Programming for Biology
Protein Evolution / Similarity Searching

What BLAST Does / Why BLAST works

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Protein Evolution/ Similarity Searching

- 9:00 – Homology and Expectation value
- 10:30 – Similarity searching workshop I
- 1:30 – Practical Similarity Searching, improving sensitivity
- 3:00 – Workshop II – investigating scoring matrices with scripts

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Goals of this lecture:

- understand why and how homology is inferred; the meaning of "expectation value"
- significance => homology, but non-significance ≠> non-homology
- understand sequence similarity, and why protein comparison is more sensitive than DNA sequence comparison

Similarity searching is POWERFUL, but not MAGIC. There are characteristic errors, and simple strategies to reduce them.

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Why is this material important?

- Most information in biological databases is based on a BLAST search
  - all functional information except for a few model organisms (mostly rats, mice, humans, and yeast)
  - Most genetic information except for E. coli, yeast, Drosophila
- The information is (usually) *correct*, but *incomplete*
- My goal: what to trust? and when to be skeptical, when using sequence names/annotations, functions, etc.
  - Trust E()-values for proteins to infer homology (common ancestry; thus common structure)
  - Understand that search results and alignment boundaries are often incomplete – the absence of a result is not a negative result
    - this can be very difficult to accept

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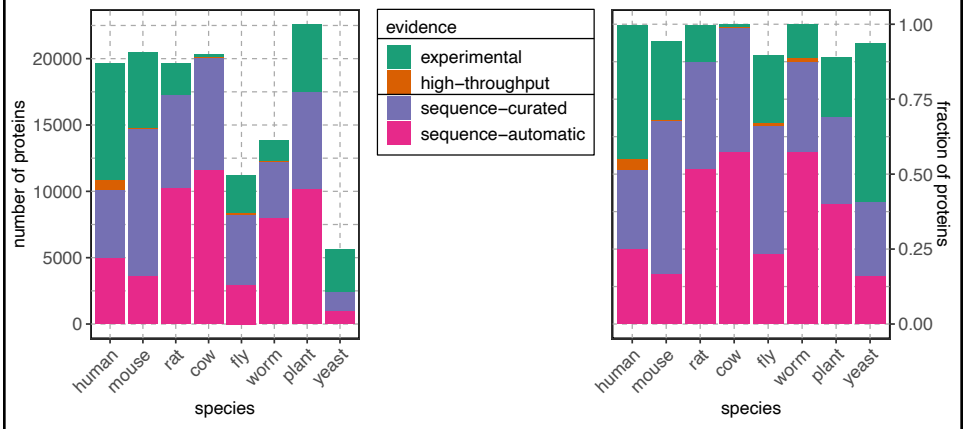
Biological (functional) knowledge is based on sequence comparison



Gene Ontology evidence codes for reference proteomes

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Effective Similarity Searching

1. Always search protein databases (possibly with DNA – blastx, fastx)
2. Use E()-values, not percent identity, to infer homology
   - E() < 0.001 is biologically/statistically significant in a single search

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1. Search smaller (comprehensive) proteome sets
   - Less redundancy; better sensitivity
2. Change the scoring matrix for:
   - short evolutionary distances (mammals, vertebrates, a-proteobacteria)
   - short sequences (exons, reads)
   - high identity (>50% alignments) to reduce over-extension

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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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Protein Evolution and Sequence Similarity

Similarity Searching I

- What is Homology and how do we recognize it?
- How do we measure sequence similarity – alignments and scoring matrices?
- DNA vs protein comparison

Similarity Searching II

- More effective similarity searching
  - Smaller databases
  - Appropriate scoring matrices
  - Using annotation/domain information

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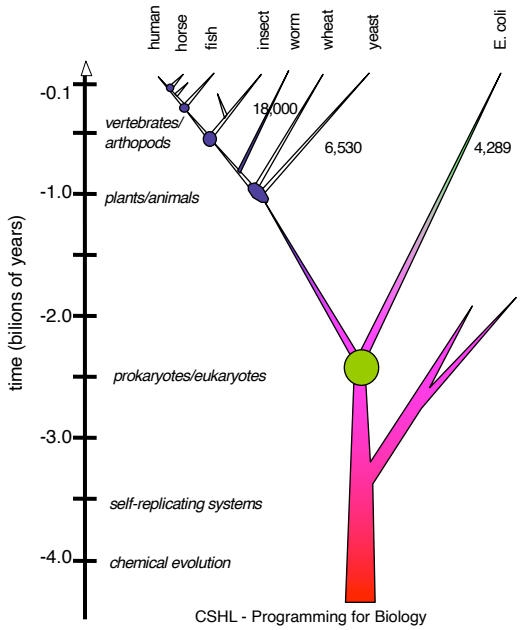
Homologues share a common ancestor



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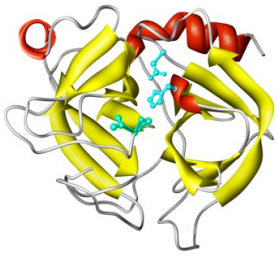
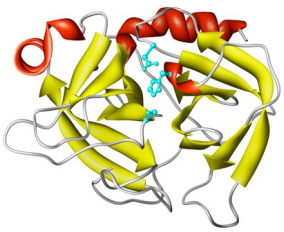
When do we infer homology?

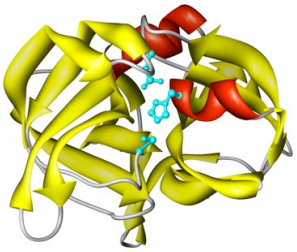
Homology <=> structural similarity
? sequence similarity



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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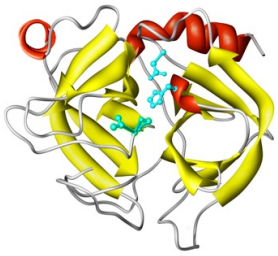
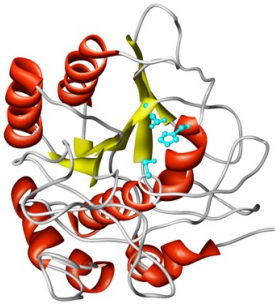
When can we infer non-homology?



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

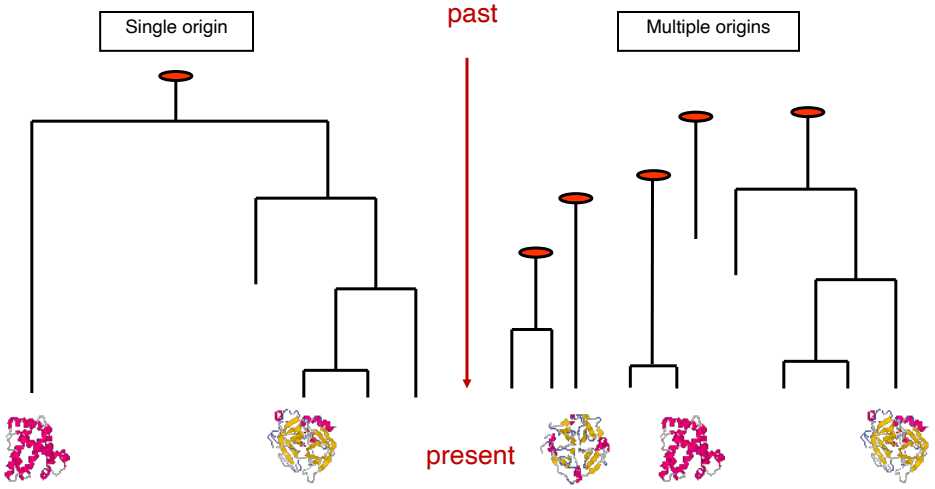
Cytochrome c4 (1etp)
E() > 100
E()<5.5; 23% 171/190

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

- Proteins/genes/DNA that share a common ancestor (this lecture)
- Specific positions/columns in a multiple sequence alignment that have a 1:1 relationship over evolutionary history
  - sequences are *50% homologous* ??? (NO)
- Specific (morphological/functional) characters that share a recent divergence (clade)
  - bird/bat/butterfly wings are/are not homologous (only in Natural History Museums)

Homology is confusing II: Are All Sequences Homologous?

No Homology without excess similarity



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Homology from sequence similarity

- 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 sequence 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 independence – one must weigh the evidence for each hypothesis (independence is the *null* hypothesis).

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BLAST works because there is a lot of excess similarity

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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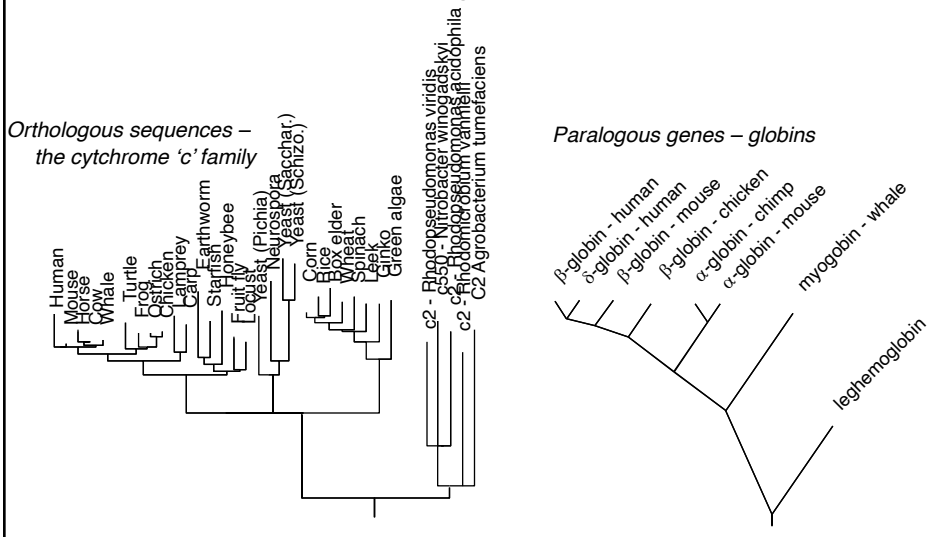
Orthologs and Paralogs – Inferring Function



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Protein Evolution and Sequence Similarity

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- DNA vs protein comparison

- More effective similarity searching
  - Smaller databases
  - Appropriate scoring matrices
  - Using annotation/domain information

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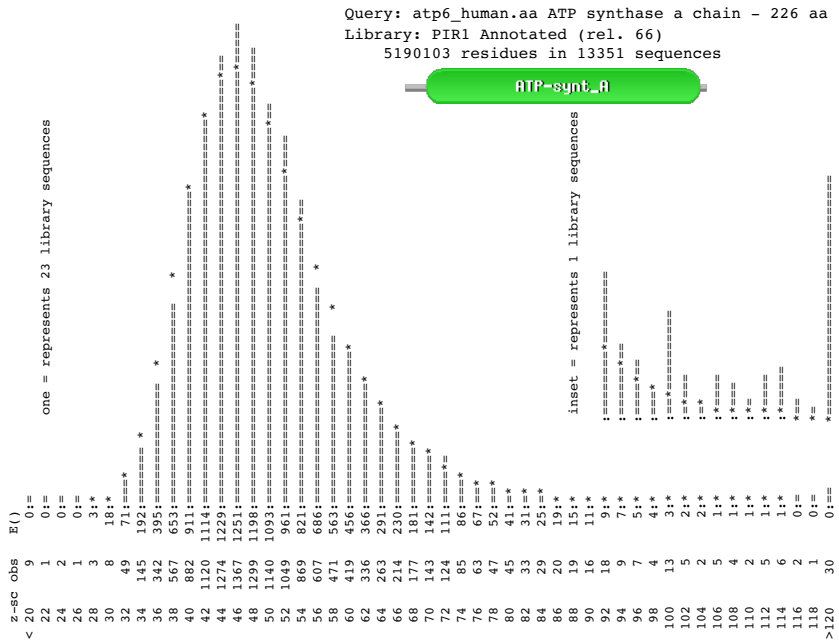

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Inferring Homology from Statistical Significance

- 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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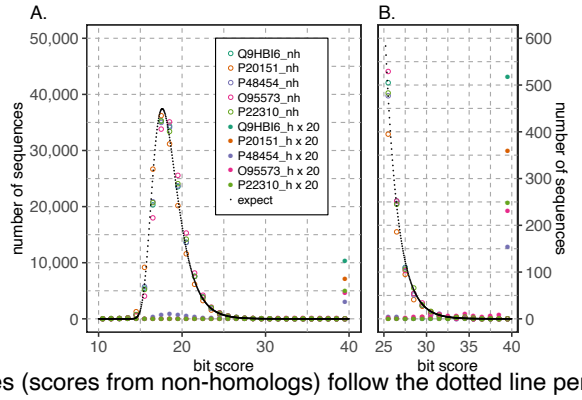
Non-homologous/homologous score distributions five proteins



1. Open circles (scores from non-homologs) follow the dotted line perfectly. Non-homologous sequences have scores that are accurately predicted by a random model (the extreme value distribution).
2. Closed circles (scores from homologs) often have scores that are much higher than expected. But some homologous sequences have non-significant (randomly expected) scores, because they are too distant from the query.

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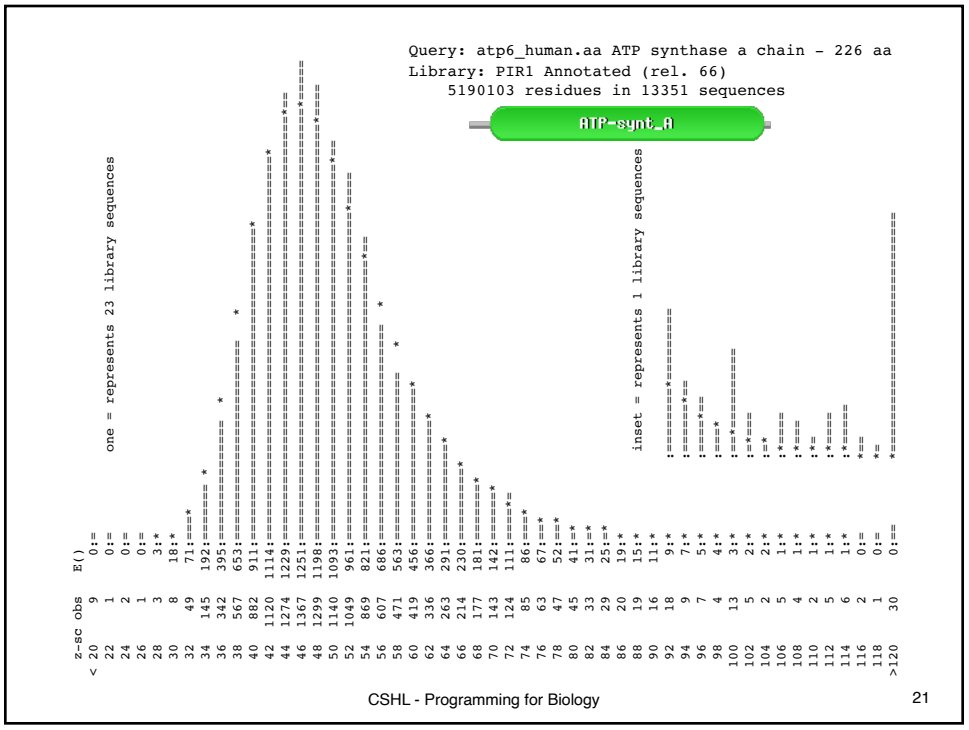

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```
                    Query: atp6_human.aa ATP synthase a chain - 226 aa
                    Library: 5190103 residues in 13351 sequences

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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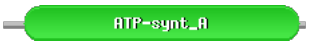


```
>sp|P00846|ATP6_HUMAN ATP synthase subunit a; F-ATPase protein 6
>sp|P0AB98|ATP6_ECOLI ATP synthase subunit a; F-ATPase subunit 6
Length=271

 Score = 47.9 bits (178),  Expect = 3e-06
 Identities = 55/199 (27%), Positives = 113/199 (56%), Gaps = 37/199 (18%)

Query  8    SFIAPTILGLPAAVLIILFPPLLIPTSKYLINNRLITTQQWLIKLTSKQMMTMHNTKGRT 67
            S     +LGL   ++++LF  +    +   +  ++ T  + +I + +  +  M++ K +
Sbjct  45   SMFFSVVLGL---LFLVLFRSVAKKATSG-VPGKFQTAIELVIGFVNGSVKDMYHGKSKL 100

Query  68   WSLMLVSLIIFIATTNLLGLLP---------HSF-------TPTTQLSMNLAMAIPLWAG 111
             + + +++ +++   NL+ LLP          H +         P+  +++ L+MA+ ++
Sbjct  101  IAPLALTIFVWVFLMNLMDLLPIDLLPYIAEHVLGLPALRVVPSADVNVTLSMALGVF-- 158

Query  112  TVIMGFRSKIKNALAHFLPQGTPTPL-----IPMLVIIETISLLIQPMALAVRLTANITA 166
             +++ F S     +  F  + T  P+     IP+ +I+E +SLL +P++L +RL  N+ A
Sbjct  159  -ILILFYSIKMKGIGGFTKELTLQPFNHWAFIPVNLILEGVSLLSKPVSLGLRLFGNMYA 217

Query  167  GHLLMHLIGSATLAMSTINLPSTLIIFTILILLTILEIAVALIQAYVFTLLVSLYL 222
            G L+  LI       S   L     IF ILI+          +QA++F +L  +YL
Sbjct  218  GELIFILIAGLLPWWSQWILNVPWAIFHILIIT---------LQAFIFMVLTIVYL 264
```


The PAM250 matrix

```
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
      C  S  T  P  A  G  N  D  E  Q  H  R  K  M  I  L  V  F  Y  W
```

frequency of replacement in homologs

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

frequency of alignment by chance

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

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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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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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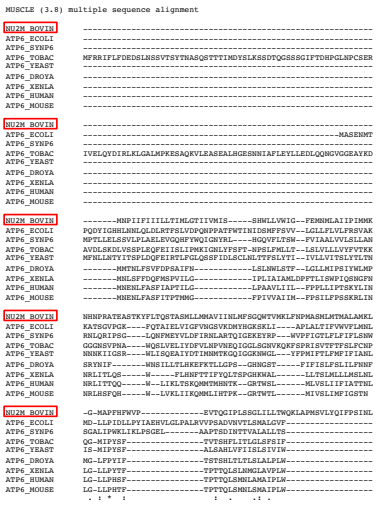
Homology from Multiple Alignment??

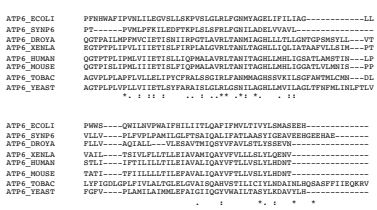
All homologous

One unrelated

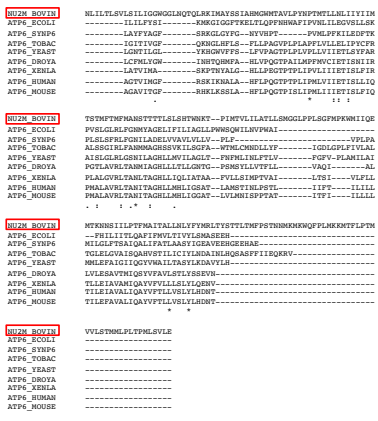


Homology from Multiple Alignment??

All homologous



One unrelated



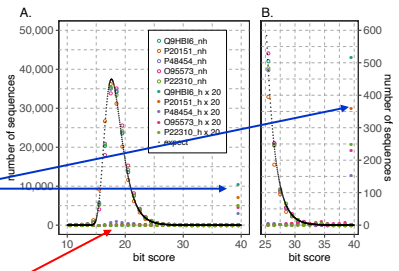
1. Multiple Alignment Programs *ASSUME* homology. (It makes no sense to align non-homologous sequences.)
2. MSA programs will *ALWAYS* provide a compact alignment

3. MSA programs do not provide any estimates of excess similarity
4. Multiple Sequence Alignments *CANNOT* be used to infer homology

Homology from significant similarity

Unrelated sequences have similarity scores that are indistinguishable from random sequences

- We infer homology (common ancestry) from excess (significant) similarity (E()-values)
- We DO NOT infer non-homology from the lack of similarity



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
- More effective similarity searching
  - Smaller databases
  - Appropriate scoring matrices

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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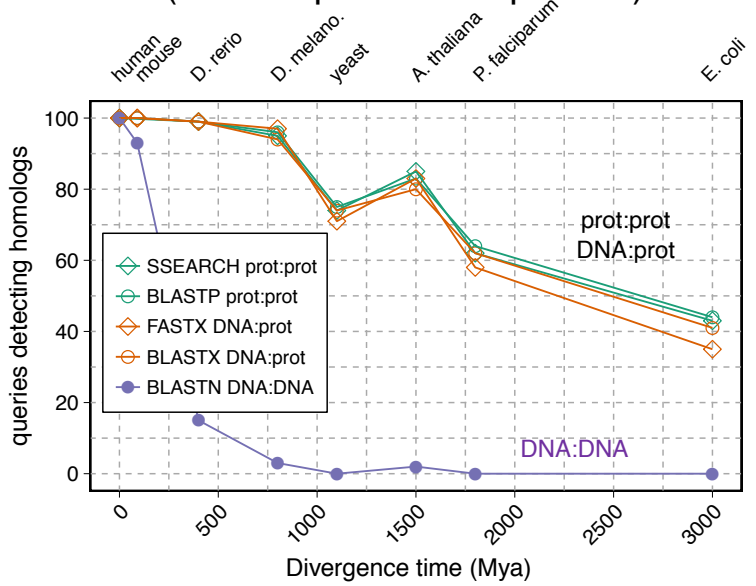
Detectable homologs to human enzymes (DNA vs protein comparison)



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Why is protein comparison more sensitive?

- Larger alphabet: 20 aa vs 4 nt, means long alignments less likely by chance
- similarity scoring matrix
  - proteins have BLOSUM62: L ~ (V,I)
  - DNA typically match/mismatch A ≠ G
  - in 3rd codon position, DNA mismatch can be amino acid identity
- Smaller databases
- Better statistics
  - for proteins, E() < 0.001 is 1/1000 (unrelated looks like random)
  - for DNA, E() < 10^{-10} a more reliable threshold (unrelated doesn't always look random)

Effective Similarity Searching

1. Always search protein databases (possibly with translated DNA)
2. Use E()-values, not percent identity, to infer homology
   - E() < 0.001 is significant in a single search (proteins)

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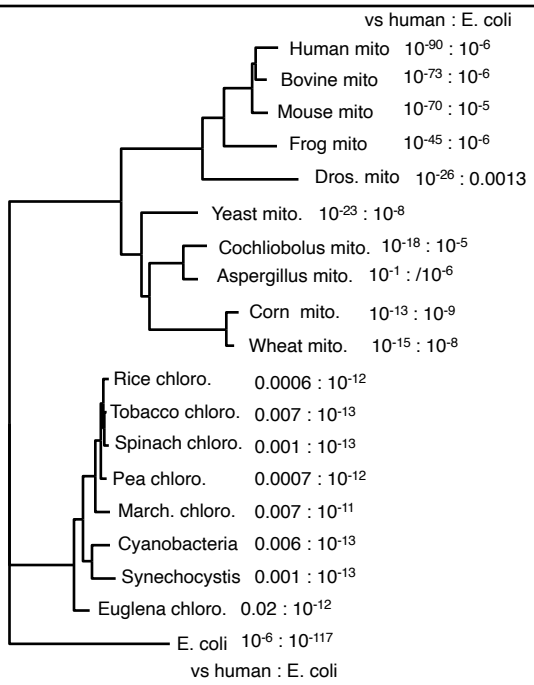
1. Search smaller (comprehensive) databases
2. Change the scoring matrix for:
   - short sequences (exons, reads)
   - short evolutionary distances (mammals, vertebrates, a-proteobacteria)
   - high identity (>50% alignments) to reduce over-extension
3. All methods (pairwise, HMM, PSSM) miss homologs, and find homologs the other methods miss

Homology is Transitive (on domains)



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Homology and Domains – Histone acetyltransferase KAT2B

```
The best scores are:                                      s-w bits E(454402) %_id  %_sim  alen
KAT2B_HUMAN Histone acetyltransferase KAT2B ( 832) 3820 1456.       0 1.000 1.000  832
KAT2A_HUMAN Histone acetyltransferase KAT2A ( 837) 2747 1049.       0 0.721 0.870  813
GCN5_SCHPO Histone acetyltransferase gcn5   ( 454)  867 334.7   3e-90 0.483 0.768  354
GCN5_YEAST Histone acetyltransferase GCN5   ( 439)  792 306.2 1.1e-81 0.469 0.760  354
GCN5_ORYSJ Histone acetyltransferase GCN5   ( 511)  760 294.0 5.9e-78 0.436 0.755  376
GCN5_ARATH Histone acetyltransferase GCN5;  ( 568)  719 278.4 3.3e-73 0.434 0.740  369
BPTF_HUMAN Nucleosome-remodeling factor sub (3046)  286 113.6 7.6e-23 0.495 0.804   97
NU301_DROME Nucleosome-remodeling factor su (2669)  276 109.8 9.1e-22 0.511 0.819   94
CECR2_HUMAN Cat eye syndrome critical regio (1484)  232  93.2   5e-17 0.371 0.790  105
BRD4_HUMAN Bromodomain-containing protein 4 (1362)  214  86.4 5.2e-15 0.379 0.698  116
BRD4_MOUSE Bromodomain-containing protein 4 (1400)  214  86.4 5.3e-15 0.379 0.698  116
BAZ2A_HUMAN Bromodomain adjacent to zinc fi (1905)  211  85.2 1.7e-14 0.382 0.683  123
BAZ2A_XENLA Bromodomain adjacent to zinc fi (1698)  206  83.3 5.5e-14 0.350 0.684  117
FSH_DROME Homeotic protein female sterile;  (2038)  205  82.9 8.8e-14 0.341 0.667  129
BAZ2A_MOUSE Bromodomain adjacent to zinc fi (1889)  204  82.5   1e-13 0.368 0.680  125
BRDT_MACFA Bromodomain testis-specific prot ( 947)  197  80.0   3e-13 0.367 0.697  109
BRD3_HUMAN Bromodomain-containing protein 3 ( 726)  194  78.9 4.9e-13 0.362 0.664  116
```


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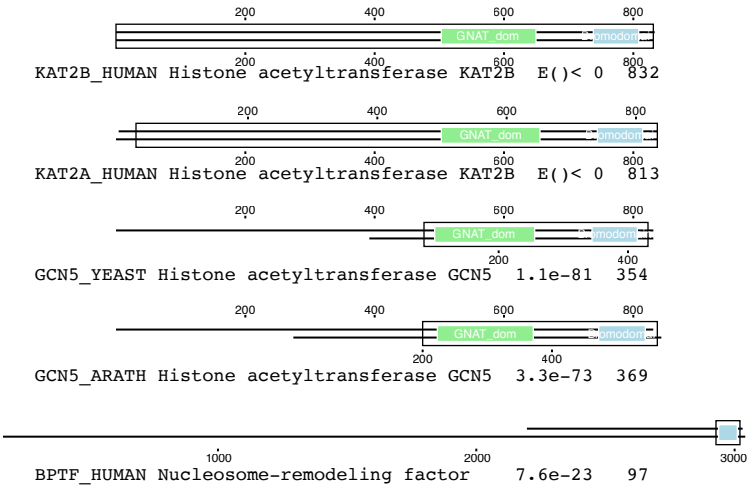
Homology and Domains – Histone deacetylase KAT2B



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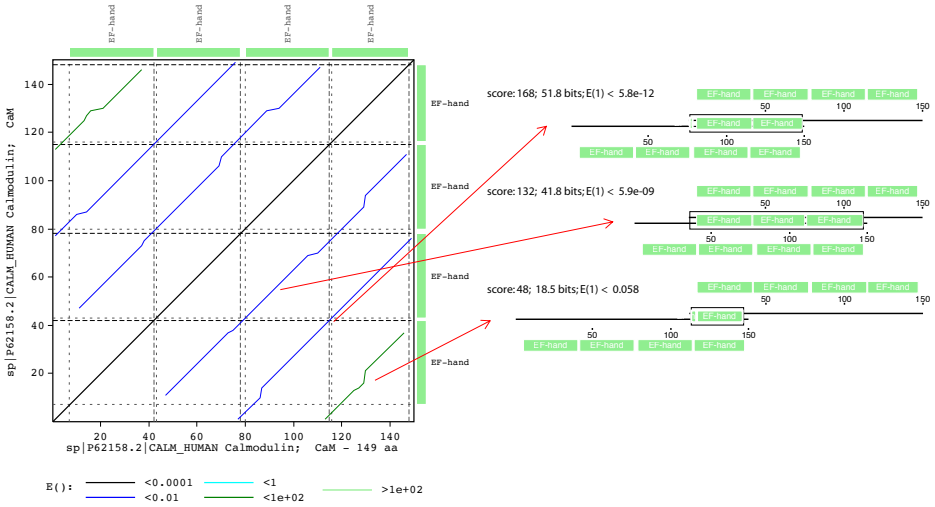
LALIGN – Identifying mobile domains: mobile (duplicated) domains in local alignments



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Computer lab: fasta.bioch.virginia.edu/mol_evol

- Significant hits are homologous
- Non-significant hits? Homologous or not?
- Are *all* aligned residues homologous
- Are *unaligned* residues non-homologous
- Are domains really missing?

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