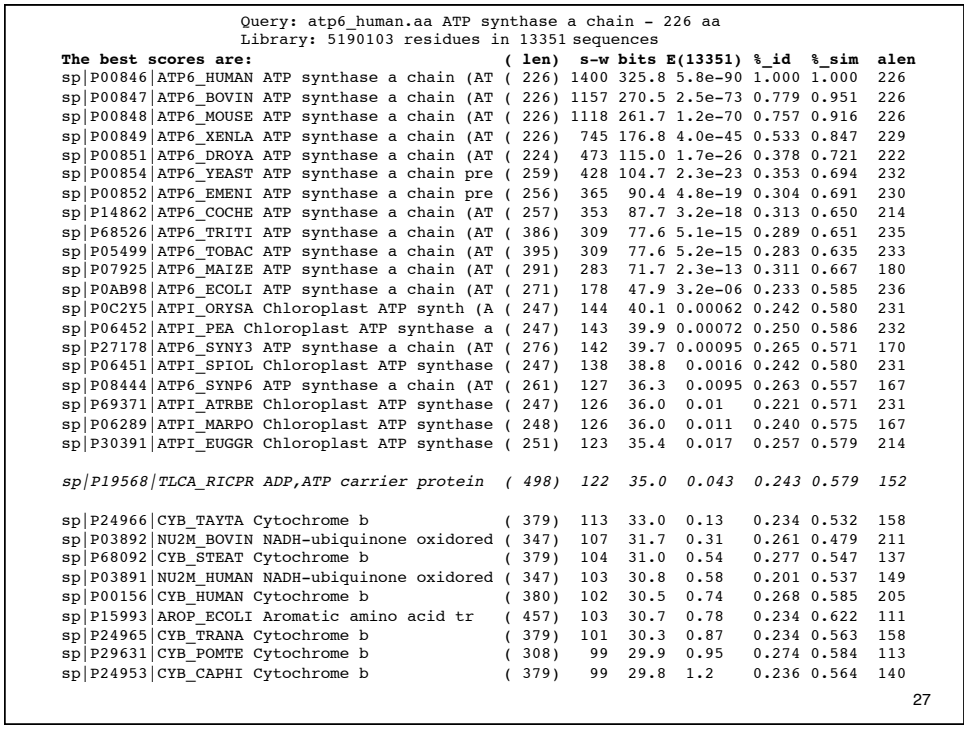
```
>>sp|P30391|ATPI_EUGGR Chloroplast ATP synthase a chain precursor   (251 aa)
 s-w opt: 123  Z-score: 151.3  bits: 35.4 E(): 0.017
Smith-Waterman score: 123; 25.7% identity (57.9% similar) in 214 aa overlap (21-222:50-243)

                                    10        20        30        40        50        60
human                          MNENLFASFIAPTILGLPAAVLIILFPPLLIPTSKYLINNRLITTQQWLIKLTSKQMMTM
                                                    .::: :  : : :.: :  . . ...: .:.:... .  .
Euglena VNMFISGIFQIANVEVGQHFYWSILGFQIHGQVLINSWIVILIIGF--LSIYTTKNL--TLVPANKQIFIELVTEFITDI
       10        20        30        40        50          60          70        80

                      70        80         90            100       110       120
human   HNTK-GRT----WSLMLVSLIIFIATTNLLG-LLPHSFT--PTTQL---SMNLAMAIPLWAGTVIMGFRSKI-KNALAHF
         .:. :.     :  .. ....:: ..:  : :.: ..   :. .:   . ..  .  :   : .  : . . :..:..:
Euglena SKTQIGEKEYSKWVPYIGTMFLFIFVSNWSGALIPWKIIELPNGELGAPTNDINTTAGLAILTSLAYFYAGLNKKGLTYF
          90        100       110       120       130       140       150       160

       130       140       150       160       170       180       190       200
Human   LPQGTPTPLIPMLVIIETISLLIQPMALAVRLTANITAGHLLMHLIGSATLAMSTINLPSTLIIFTILILLTILEIAVAL
            :::..  . :.: ..   .:..:. :: .:: : .:.. .. :         .:  ::. . ::.: ..   ..
Euglena KKYVQPTPILLPINILEDFT---KPLSLSFRLFGNILADELVVAVLVSL--------VP--LIVPVPLIFLGLF---TSG
         170       180          190       200       210               220

       210       220
human   IQAYVFTLLVSLYLHDNT
         ::: .:. : . :.
Euglena IQALIFATLSGSYIGEAMEGHH
      230       240       250
```

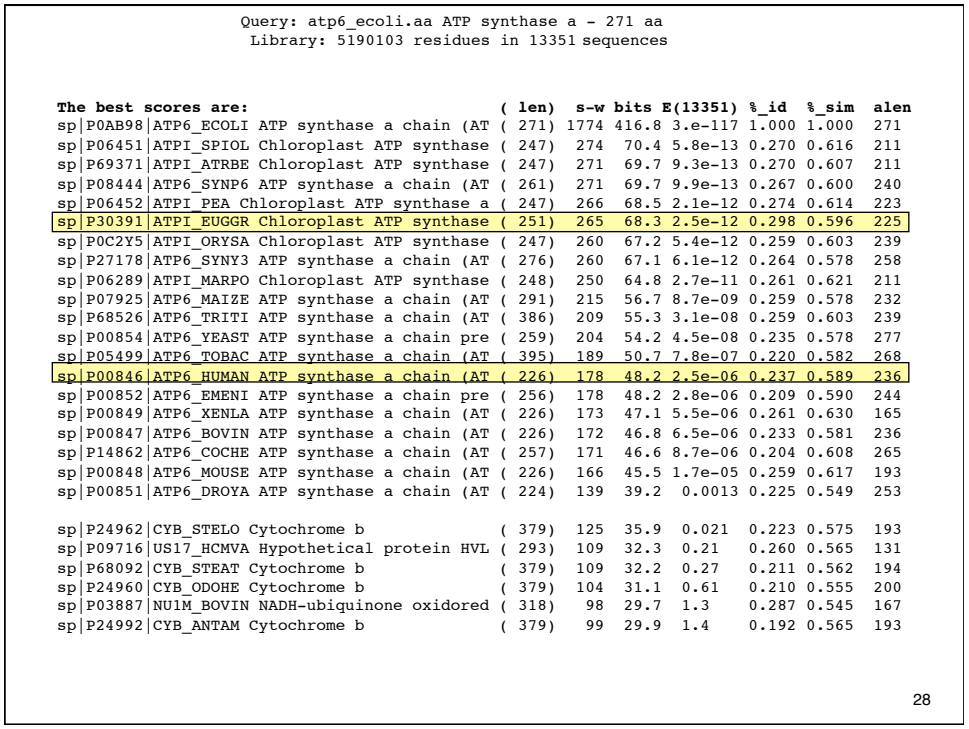
26



Query: atp6_human.aa ATP synthase a chain - 226 aa
Library: 5190103 residues in 13351 sequences

The best scores are:	(len)	s-w	bits	E(13351)	%_id	%_sim	alen
sp|P00846|ATP6_HUMAN ATP synthase a chain (AT	(226)	1400	325.8	5.8e-90	1.000	1.000	226
sp|P00847|ATP6_BOVIN ATP synthase a chain (AT	(226)	1157	270.5	2.5e-73	0.779	0.951	226
sp|P00848|ATP6_MOUSE ATP synthase a chain (AT	(226)	1118	261.7	1.2e-70	0.757	0.916	226
sp|P00849|ATP6_XENLA ATP synthase a chain (AT	(226)	745	176.8	4.0e-45	0.533	0.847	229
sp|P00851|ATP6_DROYA ATP synthase a chain (AT	(224)	473	115.0	1.7e-26	0.378	0.721	222
sp|P00854|ATP6_YEAST ATP synthase a chain pre	(259)	428	104.7	2.3e-23	0.353	0.694	232
sp|P00852|ATP6_EMENI ATP synthase a chain pre	(256)	365	90.4	4.8e-19	0.304	0.691	230
sp|P14862|ATP6_COCHE ATP synthase a chain (AT	(257)	353	87.7	3.2e-18	0.313	0.650	214
sp|P68526|ATP6_TRITI ATP synthase a chain (AT	(386)	309	77.6	5.1e-15	0.289	0.651	235
sp|P05499|ATP6_TOBAC ATP synthase a chain (AT	(395)	309	77.6	5.2e-15	0.283	0.635	233
sp|P07925|ATP6_MAIZE ATP synthase a chain (AT	(291)	283	71.7	2.3e-13	0.311	0.667	180
sp|P0AB98|ATP6_ECOLI ATP synthase a chain (AT	(271)	178	47.9	3.2e-06	0.233	0.585	236
sp|P0C2Y5|ATPI_ORYSA Chloroplast ATP synth (A	(247)	144	40.1	0.00062	0.242	0.580	231
sp|P06452|ATPI_PEA Chloroplast ATP synthase a	(247)	143	39.9	0.00072	0.250	0.586	232
sp|P27178|ATP6_SYNY3 ATP synthase a chain (AT	(276)	142	39.7	0.00095	0.265	0.571	170
sp|P06451|ATPI_SPIOL Chloroplast ATP synthase	(247)	138	38.8	0.0016	0.242	0.580	231
sp|P08444|ATP6_SYNP6 ATP synthase a chain (AT	(261)	127	36.3	0.0095	0.263	0.557	167
sp|P69371|ATPI_ATRBE Chloroplast ATP synthase	(247)	126	36.0	0.01	0.221	0.571	231
sp|P06289|ATPI_MARPO Chloroplast ATP synthase	(248)	126	36.0	0.011	0.240	0.575	167
sp|P30391|ATPI_EUGGR Chloroplast ATP synthase	(251)	123	35.4	0.017	0.257	0.579	214
sp|P19568|TLCA_RICPR ADP,ATP carrier protein	*(498)*	*122*	*35.0*	*0.043*	*0.243*	*0.579*	*152*
sp|P24966|CYB_TAYTA Cytochrome b	(379)	113	33.0	0.13	0.234	0.532	158
sp|P03892|NU2M_BOVIN NADH-ubiquinone oxidored	(347)	107	31.7	0.31	0.261	0.479	211
sp|P68092|CYB_STEAT Cytochrome b	(379)	104	31.0	0.54	0.277	0.547	137
sp|P03891|NU2M_HUMAN NADH-ubiquinone oxidored	(347)	103	30.8	0.58	0.201	0.537	149
sp|P00156|CYB_HUMAN Cytochrome b	(380)	102	30.5	0.74	0.268	0.585	205
sp|P15993|AROP_ECOLI Aromatic amino acid tr	(457)	103	30.7	0.78	0.234	0.622	111
sp|P24965|CYB_TRANA Cytochrome b	(379)	101	30.3	0.87	0.234	0.563	158
sp|P29631|CYB_POMTE Cytochrome b	(308)	99	29.9	0.95	0.274	0.584	113
sp|P24953|CYB_CAPHI Cytochrome b	(379)	99	29.8	1.2	0.236	0.564	140

27



Query: atp6_ecoli.aa ATP synthase a - 271 aa
Library: 5190103 residues in 13351 sequences

The best scores are:	(len)	s-w	bits	E(13351)	%_id	%_sim	alen
sp|P0AB98|ATP6_ECOLI ATP synthase a chain (AT	(271)	1774	416.8	3.e-117	1.000	1.000	271
sp|P06451|ATPI_SPIOL Chloroplast ATP synthase	(247)	274	70.4	5.8e-13	0.270	0.616	211
sp|P69371|ATPI_ATRBE Chloroplast ATP synthase	(247)	271	69.7	9.3e-13	0.270	0.607	211
sp|P08444|ATP6_SYNP6 ATP synthase a chain (AT	(261)	271	69.7	9.9e-13	0.267	0.600	240
sp|P06452|ATPI_PEA Chloroplast ATP synthase a	(247)	266	68.5	2.1e-12	0.274	0.614	223
sp|P30391|ATPI_EUGGR Chloroplast ATP synthase	(251)	265	68.3	2.5e-12	0.298	0.596	225
sp|P0C2Y5|ATPI_ORYSA Chloroplast ATP synthase	(247)	260	67.2	5.4e-12	0.259	0.603	239
sp|P27178|ATP6_SYNY3 ATP synthase a chain (AT	(276)	260	67.1	6.1e-12	0.264	0.578	258
sp|P06289|ATPI_MARPO Chloroplast ATP synthase	(248)	250	64.8	2.7e-11	0.261	0.621	211
sp|P07925|ATP6_MAIZE ATP synthase a chain (AT	(291)	215	56.7	8.7e-09	0.259	0.578	232
sp|P68526|ATP6_TRITI ATP synthase a chain (AT	(386)	209	55.3	3.1e-08	0.259	0.603	239
sp|P00854|ATP6_YEAST ATP synthase a chain pre	(259)	204	54.2	4.5e-08	0.235	0.578	277
sp|P05499|ATP6_TOBAC ATP synthase a chain (AT	(395)	189	50.7	7.8e-07	0.220	0.582	268
sp|P00846|ATP6_HUMAN ATP synthase a chain (AT	(226)	178	48.2	2.5e-06	0.237	0.589	236
sp|P00852|ATP6_EMENI ATP synthase a chain pre	(256)	178	48.2	2.8e-06	0.209	0.590	244
sp|P00849|ATP6_XENLA ATP synthase a chain (AT	(226)	173	47.1	5.5e-06	0.261	0.630	165
sp|P00847|ATP6_BOVIN ATP synthase a chain (AT	(226)	172	46.8	6.5e-06	0.233	0.581	236
sp|P14862|ATP6_COCHE ATP synthase a chain (AT	(257)	171	46.6	8.7e-06	0.204	0.608	265
sp|P00848|ATP6_MOUSE ATP synthase a chain (AT	(226)	166	45.5	1.7e-05	0.259	0.617	193
sp|P00851|ATP6_DROYA ATP synthase a chain (AT	(224)	139	39.2	0.0013	0.225	0.549	253
sp|P24962|CYB_STELO Cytochrome b	(379)	125	35.9	0.021	0.223	0.575	193
sp|P09716|US17_HCMVA Hypothetical protein HVL	(293)	109	32.3	0.21	0.260	0.565	131
sp|P68092|CYB_STEAT Cytochrome b	(379)	109	32.2	0.27	0.211	0.562	194
sp|P24960|CYB_ODOHE Cytochrome b	(379)	104	31.1	0.61	0.210	0.555	200
sp|P03887|NU1M_BOVIN NADH-ubiquinone oxidored	(318)	98	29.7	1.3	0.287	0.545	167
sp|P24992|CYB_ANTAM Cytochrome b	(379)	99	29.9	1.4	0.192	0.565	193

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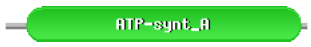
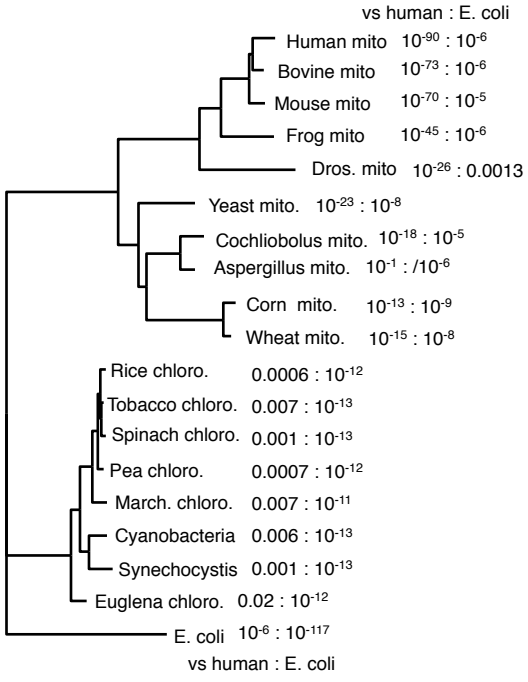
Homology is Transitive (on domains)





29

Homology and Domains – Histone deacetylase PCAF

```
The best scores are:                                                    s-w bits E(362341) %_id %_sim  alen
PCAF_HUMAN Histone acetyltransferase PCAF;                      ( 832) 4876 1092       0 1.000 1.000  832
PCAF_MOUSE Histone acetyltransferase PCAF;                      ( 813) 4507 1010       0 0.929 0.974  817
GCNL2_HUMAN General control of amino acid synthesis protein 5-l ( 837) 3535 793.       0 0.716 0.864  821
GCN5_YEAST Histone acetyltransferase GCN5                       ( 439) 1049 240. 5.2e-62 0.469 0.743  354
GCN5_ARATH Histone acetyltransferase GCN5;  AtGCN5              ( 568)  956 219. 1.2e-55 0.435 0.733  375
BPTF_HUMAN Nucleosome-remodeling factor subunit BPTF            (3046)  369 88.3 2.4e-15 0.495 0.773   97
NU301_DROME Nucleosome-remodeling factor subunit NURF301        (2669)  359 86.2 9.3e-15 0.511 0.787   94
CECR2_HUMAN Cat eye syndrome critical region protein 2          (1484)  306 74.6 1.6e-11 0.371 0.771  105
BRD4_HUMAN Bromodomain-containing protein 4; HUNK1 protein      (1362)  288 70.6 2.3e-10 0.379 0.681  116
BRDT_MACFA Bromodomain testis-specific protein                  ( 947)  270 66.7 2.3e-09 0.353 0.690  116
FSH_DROME Homeotic protein female sterile; Fragile-chorion memb (2038)  276 67.8 2.4e-09 0.341 0.651  129
BRDT_HUMAN Bromodomain testis-specific protein; RING3-like prot ( 947)  266 65.9 4.3e-09 0.345 0.690  116
Y0777_DICDI Bromodomain-containing protein DDB_G0280777         (1823)  260 64.3 2.5e-08 0.385 0.725  109
BRDT_MOUSE Bromodomain testis-specific protein; RING3-like prot ( 956)  247 61.6 8.1e-08 0.328 0.647  116
BAZ2B_HUMAN Bromodomain adjacent to zinc finger domain protein  (1972)  247 61.3   2e-07 0.343 0.695  105
TAF1_DROME Transcription initiation factor TFIID subunit 1; Tra (2129)  230 57.5 3.1e-06 0.349 0.689  106
82_SCHPO Bromodomain-containing protein C631.02                 ( 727)  217 55.0 5.9e-06 0.320 0.587  172
BRD9_XENLA Bromodomain-containing protein 9                     ( 527)  214 54.5 6.2e-06 0.292 0.579  171
GTE6_ARATH Transcription factor GTE6; Protein GENERAL TRANSCRIP ( 369)  201 51.7 2.9e-05 0.290 0.601  183
BAZ1B_MOUSE Bromodomain adjacent to zinc finger domain protein  (1479)  212 53.7 3.1e-05 0.302 0.583  139
K2_SCHPO Bromodomain-containing protein C1450.02                ( 578)  204 52.2 3.3e-05 0.310 0.628  113
TAF1_HUMAN Transcription initiation factor TFIID subunit 1; Tra (1872)  212 53.6 4.2e-05 0.339 0.678  115
BAZ1B_HUMAN Bromodomain adjacent to zinc finger domain protein  (1483)  209 53.0   5e-05 0.397 0.705   78
TIF1A_HUMAN Transcription intermediary factor 1-alpha;  TIF1-al (1050)  206 52.5 5.1e-05 0.384 0.698   86
BDF2_YEAST Bromodomain-containing factor 2                      ( 638)  200 51.3 6.9e-05 0.304 0.607  168
```

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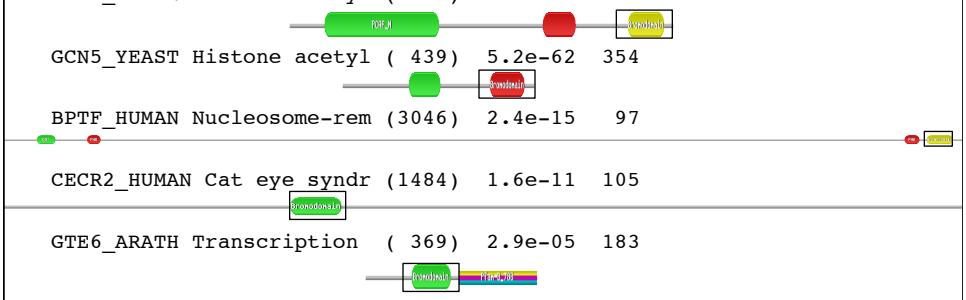
Homology and Domains – Histone deacetylase PCAF

```
The best scores are:                E(362341) alen
PCAF_HUMAN Histone acetyl ( 832)          0   832
GCN5_YEAST Histone acetyl ( 439)   5.2e-62   354
BPTF_HUMAN Nucleosome-rem (3046)   2.4e-15    97
CECR2_HUMAN Cat eye syndr (1484)   1.6e-11   105
GTE6_ARATH Transcription  ( 369)   2.9e-05   183
```



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Protein Evolution and Sequence Similarity

- What is Homology and how do we recognize it?
- How do we measure sequence similarity – alignments and scoring matrices?
- DNA vs protein comparison
- Practical Similarity Searching
- Similarity scoring matrices
- When are we certain that an alignment is significant - similarity score statistics?
- When to trust similarity statistics?
- Improving sensitivity with PSI-BLAST

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DNA vs protein sequence comparison

The best scores are:		DNA E(188,018)	tfastx3 E(187,524)	prot. E(331,956)
DMGST	D.melanogaster GST1-1	1.3e-164	4.1e-109	1.0e-109
MDGST1	M.domestica GST-1 gene	2e-77	3.0e-95	1.9e-76
LUCGLTR	Lucilia cuprina GST	1.5e-72	5.2e-91	3.3e-73
MDGST2A	M.domesticus GST-2 mRNA	9.3e-53	1.4e-77	1.6e-62
MDNF1	M.domestica nf1 gene. 10	4.6e-51	2.8e-77	2.2e-62
MDNF6	M.domestica nf6 gene. 10	2.8e-51	4.2e-77	3.1e-62
MDNF7	M.domestica nf7 gene. 10	6.1e-47	9.2e-77	6.7e-62
AGGST15	A.gambiae GST mRNA	3.1e-58	4.2e-76	4.3e-61
CVU87958	Culicoides GST	1.8e-41	4.0e-73	3.6e-58
AGG3GST11	A.gambiae GST1-1 mRNA	1.5e-46	2.8e-55	1.1e-43
BMO6502	Bombyx mori GST mRNA	1.1e-23	8.8e-50	5.7e-40
AGSUGST12	A.gambiae GST1-1 gene	2.3e-16	4.5e-46	5.1e-37
MOTGLUSTRA	Manduca sexta GST	5.7e-07	2.5e-30	8.0e-25
RLGSTARGN	R.legominosarum *gstA*	0.0029	3.2e-13	1.4e-10
HUMGSTT2A	H. sapiens GSTT2	0.32	3.3e-10	2.0e-09
HSGSTT1	H.sapiens GSTT1 mRNA	7.2	8.4e-13	3.6e-10
ECAE000319	E. coli hypothet. prot.	—	4.7e-10	1.1e-09
MYMDCMA	Methyl. dichlorometh. DH	—	1.1e-09	6.9e-07
BCU19883	Burkholderia maleylacetate red.	—	1.2e-09	1.1e-08
NFU43126	Naegleria fowleri GST	—	3.2e-07	0.0056
SP505GST	Sphingomonas paucim	—	1.8e-06	0.0002
EN1838	H. sapiens maleylaceto. iso.	—	2.1e-06	5.9e-06
HSU86529	Human GSTZ1	—	3.0e-06	8.0e-06
SYCCPNC	Synechocystis GST	—	1.2e-05	9.5e-06
HSEF1GMR	H.sapiens EF1g mRNA	—	9.0e-05	0.00065

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Sequence Similarity - Conclusions

- *Homologous* sequences share a common ancestor, but most sequences are *non-homologous*
- Always compare Protein Sequences
- Sequence Homology can be reliably inferred from statistically significant similarity (non-homology cannot from non-similarity)
- Homologous proteins share common structures, but not necessarily common functions
- Sequence statistical significance estimates are accurate (verify this yourself)$10^{-6} < E() < 10^{-3}$ is statistically significant
- Scoring matrices set evolutionary look back horizons - not every discovery is distant
- PSI-BLAST can be more sensitive, but with lower statistical accuracy

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Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. How to avoid mistakes (what to look out for)
5. When to do something different
6. PSI-BLAST – the most sensitive method

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1. What question to ask?

- Is there an homologous protein (a protein with a similar structure)?
- Does that homologous protein have a similar function?
- Does XXX genome have YYY (kinase, GPCR, …)?

Questions not to ask:

- Does this DNA sequence have a similar regulatory element (too short – never significant)?
- Does (non-significant) protein have a similar function/modification/antigenic site?

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2. What program to run?

- What is your query sequence?
  - protein – BLAST (NCBI), SSEARCH (EBI)
  - protein coding DNA (EST) – BLASTX (NCBI), FASTX (EBI)
  - DNA (structural RNA, repeat family) – BLASTN (NCBI), FASTA (EBI)
- Does XXX genome have YYY (protein)?
  - TBLASTX YYY vs XXX genome
  - TFASTX YYY vs XXX genome
- Does my protein contain repeated domains?
  - LALIGN (UVa http://fasta.bioch.virginia.edu)

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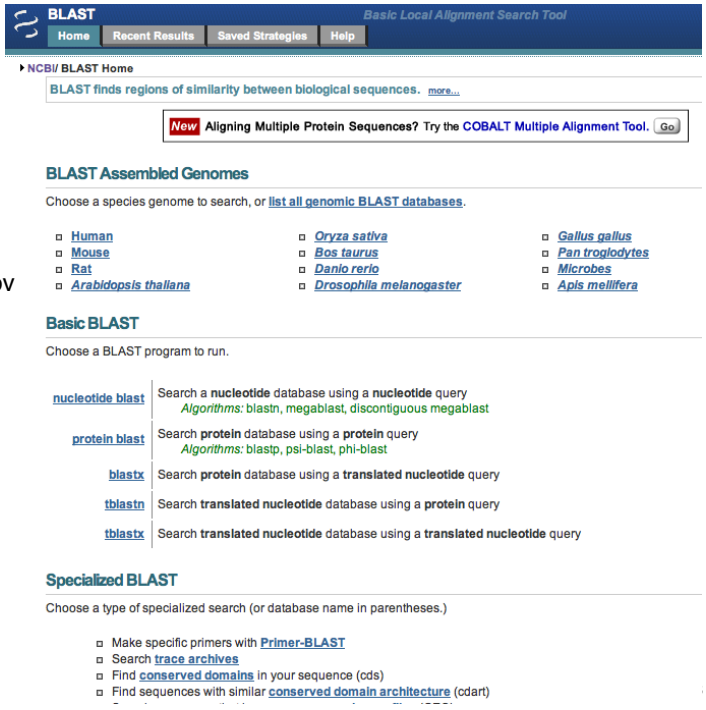
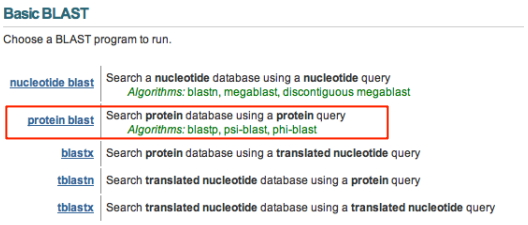
NCBI BLAST Server

blast.ncbi.nlm.nih.gov



NCBI BLAST Server

blast.ncbi.nlm.nih.gov



What is wrong with this picture?

Always compare protein sequences

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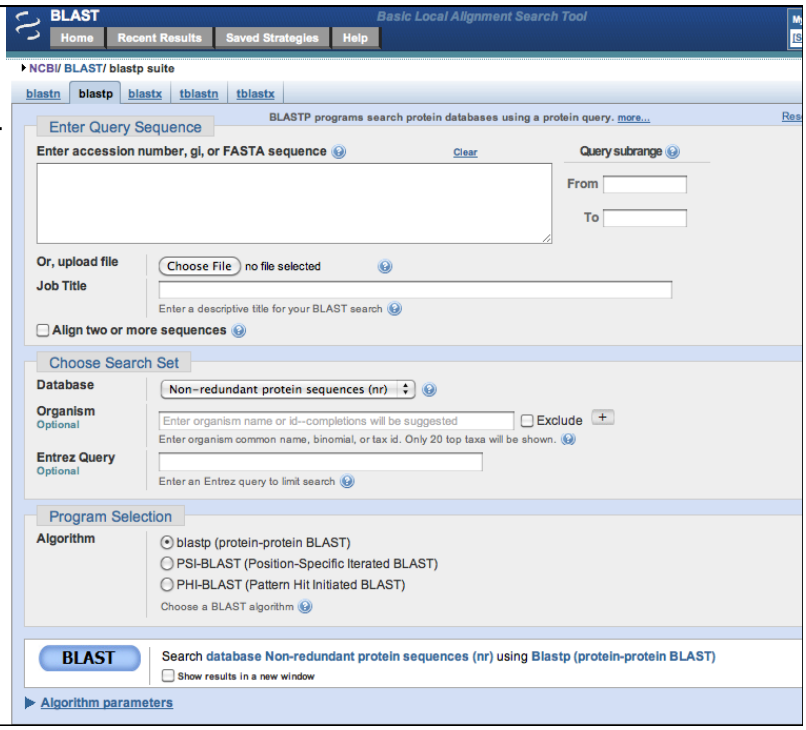
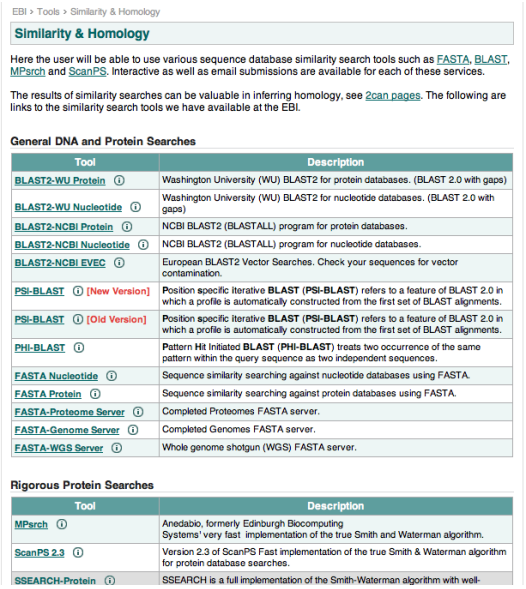
NCBI BLAST Server



Searching at the EBI
www.ebi.ac.uk/Tools/similarity.html



EBI > Tools > Similarity & Homology

Similarity & Homology

Here the user will be able to use various sequence database similarity search tools such as FASTA, BLAST, MPsrch and ScanPS. Interactive as well as email submissions are available for each of these services.

The results of similarity searches can be valuable in inferring homology, see 2can pages. The following are links to the similarity search tools we have available at the EBI.

General DNA and Protein Searches

Tool	Description
BLAST2-WU Protein	Washington University (WU) BLAST2 for protein databases. (BLAST 2.0 with gaps)
BLAST2-WU Nucleotide	Washington University (WU) BLAST2 for nucleotide databases. (BLAST 2.0 with gaps)
BLAST2-NCBI Protein	NCBI BLAST2 (BLASTALL) program for protein databases.
BLAST2-NCBI Nucleotide	NCBI BLAST2 (BLASTALL) program for nucleotide databases.
BLAST2-NCBI EVEC	European BLAST2 Vector Searches. Check your sequences for vector contamination.
PSI-BLAST [New Version]	Position specific iterative **BLAST (PSI-BLAST)** refers to a feature of BLAST 2.0 in which a profile is automatically constructed from the first set of BLAST alignments.
PSI-BLAST [Old Version]	Position specific iterative **BLAST (PSI-BLAST)** refers to a feature of BLAST 2.0 in which a profile is automatically constructed from the first set of BLAST alignments.
PHI-BLAST	Pattern Hit Initiated **BLAST (PHI-BLAST)** treats two occurrence of the same pattern within the query sequence as two independent sequences.
FASTA Nucleotide	Sequence similarity searching against nucleotide databases using FASTA.
FASTA Protein	Sequence similarity searching against protein databases using FASTA.
FASTA-Proteome Server	Completed Proteomes FASTA server.
FASTA-Genome Server	Completed Genomes FASTA server.
FASTA-WGS Server	Whole genome shotgun (WGS) FASTA server.

Rigorous Protein Searches

Tool	Description
MPsrch	Anedabio, formerly Edinburgh Biocomputing Systems' very fast implementation of the true Smith and Waterman algorithm.
ScanPS 2.3	Version 2.3 of ScanPS Fast implementation of the true Smith & Waterman algorithm for protein database searches.
SSEARCH-Protein	SSEARCH is a full implementation of the Smith-Waterman algorithm with well-

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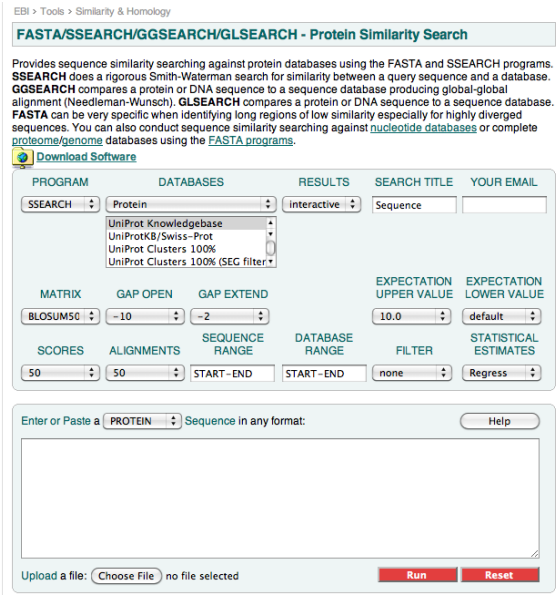
Searching at the EBI – ssearch



EBI > Tools > Similarity & Homology

FASTA/SSEARCH/GGSEARCH/GLSEARCH - Protein Similarity Search

Provides sequence similarity searching against protein databases using the FASTA and SSEARCH programs. **SSEARCH** does a rigorous Smith-Waterman search for similarity between a query sequence and a database. **GGSEARCH** compares a protein or DNA sequence to a sequence database producing global-global alignment (Needleman-Wunsch). **GLSEARCH** compares a protein or DNA sequence to a sequence database. **FASTA** can be very specific when identifying long regions of low similarity especially for highly diverged sequences. You can also conduct sequence similarity searching against nucleotide databases or complete proteome/genome databases using the FASTA programs.

Download Software

PROGRAM: SSEARCH | DATABASES: Protein (UniProt Knowledgebase, UniProtKB/Swiss-Prot, UniProt Clusters 100%, UniProt Clusters 100% (SEG filter) | RESULTS: interactive | SEARCH TITLE: Sequence | YOUR EMAIL:

MATRIX: BLOSUM50 | GAP OPEN: -10 | GAP EXTEND: -2 | EXPECTATION UPPER VALUE: 10.0 | EXPECTATION LOWER VALUE: default

SCORES: 50 | ALIGNMENTS: 50 | SEQUENCE RANGE: START-END | DATABASE RANGE: START-END | FILTER: none | STATISTICAL ESTIMATES: Regress

Enter or Paste a PROTEIN Sequence in any format: Help

Upload a file: Choose File no file selected Run Reset

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3. What database to search?

- Search the smallest comprehensive database likely to contain your protein
  - vertebrates – human proteins (40,000)
  - fungi – S. cerevisiae (6,000)
  - bacteria – E. coli, gram positive, etc. (<100,000)
- Search a richly annotated protein set (SwissProt, 450,000)
- Always search NR (~10 million) *LAST*
- Never Search "GenBank" (DNA)

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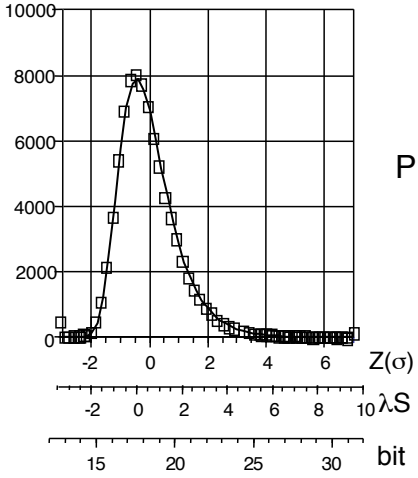
Extreme value distribution



$S' = \lambda S_{raw} - \ln K\ m\ n$

$S_{bit} = (\lambda S_{raw} - \ln K)/\ln(2)$

$P(S'>x) = 1 - \exp(-e^{-x})$

$P(S_{bit} > x) = 1 - \exp(-mn2^{-x})$

$E(S'>x \mid D) = P\ D$

$P(B \text{ bits}) = m\ n\ 2^{-B}$

$P(40 \text{ bits}) = 1.5 \times 10^{-7}$

$E(40 \mid D=4000) = 6 \times 10^{-4}$

$E(40 \mid D=10E6) = 1.5$

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Statistical Significance and Database Size

atp6_human vs E. coli
>>refINP_290377.1l F0F1 ATP synthase subunit [E. ecoli] (271 aa)
 s-w opt: 178 Z-score: 188.8 bits: 42.4 E(): 4.4e-05
Smith-Waterman score: 178; 23.3% identity (58.5% similar) in 236 aa overlap (8-222:45-264)

Database	Entries	Length	E()	hits	time (s)
E. coli	4,237	1.3 E 06	1.5 E-06*	1	< 0.5
S. cerevisiae	5,866	2.9 E 06	2.1 E-06	1	< 0.5
Human	38,114	18.4 E 06	1.2 E-05	1	1.1
Swiss Prot	4.3 E 05	1.5 E 08	2.4 E-05*	393	7.1
Refseq NP only	7.1 E 05	2.6 E 08	0.00017*	504	10.8
Refseq	7.3 E 06	2.5 E 09	0.0017*	2767	124
NR	9.9 E 06	3.4 E 09	0.0032*	7773	151

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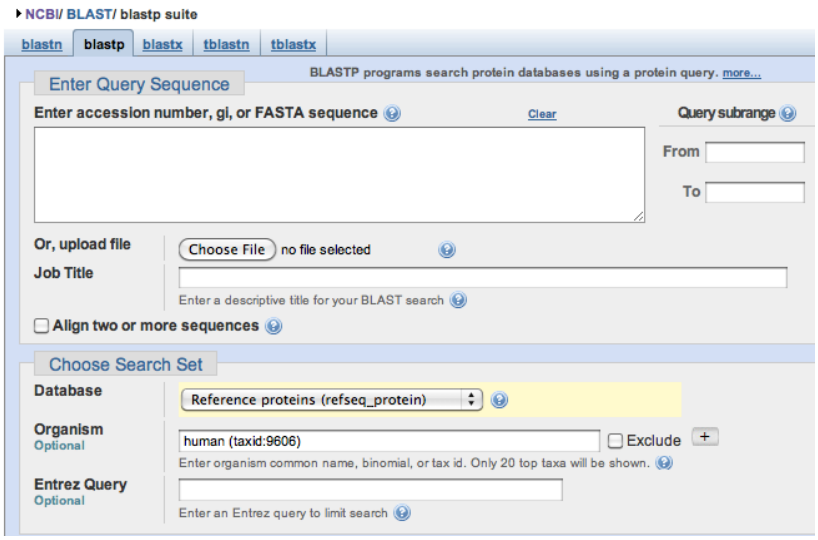
NCBI – selecting sequences with Entrez



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Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. How to avoid mistakes (what to look out for)
5. When to do something different
6. PSI-BLAST – the most sensitive method

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Inferring Homology from Statistical Significance

- Real *UNRELATED* sequences have similarity scores that are indistinguishable from *RANDOM* sequences
- If a similarity is NOT *RANDOM*, then it must be NOT *UNRELATED*
- Therefore, NOT *RANDOM* (statistically significant) similarity must reflect *RELATED* sequences

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Smith-Waterman (ssearch)

```
The best scores are:                      s-w bits E(115640)  %_id alen
GTM1_MOUSE Glutathione S-trans ( 218) 1497 363.5  2e-100  1.000  218
GTM2_CHICK Glutathione S-trans ( 220)  958 234.9 1.1e-61  0.619  218
GTP_HUMAN  Glutathione S-trans ( 210)  356  91.2 1.8e-18  0.308  211
PGD2_MOUSE Glutathione-req.    ( 199)  262  68.8 9.7e-12  0.319  204
GTA1_MOUSE Glutathione S-trans ( 223)  229  60.9 2.6e-09  0.284  225
SC1_OCTDO  S-crystallin 1 OL1  ( 215)  228  60.7 3.0e-09  0.269  219
GTS_MUSDO  Glutathione S-trans ( 241)  228  60.6 3.4e-09  0.264  201
GTS1_CAEEL Prob. Glut. S-trans ( 210)  220  58.8 1.1e-08  0.284  225
GTS_OMMSL  Glutathione S-trans ( 203)  196  53.0 5.5e-07  0.258  209
GTH3_ARATH Glutathione S-trans ( 215)  142  40.1  0.0045  0.310  126
GTT2_HUMAN Glutathione S-trans ( 244)  132  37.7   0.027  0.257  167
GT24_DROME Glutathione S-trans ( 216)  131  37.5   0.028  0.255  153
YFCG_ECOLI Hypothetical GST    ( 215)  112  33.0    0.64  0.235  187
YJY1_YEAST hypothetical 30.5   ( 261)  110  32.4  *1.1*   0.248  149
DCMA_METS1 dichloromethane DM  ( 267)  103  30.8    3.7   0.214  210
YA42_HAEIN Hypothetical prot.  ( 617)  108  31.7  *4.6*   0.283  120
GTO1_RAT   Glutathione trans   ( 241)  100  30.1    5.4   0.234  158
DP41_BACHD DNA polymerase I    ( 413)  104  30.8  *5.4*   0.234  184
GTH1_WHEAT Glutathione S-trans ( 229)   98  29.6    7.0   0.246  171
LGUL_SOYBN Lactoylglutathione  ( 219)   97  29.4    7.8   0.200  190
```

Highest scoring unrelated sequence E() ~ 1.0

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Unrelated ≠ Random (low complexity)

Search with complete grou_drome:

```
The best scores are:                                      opt  bits  E(14548)
RGHUB1 GTP-binding regulatory protein beta-1 chai  ( 341)  237  46.6  3.5e-05
RGBOB1 GTP-binding regulatory protein beta-1 chai  ( 341)  237  46.6  3.5e-05
RGHUB3 GTP-binding regulatory protein beta-3 chai  ( 341)  233  46.0  5.2e-05
RGMSB4 GTP-binding regulatory protein beta-4 chai  ( 341)  232  45.8  5.7e-05
PIHUPF salivary proline-rich glycoprotein precurs  ( 252)  224  44.5 *0.00010*
RGFFB  GTP-binding regulatory protein beta chain   ( 347)  223  44.5  0.00014
PIRT3  acidic proline-rich protein precursor - rat ( 207)  199  40.8 *0.0011*
PIHUB6 salivary proline-rich protein precursor PR  ( 393)  203  41.6 *0.0012*
CGBO2S collagen alpha 2(I) chain - bovine (fragme  ( 403)  195  40.5 *0.0027*
WMBEW6 capsid protein - human herpesvirus 1 (stra  ( 636)  192  40.2 *0.0051*
```

Search with seg-ed grou_drome: (low complexity regions removed)

```
The best scores are:                                      opt bits E(14548)
RGHUB3 GTP-binding regulatory protein beta-3 chai  ( 341)  233  56.5 3.6e-08
RGMSB4 GTP-binding regulatory protein beta-4 chai  ( 341)  232  56.3 4.1e-08
RGHUB2 GTP-binding regulatory protein beta-2 chai  ( 341)  228  55.5 7.2e-08
RGBOB1 GTP-binding regulatory protein beta-1 chai  ( 341)  225  54.9 1.1e-07
RGFFB  GTP-binding regulatory protein beta chain   ( 347)  223  54.5 1.5e-07
BVBYMS MSI1 protein - yeast (Saccharomyces cerevi  ( 423)  135  37.0 *0.033*
ERHUAH coatomer complex alpha chain homolog - hum  (1225)  134  37.1 *0.088*
A28468 chromogranin A precursor - human            ( 458)  122  34.4 *0.21*
RGOOBE GTP-binding regulatory protein beta chain   ( 342)  120  33.9  0.22
```

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`pseg` removes low-complexity regions

```
>gi|17380405|sp|P16371|GROU_DROME Groucho protein (Enhancer of split M9/10)

                              1-8    MYPSPVRH
                    paaggpppqgp     9-19
                             20-131  IKFTIADTLERIKEEFNFLQAQYHSIKLEC
                                     EKLSNEKTEMQRHYVMYYEMSYGLNVEMHK
                                     QTEIAKRLNTLINQLLPFLQADHQQQVLQA
                                     VERAKQVTMQELNLIIGQQIHA
                   qqvpggppqpmg  132-143
                             144-281 ALNPFGALGATMGLPHGPQGLLNKPPEHHR
                                     PDIKPTGLEGPAAAEERLRNSVSPADREKY
                                     RTRSPLDIENDSKRRKDEKLQEDEGEKSDQ
                                     DLVVDVANEMESHSPRPNGEHVSMEVRDRE
                                     SLNGERLEKPSSSGIKQE
               rppsrsgssssrstps  282-297
                             298-310 LKTKDMEKPGTPG
             akartptpnaaapapgvnpk  311-330
            qmmpqgpppagypgapyqrpa  331-351
                             352-719 DPYQRPPSDPAYGRPPPMPYDPHAHVRTNG
                                     IPHPSALTGGKPAYSFHMNGEGSLQPVPFP
                                     PDALVGVGIPRHARQINTLSHGEVVCAVTI
                                     SNPTKYVYTGGKGCVKVWDISQPGNKNPVS
                                     QLDCLQRDNYIRSVKLLPDGRTLIVGGEAS
                                     NLSIWDLASPTPRIKAELTSAAPACYALAI
                                     SPDSKVCFSCCSDGNIAVWDLHNEILVRQF
                                     QGHTDGASCIDISPDGSRLWTGGLDNTVRS
                                     WDLREGRQLQQHDFSSQIFSLGYCPTGDWL
                                     AVGMENSHVEVLHASKPDKYQLHLHESCVL
                                     SLRFAACGKWFVSTGKDNLLNAWRTPYGAS
                                     IFQSKETSSVLSCDISTDDKYIVTGSGDKK
                                     ATVYEVIY
```

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Statistical estimates from random shuffles

- BLAST estimates statistical significance from simulations of “normal” (average composition) proteins
- FASTA estimates statistical significance from the distribution of similarity scores obtained during the database search (selects 60,000 unrelated sequence scores from the database of *real* proteins)
- What if the sequences are different from most proteins, but similar to each other, e.g. membrane proteins?
- PRSS estimates statistical significance by producing hundreds of shuffled (random) sequences with the same length and composition, and then estimates λ and K from comparisons against those proteins

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prss - uniform and window shuffle

```
>LWEC6_H+-transporting ATP synthase (EC 3.6.1.34) protein 6 - Escherichia coli
MASENMTPQD YIGHHLNNLQ LDLRTFSLVD PQNPPATFWT INIDSMFFSV VLGLLFLVLF
RSVAKKATSG VPGKFQTAIE LVIGFVNGSV KDMYHGKSKL IAPLALTIFV WVFLMNLMDL
LPIDLLPYIA EHVLGLPALR VVPSADVNVT LSMALGVFIL ILFYSIKMKG IGGFTKELTL
QPFNHWAFIP VNLILEGVSL LSKPVSLGLR LFGNMYAGEL IFILIAGLLP WWSQWILNVP
WAIFHILIIT LQAFIFMVLT IVYLSMASEE H

>lwec6_0 shuffled
GMPISVLLFK PPEVLLVFLL SVMGTNFPAW GGFIMKGFKI VSFVGWVRFV AVAGHLALYK
ITRDVNIVKS AVFGSALLHP LLLQLSELNL VFVNLLNIKI RTAYVHGMTL LSHIPLFPAS
GEGVFSDMLM IITWNSASVL SGLDMFANIA LLGNPLLMTN IVIILQRKFI ATTKFSLADI
HLHKQYSWDG MMSHTLIIFS ALELWVQNGD IFIPLNEYIL PFTLYVPNWL ITQALVVALV
ELPGQQIDAE PLFLLPIPFS EKTWYGDIMF L

PRSS34 - 1000 shuffles;  uniform shuffle
 unshuffled s-w score: 178; bits(s=178|n_l=271): 34.8 p(178) < 2.005e-06
For 10000 sequences, a score >= 178 is expected 0.02005 times

>lwec6_0 shuffled window: 10
EDSMANTMPQ HQNILGYHLN DLRTSDFVLL FTQAPWPTPN SMNIDIVFSF VLLVLLFFGL
SRGAVKATKS EQVTGIKFAP VVSGVILGFN HDKGMSLYKK VLPIIFLAAT DWLMNFVLLM
IIDLYLLAPP ERVGHPLLAL APNVVVSVDT MLFLIGSALV IFSLMKGIKY TTIFGLEKGL
QAWNFFPHIP NLSVEVGLLI GLPVRSSLKL MFLELAGNGY PFGILILILA SLINVWPWQW
IAIIWTIFHL VQMTFFLAIL VSESELMIYA H

PRSS34 - 1000 shuffles;  window shuffle, window size: 20
 unshuffled s-w score: 178; bits(s=178|n_l=271): 34.5 p(178) < 2.601e-06
For 10000 sequences, a score >= 178 is expected 0.02602 times
```

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Similarity Searching II

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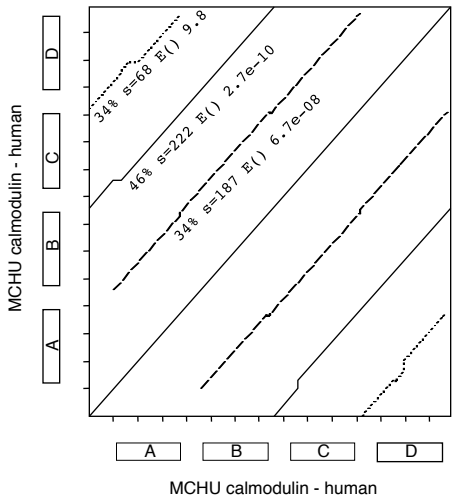
Local alignments - calmodulin

```
 46.1% identity in 76 aa overlap (1-76:77-149); score:  222 E(10000): 2.7e-10
               10        20        30        40        50        60
mchu    MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADG
        : :  .::.:   .::: .:::::.: :.. ::  :: .::.. :. :...:: :.: ::
mchu    MKDTDSEEEI---REAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDG
          80           90        100       110       120       130
                70
mchu    NGTIDFPEFLTMMARK
        .: ... ::. ::. :
mchu    DGQVNYEEFVQMMTAK
            140

  34.3% identity in 105 aa overlap (11-111:47-147); score:  187 E(10000): 6.7e-08
                20        30         40        50        60
mchu    AEFKEAFSLFDKDGDGTITTKELGTVM-RSLGQNPTEAELQDMINEVDADGNGTIDFPEF
        ::... ..  : ::.:::   :. :.: :.. .. .: :... .   : :::: :.  :.
mchu    AELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAEL
          50        60        70        80        90       100
          70        80        90       100       110
mchu    ---LTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMT
           .: ...:. : . .: ::::    : ::.: ..  :. ..::
mchu    RHVMTNLGEKLTDEEVDEMIREA----DIDGDGQVNYEEFVQMMT
         110       120           130       140

  34.2% identity in 38 aa overlap (1-37:113-146); score:   68 E(10000):    9.8
               10        20        30
mchu    MADQLTEEQIAEF-KEAFSLFDKDGDGTITTKELGTVM
        ....:.:.. :. .::    : :::: .. .:.  .:
mchu    LGEKLTDEEVDEMIREA----DIDGDGQVNYEEFVQMM
             120           130       140
```


Repeated domains with local alignments



More about scoring matrices ...

PAM series:

- Evolutionary model - extrapolated from PAM1
- PAM20: 20% change (mammals)
- PAM250: 250% change (<20% identity)
- Gap penalties should vary
- shallow matrices (PAM10-40) for short sequences and short distances

BLOSUM series

- Empirically determined, no extrapolation (no model)
- BLOSUM45-50 - distant (1/3 bits)
- BLOSUM80 -very highly conserved (not small change), high info/position
- BLOSUM62 - 1/2 bits

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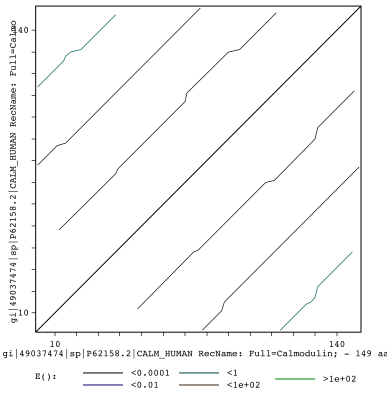
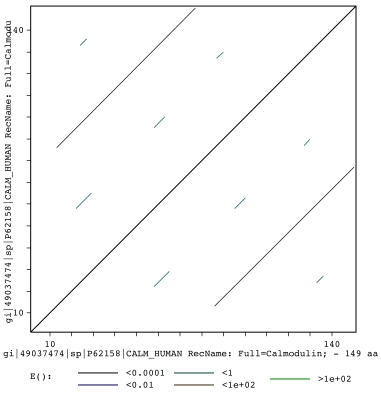
Scoring matrices set look back time

BLOSUM50 -10/-2



MD20 -26/-4



Where do scoring matrices come from?

Pam40

	A	R	N	D	E	I	L
A	8						
R	-9	12					
N	-4	-7	11				
D	-4	-13	3	11			
E	-3	-11	-2	4	11		
I	-6	-7	-7	-10	-7	12	
L	-8	-11	-9	-16	-12	-1	10

Pam250

	A	R	N	D	E	I	L
A	2						
R	-2	6					
N	0	0	2				
D	0	-1	2	4			
E	0	-1	1	3	4		
I	-1	-2	-2	-2	-2	5	
L	-2	-3	-3	-4	-3	2	6

q_{ij} : replacement frequency at PAM40, 250

$q_{R:N\,(40)} = 0.000435$ $\quad p_R = 0.051$

$q_{R:N\,(250)} = 0.002193$ $\quad p_N = 0.043$

$\lambda_2\, S_{ij} = \lg_2 (q_{ij}/p_i p_j)$ $\quad \lambda_e\, S_{ij} = \ln(q_{ij}/p_i p_j)$ $\quad p_R p_N = 0.002193$

$\lambda_2\, S_{R:N(40)} = \lg_2 (0.000435/0.00219) = -2.333$

$\lambda_2 = 1/3$; $S_{R:N(40)} = -2.333/\lambda_2 = -7$

$\lambda\, S_{R:N(250)} = \lg2\ (0.002193/0.002193) = 0$

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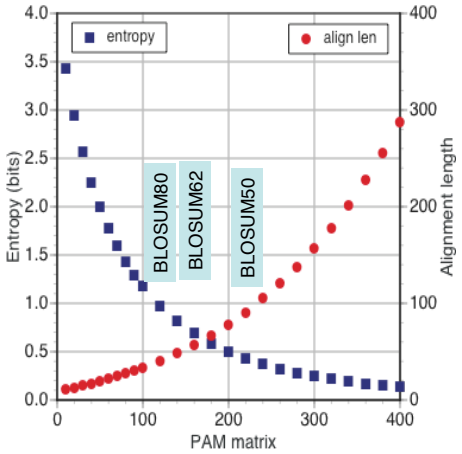
PAM matrices and alignment length



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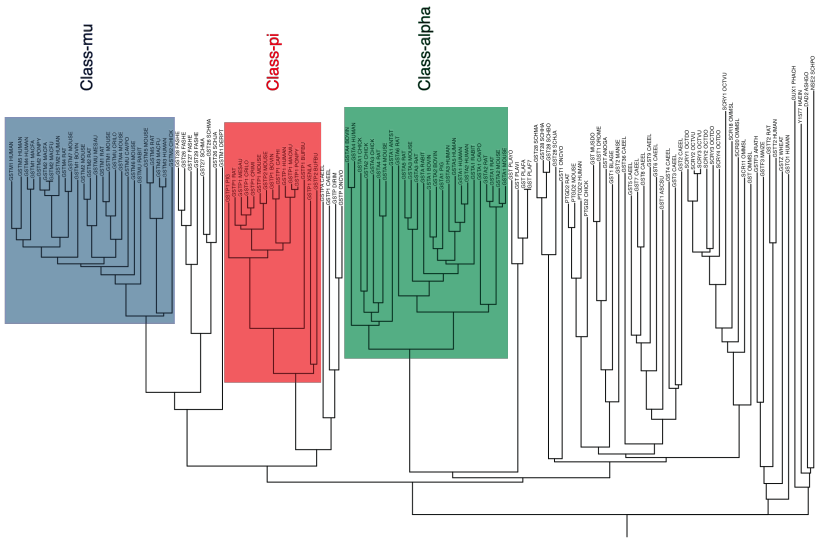
Glutathione Transferases (gstm1_human)



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		BLOSUM50-10/-2 E(320363)	f_id	BLOSUM62-11/-1 E(320363)	f_id	MD40 -21/-4 E(320363)	f_id	MD10 -23/-4 E(320363)	f_id
Class-mu	GSTM1_HUMAN	1.3e-101	1.00	5.1e-132	1.000	0	1.000	0	1.000
	GSTM4_HUMAN	1.9e-89	0.867	1.1e-115	0.867	2.2e-188	0.867	1.9e-193	0.867
	GSTM2_MOUSE	3.0e-87	0.839	3.6e-113	0.839	1.4e-184	0.847	2.5e-187	0.847
	GSTM5_HUMAN	4.9e-87	0.876	6.9e-114	0.876	4.7e-187	0.876	7.2e-195	0.912
	GSTM2_HUMAN	8.2e-87	0.844	8.2e-113	0.844	2.6e-182	0.844	1.3e-184	0.844
	GSTM1_MOUSE	7.0e-83	0.780	2.5e-107	0.780	4.7e-169	0.780	1.5e-162	0.780
	GSTM6_MOUSE	1.9e-82	0.775	1.0e-106	0.775	5.1e-168	0.779	1.3e-161	0.779
	GSTM4_MOUSE	8.7e-82	0.769	4.7e-105	0.769	7.7e-166	0.769	2.1e-158	0.769
	GSTM5_MOUSE	6.9e-73	0.727	3.5e-94	0.727	1.3e-142	0.727	3.7e-128	0.727
	GSTM3_HUMAN	8.2e-73	0.731	6.7e-95	0.731	3.4e-143	0.731	8.2e-129	0.731
	GSTM2_CHICK	9.8e-65	0.656	4.7e-84	0.656	3.0e-117	0.656	1.4e-93	0.675
	GST26_FASHE	2.9e-44	0.495	1.3e-56	0.491	2.7e-59	0.502	3.2e-18	0.510
	GSTM1_DERPT	5.2e-42	0.467	1.6e-53	0.487	5.1e-57	0.505	2.4e-29	0.651
	GST27_SCHMA	2.4e-37	0.467	9.5e-49	0.458	4.7e-42	0.470	5.1e-20	0.607
Class-pi	GSTP1_PIG	2.9e-20	0.327	1.2e-25	0.327	0.00034	0.409		
	GSTP1_XENLA	5.2e-19	0.333	6.0e-24	0.330	0.12	0.464		
	GSTP2_MOUSE	8.0e-17	0.294	1.3e-20	0.294	1.1	0.395		
	GSTP1_CAEEL	1.1e-16	0.324	4.3e-21	0.319	1.1	0.706		
	GSTP1_HUMAN	3.0e-16	0.284	2.2e-20	0.284	0.29	0.467		
	GSTP1_BUFBU	1.2e-14	0.285	7.2e-18	0.272	9.7	0.588		
	GSTPA_CAEEL	1.1e-13	0.298	2.8e-17	0.284	0.002	0.400		
	PTGD2_MOUSE	4.8e-12	0.302	2.6e-14	0.293				
	PTGD2_RAT	4.8e-12	0.302	1.5e-14	0.293				
	PTGD2_HUMAN	1.1e-11	0.292	4.0e-13	0.281				
	PTGD2_CHICK	9.8e-11	0.304	6.9e-13	0.302				
	GSTP2_BUFBU	2.0e-10	0.288	2.2e-12	0.307				
	GST_MUSDO	5.8e-09	0.257	2.3e-11	0.251				
	GST1_DROME	1.0e-08	0.255	2.9e-10	0.237				
Class-alpha	GSTA1_MOUSE	1.5e-08	0.279	4.9e-11	0.264				
	GSTA2_HUMAN	6.6e-08	0.286	1.2e-08	0.273				
	GSTA5_HUMAN	7.8e-08	0.275	1.2e-08	0.259				
	GSTA2_MOUSE	1.1e-07	0.269	9.9e-10	0.255				
	GSTA3_MOUSE	1.3e-07	0.278	8.9e-09	0.258				
	GSTA1_HUMAN	3.0e-07	0.272	8.0e-08	0.259				
	GST36_CAEEL	3.3e-07	0.256	1.1e-08	0.264				
	GSTA2_CHICK	4.2e-07	0.279	8.0e-08	0.266				

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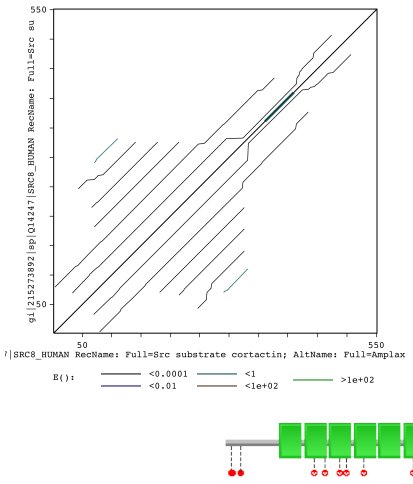
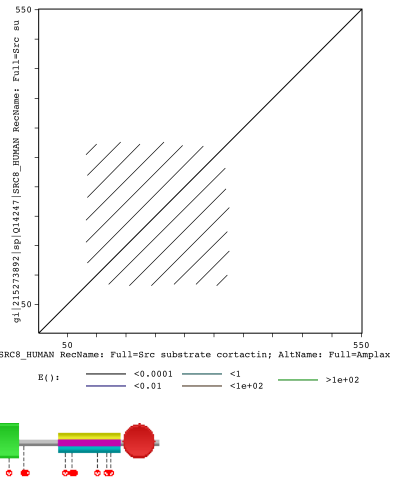
Scoring matrices influence alignment lengths

BLOSUM62 -11/-1



MD20 -26/-4



Scoring Matrices - Summary

- PAM and BLOSUM matrices greatly improve the sensitivity of protein sequence comparison – low identity with significant similarity
- PAM matrices have an evolutionary model - lower number, less divergence – lower=closer; higher=more distant
- BLOSUM matrices are sampled from conserved regions at different average identity – higher=more conservation
- Short alignments require shallow matrices
- Shallow matrices set maximum look-back time

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Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. How to avoid mistakes (what to look out for)
5. When to do something different
6. PSI-BLAST – the most sensitive method

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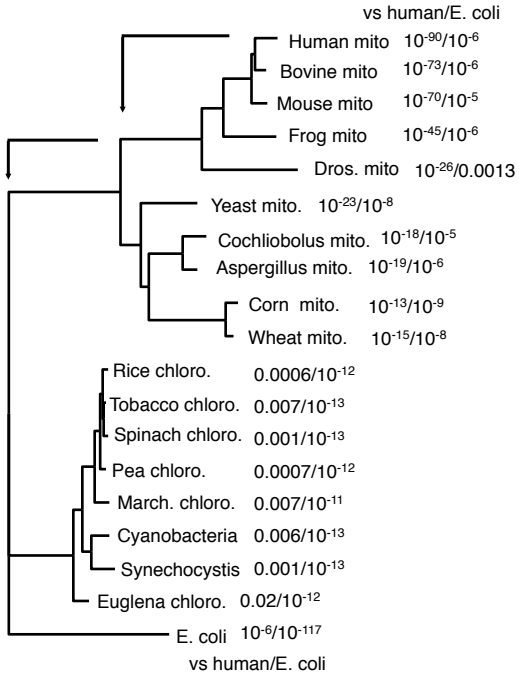

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ATP synthase - matrices, gaps, algorithms

Matrix:	BLOSUM50		BLOSUM62		BLASTP	
Gap open/extend	-10/-2		-11/-1		-11/-1	
The best scores are:	**bits**	**E(13351)**	**bits**	**E(13351)**	**bits**	**E()**
ATP6_HUMAN ATP synthase a chai	297.7	1.7e-81	373.6	2.4e-104	296	3e-81
ATP6_BOVIN ATP synthase a chai	252.4	7.2e-68	310.7	2.0e-85	253	2e-68
ATP6_MOUSE ATP synthase a chai	246.4	4.5e-66	302.9	4.4e-83	245	5e-66
ATP6_XENLA ATP synthase a chai	111.9	1.4e-25	125.9	8.7e-30	142	9e-35
ATP6_YEAST ATP synthase a ch	78.7	1.6e-15	90.1	5.7e-19	93	5e-20
ATP6_EMENI ATP synthase a chai	66.3	8.4e-12	76.6	6.8e-15	75	2e-14
ATP6_DROYA ATP synthase a chai	65.6	1.2e-11	75.4	1.4e-14	101	2e-22
ATP6_COCHE ATP synthase a cha	53.6	5.5e-08	60.6	4.6e-10	75	1e-14
ATP6_ECOLI ATP synthase a ch	45.1	2.2e-05	49.1	1.4e-06	42	1e-04
ATP6_TRITI ATP synthase a ch	45.0	3.3e-05	50.7	6.5e-07	83	5e-17
ATP6_TOBAC ATP synthase a chai	40.4	0.00084	47.0	8.6e-06	80	3e-16
ATP6_MAIZE ATP synthase a chai	39.6	0.001	44.9	2.6e-05		
ATPI_PEA Chloroplast ATP syn	35.8	0.013	38.0	0.0028		
ATPI_SPIOL Chloroplast ATP syn	35.5	0.015	38.0	0.0028		
ATPI_ATRBE Chloroplast ATP s	34.0	0.044	36.3	0.0086		
ATPI_MARPO Chloroplast ATP syn	33.2	0.075	34.3	0.036		
*HBA_ODOVI Hemoglobin subunit a			31.9	0.11*		
*AROP_ECOLI Aromatic amino ac	32.1	0.31	31.4	0.5 *		
ATPI_EUGGR Chloroplast ATP syn	31.1	0.32	32.2	0.15		
ATP6_SYNP6 ATP synthase a chai	31.1	0.34	31.8	0.21		
TLCA_RICPR ADP,ATP carrier pro	31.5	0.49	29.7	1.7		
ATP6_SYNY3 ATP synthase a chai	30.6	0.51	31.8	0.22	28	1.9
ATPI_ORYSA Chloroplast ATP	30.1	0.65	32.2	0.15		
*GLUC_MYOSC Glucagon precursor	28.7	0.65	34.4	0.013*		
*VP6_BPPH6 Protein P6	29.1	0.85	28.6	1.3*		
*GLUC_LEPSP Glucagon precursor	27.7	1.	32.7	0.033*		
*ADH1_MOUSE Alcohol dehydrogena	29.8	1.2	34.4	0.013*		

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Metazoan ATP Synthases

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CLUSTAL W (1.81) multiple sequence alignment

ATP6_BOVIN  MNENLFTSFITPVILGLPLVTLIVLFPSLLF--PTSNRLVSNRFVTLQQWMLQLVSKQMMSIHNSKGQTWT-LML
ATP6_MOUSE  MNENLFASFITPTMMGFPIVVAIIMFPSILF--PSSKRLINNRLHSFQHWLVKLIIKQMMLIHTPKGRTWT-LMI
ATP6_HUMAN  MNENLFASFIAPTILGLPAAVLIILFPPLLI--PTSKYLINNRLITTQQWLIKLTSKQMMTMHNTKGRTWS-LML
ATP6_XENLA  MNLSFFDQFMSPVILGIPLIAIAMLDPFTLISWPIQSNGFNNRLITLQSWFLHNFTTIFYQLTSP-GHKWA-LLL
ATP6_DROYA  MMTNLFSVFDPSAIFNLSLNWLSTFLGLLMI--PSIYWLMPSRYNIFWNSILLTLHKEFKTLLGPSGHNGSTFIF
            *  .:*  * ...::.:.      :    ::  *     . .*       ::    . :  :  . *:. : :::

ATP6_BOVIN  MSLILFIGSTNLLGLLPHSFTPTTQLSMNLGMAIPLWAGAVITGFRNKTKASLAHFLPQGTPTPLIPMLVIIETI
ATP6_MOUSE  VSLIMFIGSTNLLGLLPHTFTPTTQLSMNLSMAIPLWAGAVITGFRHKLKSSLAHFLPQGTPISLIPMLIIIETI
ATP6_HUMAN  VSLIIFIATTNLLGLLPHSFTPTTQLSMNLAMAIPLWAGTVIMGFRSKIKNALAHFLPQGTPTPLIPMLVIIETI
ATP6_XENLA  TSLMLLLMSLNLLGLLPYTFTPTTQLSLNMGLAVPLWLATVIMASKP-TNYALGHLLPEGTPTPLIPVLIIIETI
ATP6_DROYA  ISLFSLILFNNFMGLFPYIFTSTSHLTLTLSLALPLWLCFMLYGWINHTQHMFAHLVPQGTPAILMPFMVCIETI
             **: ::   *::**:*: **.*::*::.:.:*:***   :: .     :  :.*::*:***  *:*.:: ****

ATP6_BOVIN  SLFIQPMALAVRLTANITAGHLLIHLIGGATLALMSISTTTALITFTILILLTILEFAVAMIQAYVFTLLVSLYLHDNT
ATP6_MOUSE  SLFIQPMALAVRLTANITAGHLLMHLIGGATLVLMNISPPTATITFIILLLLTILEFAVALIQAYVFTLLVSLYLHDNT
ATP6_HUMAN  SLLIQPMALAVRLTANITAGHLLMHLIGSATLAMSTINLPSTLIIFTILILLTILEIAVALIQAYVFTLLVSLYLHDNT
ATP6_XENLA  SLFIRPLALGVRLTANLTAGHLLIQLIATAAFVLLSIMPTVAILTSIVLFLLTLLEIAVAMIQAYVFVLLLSLYLQENV
ATP6_DROYA  SNIIRPGTLAVRLTANMIAGHLLLTLLGNTGPSMSYLLVTFLLVAQIALLVL---ESAVTMIQSYVFAVLSTLYSSEVN
            * :*:* :*.******: *****: *:. :   :  :  .   :    *::*   * **::**:***.:* :**  :
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PSI-BLAST ATP6_HUMAN - 4 iterations

Sequences producing significant alignments:	Results from round: (1) Score (bits)	E Value	(2) Score (bits)	E Value	(3) Score (bits)	E Value	(4) Score (bits)	E Value
ATP6_HUMAN ATP synthase a chain (ATPase protein 6)	296	3e-81	257	1e-69	241	2e-62	222	5e-59
ATP6_BOVIN ATP synthase a chain (ATPase protein 6)	253	2e-68	257	2e-69	239	8e-65	230	2e-61
ATP6_MOUSE ATP synthase a chain (ATPase protein 6)	245	5e-66	247	3e-66	234	4e-64	225	6e-60
ATP6_XENLA ATP synthase a chain (ATPase protein 6)	142	9e-35	227	1e-60	189	3e-49	177	2e-45
ATP6_DROYA ATP synthase a chain (ATPase protein 6) (2)	101	2e-22	206	3e-54	209	5e-55	196	4e-51
ATP6_YEAST ATP synthase a chain precursor (ATPase prot	93	5e-20	97	3e-21	199	4e-52	191	2e-49
ATP6_TRITI ATP synthase a chain (ATPase protein 6) (3)	83	5e-17	96	5e-21	218	1e-57	236	4e-63
ATP6_TOBAC ATP synthase a chain (ATPase protein 6)	80	3e-16	90	4e-19	200	2e-52	230	3e-61
ATP6_MAIZE ATP synthase a chain (ATPase protein 6)	76	5e-15	88	1e-18	198	1e-51	219	5e-58
ATP6_COCHE ATP synthase a chain (ATPase protein 6)	75	1e-14	86	9e-18			197	2e-51
ATP6_EMENI ATP synthase a chain precursor (ATPase prot (4)	75	2e-14	84	3e-17	123	5e-29	181	2e-46
ATP6_ECOLI ATP synthase a chain (ATPase protein 6)	42	1e-04	40	5e-04	46	8e-06	49	1e-06
ATPI_SPIOL Chloroplast ATP synthase a chain precursor			32	0.12	36	0.006	39	0.001
ATP6_SYNY3 ATP synthase a chain (ATPase protein 6)	28	1.9	32	0.16	44	5e-05	45	1e-05
ATPI_MARPO Chloroplast ATP synthase a chain precursor			31	0.21	44	4e-05	44	3e-05
ATPI_PEA Chloroplast ATP synthase a chain precursor (A			31	0.32	37	0.005		
LAMA2_MOUSE Laminin subunit alpha-2 precursor (Laminin			31	0.34				
ATPI_ATRBE Chloroplast ATP synthase a chain precursor			31	0.39	41	2e-04		
ATP6_SYNP6 ATP synthase a chain (ATPase protein 6)			28	1.7	41	2e-04		
ATPI_EUGGR Chloroplast ATP synthase a chain precursor					39	0.001		
ATPI_ORYSA Chloroplast ATP synthase a chain precursor			28	1.9	36	0.008		
ATPI_ATRBE Chloroplast ATP synthase a chain precursor					36	0.009	38	0.002
ATP6_ASPAM ATP synthase a chain (ATPase protein 6)							36	0.008
POLG_KUNJM Genome polyprotein [Contains: Capsid protei...	27	5.0						
POL_HTL1C Gag-Pro-Pol polyprotein (Pr160Gag-Pro-Pol) [...	27	5.0						
POLG_DEN2J Genome polyprotein [Contains: Capsid protei...	27	5.2	26	7.0				

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Position-Specific Scores
ATP Synthase, 4 iterations

		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	bits/pos
BL62	Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2	0.70
46	Q	-2	-1	-2	-2	-4	6	0	1	0	-4	-3	-1	-2	-1	-3	-1	-2	6	4	-3	0.74
	%	0	0	0	0	0	54	0	12	0	0	0	0	0	0	0	0	0	13	20	0	
47	Q	-1	-1	3	3	-3	3	3	-2	3	-4	-4	-1	-3	-4	-2	2	-1	-4	-2	-3	0.51
	%	0	0	13	20	0	16	19	0	8	0	0	0	0	0	0	24	0	0	0	0	
56	Q	-2	-1	-2	-2	-3	5	2	-4	-1	4	-1	-1	-1	-2	-3	-2	-2	-3	-2	0	0.51
	%	0	0	0	0	0	46	13	0	0	41	0	0	0	0	0	0	0	0	0	0	
97	Q	-2	-1	0	-2	-4	4	0	-3	8	-4	-4	-1	-2	-3	-3	-1	-2	-3	0	-4	1.11
	%	0	0	0	0	0	35	0	0	65	0	0	0	0	0	0	0	0	0	0	0	
131	Q	3	-1	-1	-1	-2	5	2	-2	-1	-3	-3	0	-2	-4	-2	1	-1	-3	-3	-2	0.52
	%	44	0	0	0	0	36	11	0	0	0	0	0	0	0	0	9	0	0	0	0	
152	Q	-2	6	-1	-2	-4	4	0	-3	-1	-4	-3	1	-2	-4	-3	-1	-2	-4	-3	-3	1.00
	%	0	77	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
210	Q	-2	0	-1	-1	-4	7	1	-3	0	-4	-3	1	-1	-4	-2	-1	-2	-3	-2	-3	1.13
	%	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

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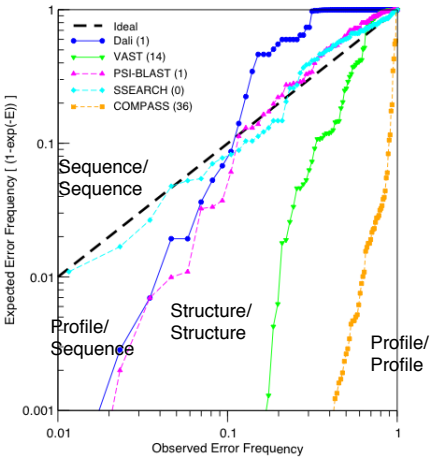
Accuracy of statistical estimates



- SSEARCH (Smith-Waterman) provides very accurate statistical estimates
- PSI-BLAST and Dali provide estimates that off by 10–100-fold
- Other structure comparison methods provide wild over estimates of statistical significance – *BEWARE of claims of significant structural similarity*

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Sensitive searches with PSI-BLAST

- PSI-BLAST improves sensitivity by building a Position Specific Scoring Matrix (PSSM)
  - models ancestral sequence (consensus distribution)
  - similar to PFAM HMM (but less sophisticated weights, gaps)
- Sensitivity improves with additional iterations
  - model moves to base of tree
- Statistical estimates are difficult
  - once a sequence is in, it is “significant” - validation must be done before a sequence is included
- Very diverse families may not produce a well defined PSSM
  - similar problems with HMMs have led to “clans”

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Sequence Similarity II - Conclusions

- Always compare Protein Sequences
  - use BLASTP or SSEARCH for protein-protein
  - blastx or fastx for DNA protein
- Search small (comprehensive) databases – never search NR or GenBank
- Scoring matrices set evolutionary look back horizons - not every discovery is distant
- Shallow scoring matrices for short domains
- Accurate statistics => highest unrelated E() ~ 1.0
- PSI-BLAST can be more sensitive, but with lower statistical accuracy

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Discussion questions

1. What is the difference between similarity and homology? When does high identity not imply homology? What conclusions can be drawn from homology?
2. What is the difference between homology and common ancestry?
3. In practical terms, how is “orthology” more useful than “homology” or “paralogy”?
4. When the *M. janaschii* genome was first sequenced, Venter and his colleagues stated that almost 60% of the open reading frames (proteins or genes) were novel to this organism. (For eubacterial like *E. coli* or *H. influenzae*, a similar number would be 20 - 40%.) On what would they base such a statement? Is it likely to be correct?
5. Name two reasons why protein sequence comparison is more effective (longer evolutionary look-back time) than DNA sequences?
6. What is the range of an expectation value (E()-value)? If you compare a sequence to 50,000 random(unrelated) sequences, what should the expectation value for the highest of the 50,000 similarity scores be (on average)?
7. In a sequence similarity database search, you identify a statistically significant similarity (*E*()<0.005), but the alignment is relatively short (50 aa). How might you determine whether the alignment reflects a genuine homology, or a random sequence match?
8. How can a sequence be homologous if you search a small database (e.g. human, 40,000 sequences), but not share significant similarity if you search a complete database (>4 million sequences)?
9. What scoring matrix should be used to identify protein orthologs that have diverged over the past 100 My (e.g. human/mouse)?

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