Sequence Similarity

Protein Sequence Comparison and Protein Evolution

(What BLAST does/Why BLAST works)

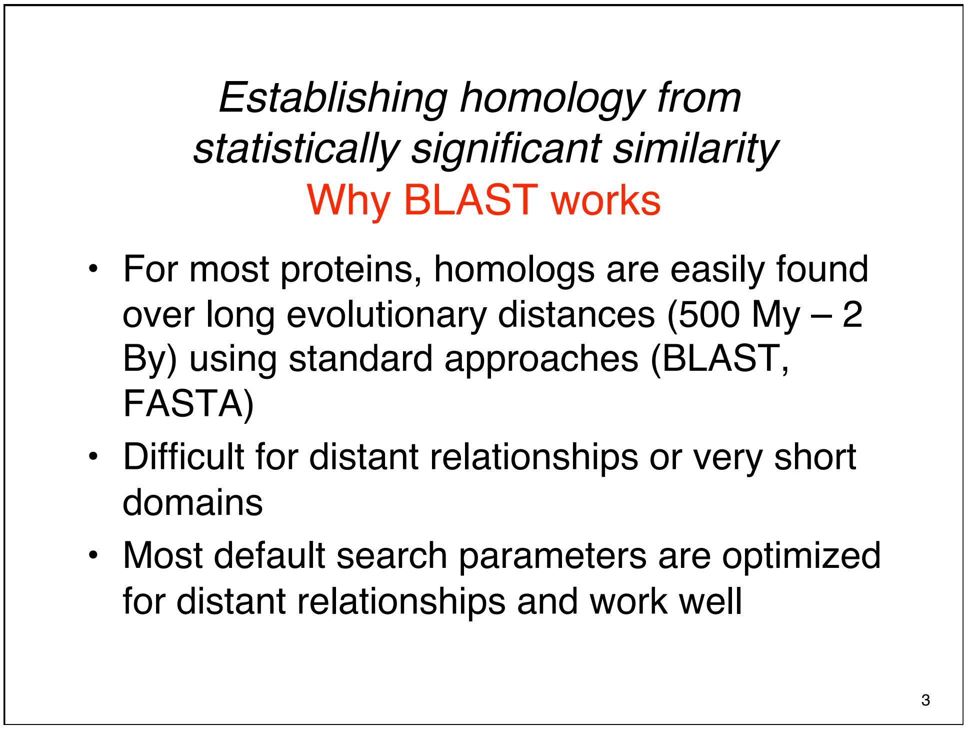
William R. Pearson

www.people.virginia.edu/~wrp

wrp@virginia.edu

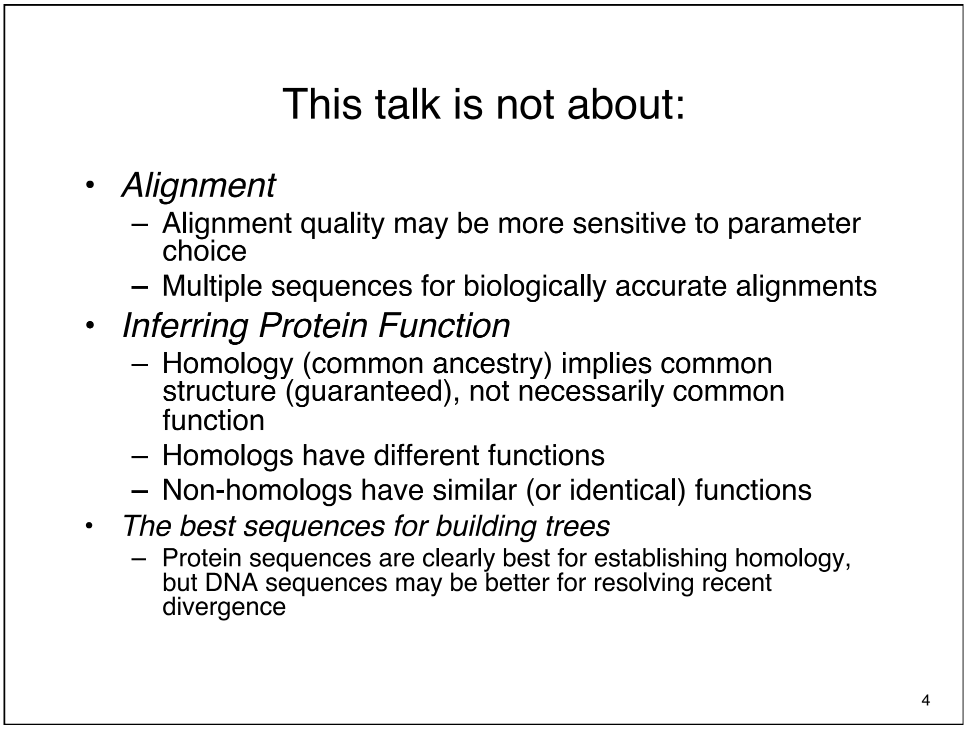
Sequence Similarity - Conclusions

- 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



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



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

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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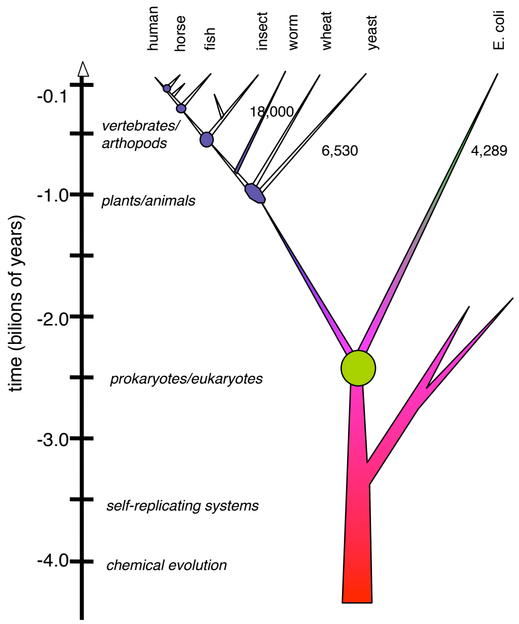

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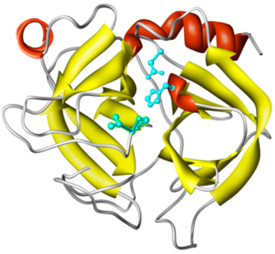
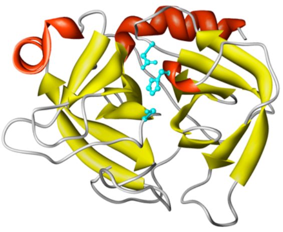
Homology <=> structural similarity
? sequence similarity

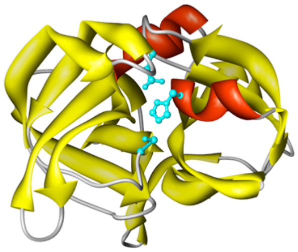


Bovine trypsin (5ptp)

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

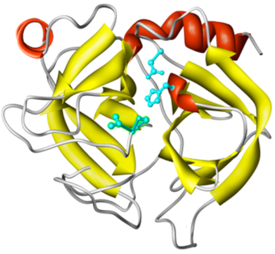


S. griseus trypsin (1sgt)
E()< 10^{-14} RMSD 1.6 A
E()< 10^{-19} 36%; 226/223



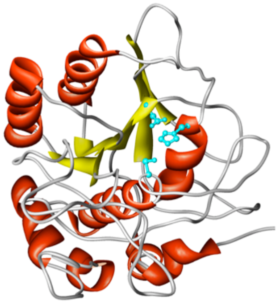
S. griseus protease A (2sga)
E()< 10^{-4}; RMSD 2.6 A
E()< 2.6 25%; 199/181

Non-homologous proteins have different structures

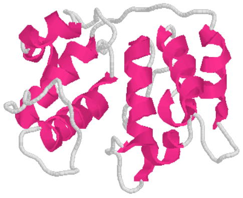


Bovine trypsin (5ptp)

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



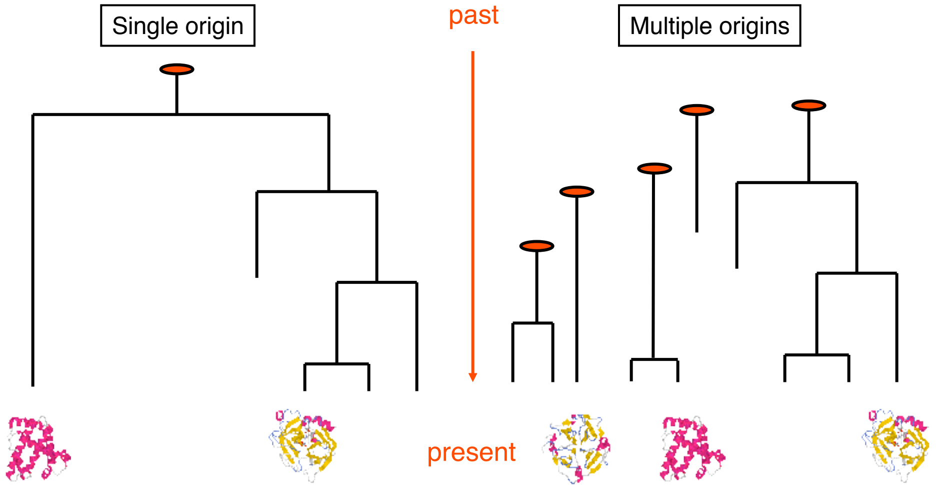
Subtilisin (1sbt)
E() >100
E()<280; 25% 159/275



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

Are All Sequences Homologous?

No Homology without excess similarity



Single origin

past

Multiple origins

present

What BLAST does:

Similarity $\overset{?}{<=>}$ Homology

Why BLAST works:

Statistical Significance $\overset{?}{<=>}$ Biological Significance

Divergence ? Convergence

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	?

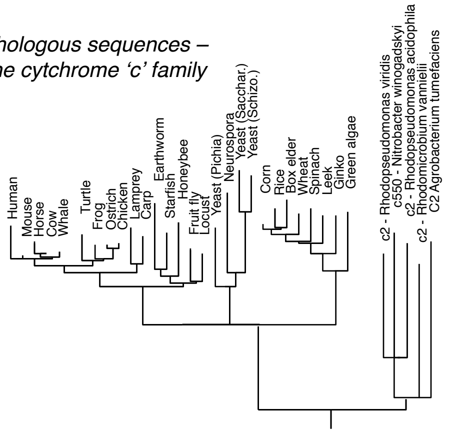
[a] PAMs, point accepted mutations. [b] Useful lookback time, 360 PAMs,15% identity. [c] Millions of years. [d] Billions of years.

E. coli proteins vs Human – Ancient Protein Domains

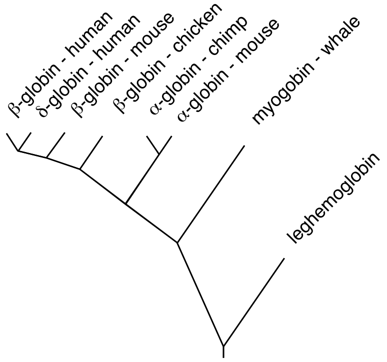
```
+----------+------+------+---------------------------+---------------------------+------------+
| expect   | %_id | alen | E coli descr              | Human descr               | sp_name    |
+----------+------+------+---------------------------+---------------------------+------------+
| 2.7e-206 | 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 |
+----------+------+------+---------------------------+---------------------------+------------+
```

Orthologs and Paralogs – Inferring Function

Orthologous sequences – the cytchrome 'c' family



Paralogous genes – globins



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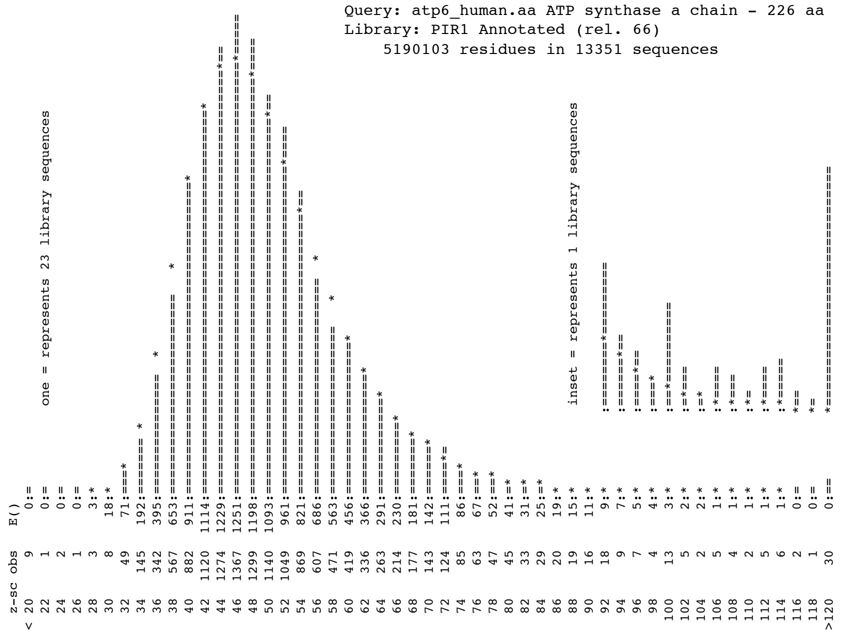

```
Query: atp6_human.aa ATP synthase a chain - 226 aa
Library: PIR1 Annotated (rel. 66)
    5190103 residues in 13351 sequences

 z-sc  obs   E()      one = represents 23 library sequences
< 20     9     0
  22     1     0
  24     2     0
  26     1     0
  28     3     3
  30     8    18
  32    49    71
  34   145   192
  36   342   395
  38   567   653
  40   882   911
  42  1120  1114
  44  1274  1229
  46  1367  1251
  48  1299  1198
  50  1140  1093
  52  1049   961
  54   869   821
  56   607   686
  58   471   563
  60   419   456
  62   336   366
  64   263   291
  66   214   230
  68   177   181
  70   143   142
  72   124   111
  74    85    86
  76    63    67
  78    47    52
  80    45    41
  82    33    31
  84    29    25
  86    20    19
  88    19    15      inset = represents 1 library sequences
  90    16    11
  92    18     9
  94     9     7
  96     7     5
  98     4     4
 100    13     3
 102     5     2
 104     2     2
 106     5     1
 108     4     1
 110     2     1
 112     5     1
 114     6     1
 116     2     0
 118     1     0
>120    30     0
```

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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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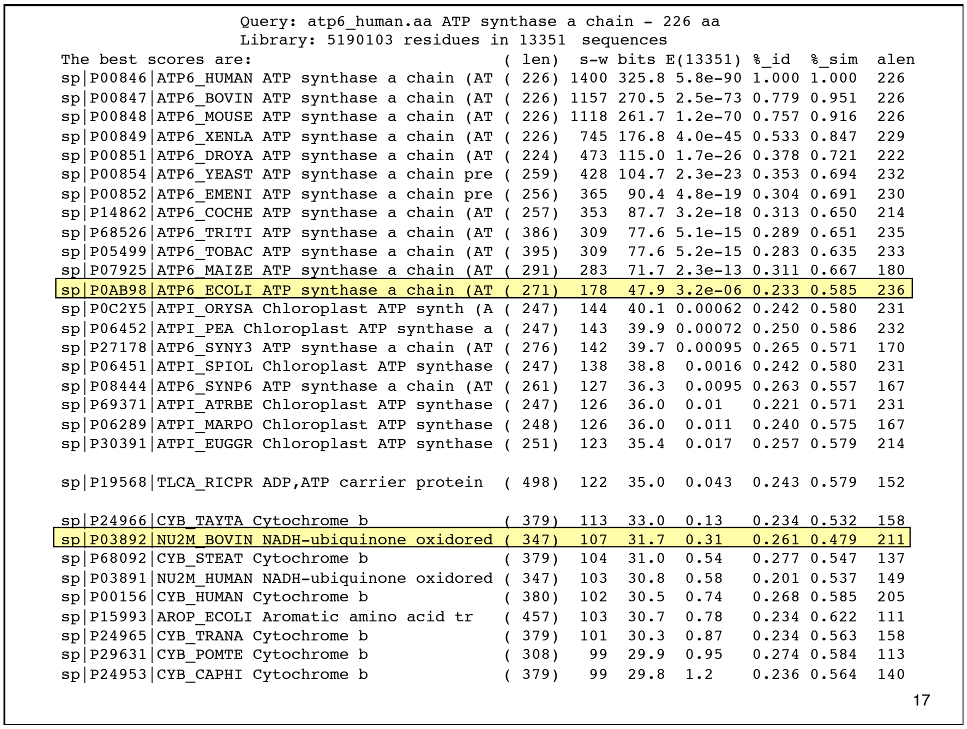

```
                   Query: atp6_human.aa ATP synthase a chain - 226 aa
                   Library: 5190103 residues in 13351  sequences
The best scores are:                          ( len)  s-w bits E(13351) %_id  %_sim  alen
sp|P00846|ATP6_HUMAN ATP synthase a chain (AT ( 226) 1400 325.8 5.8e-90 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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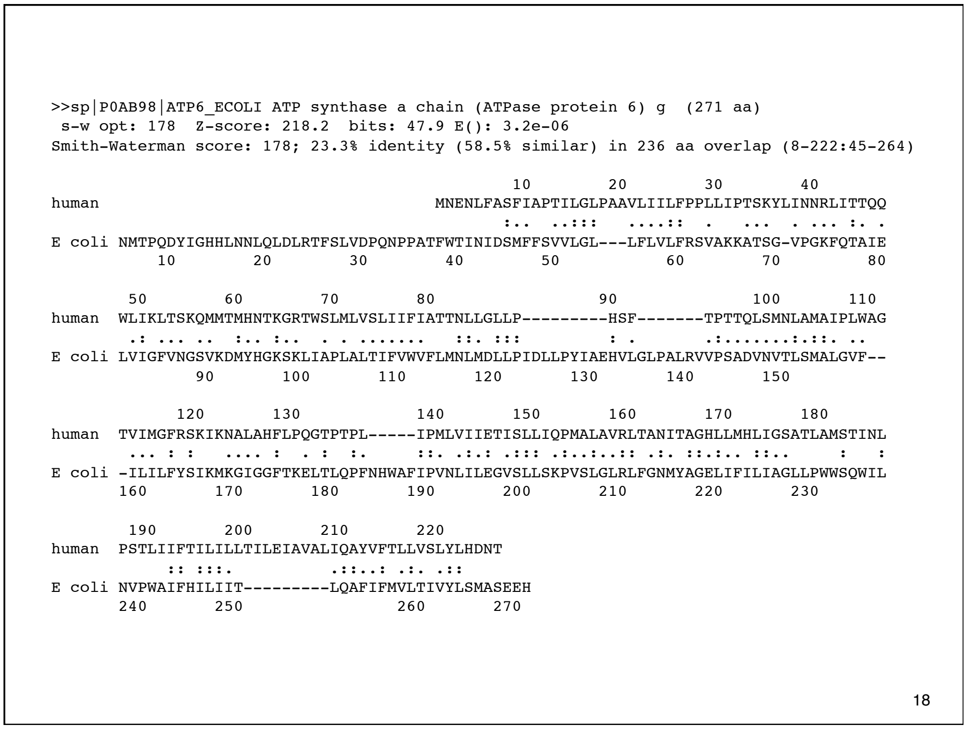

```
>>sp|P0AB98|ATP6_ECOLI ATP synthase a chain (ATPase protein 6) g  (271 aa)
 s-w opt: 178  Z-score: 218.2  bits: 47.9 E(): 3.2e-06
Smith-Waterman score: 178; 23.3% identity (58.5% similar) in 236 aa overlap (8-222:45-264)

                                                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
```

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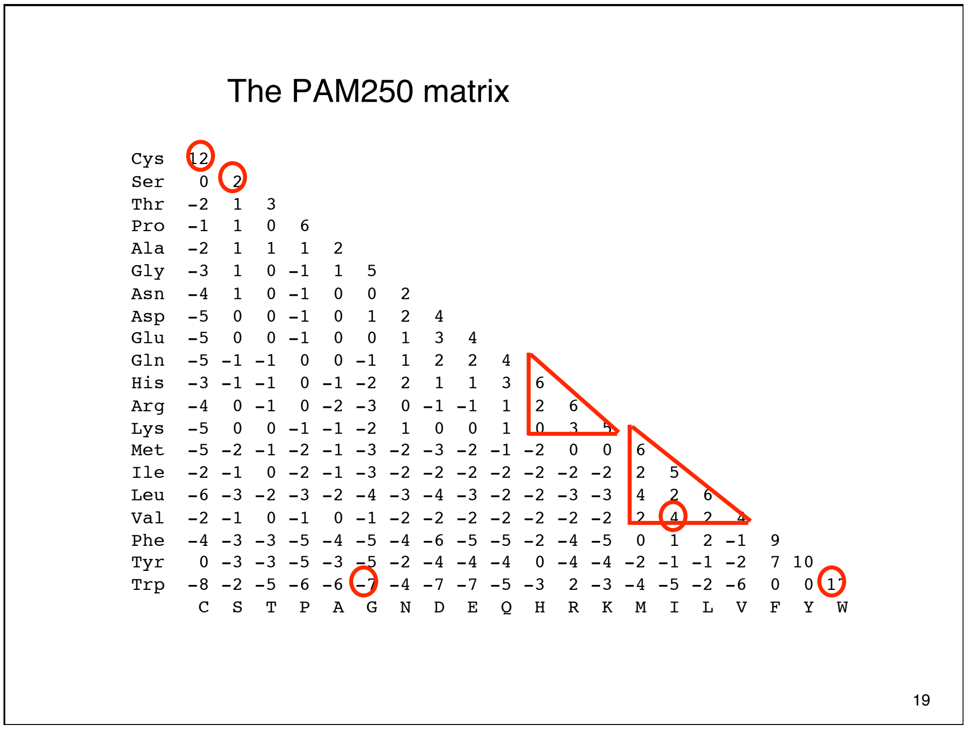
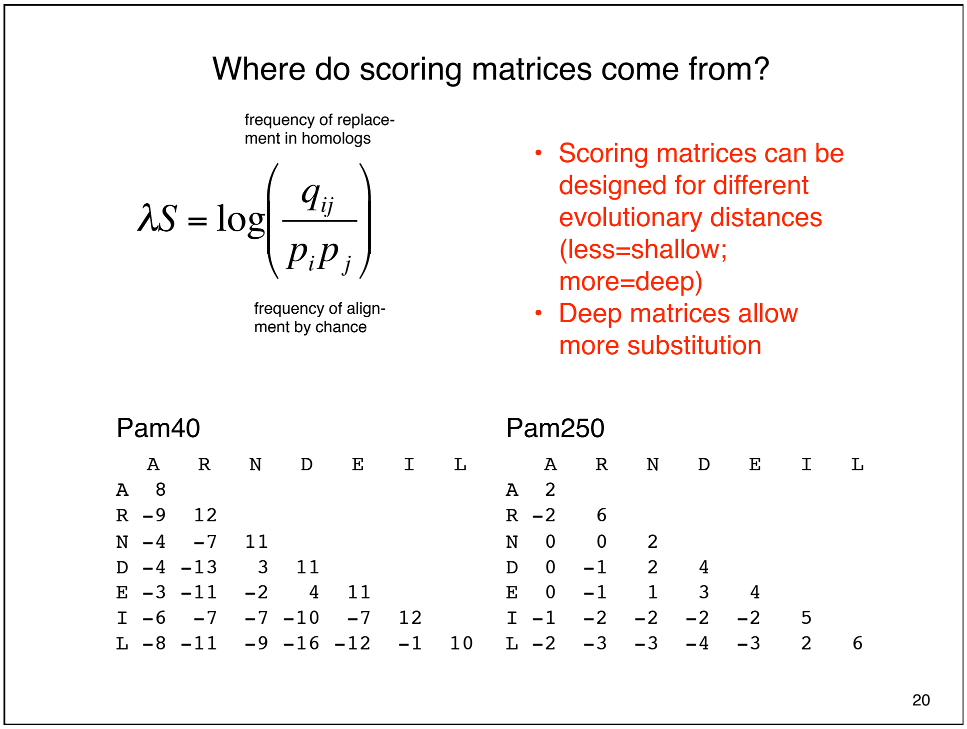


The PAM250 matrix

	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

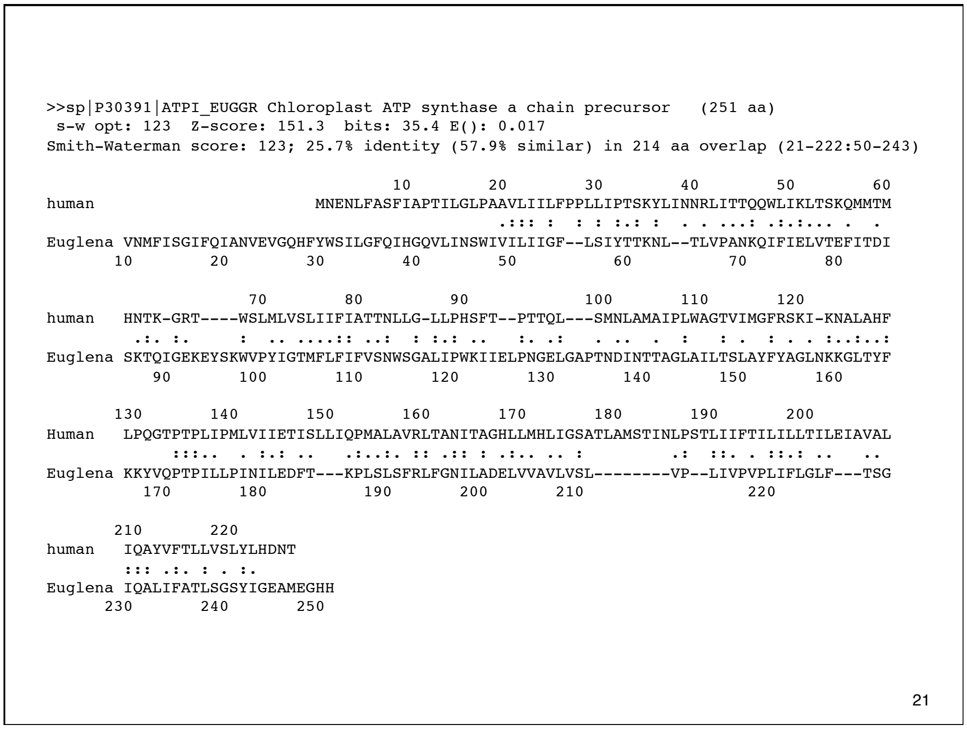
- Scoring matrices can be designed for different evolutionary distances (less=shallow; more=deep)
- Deep matrices allow more substitution

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

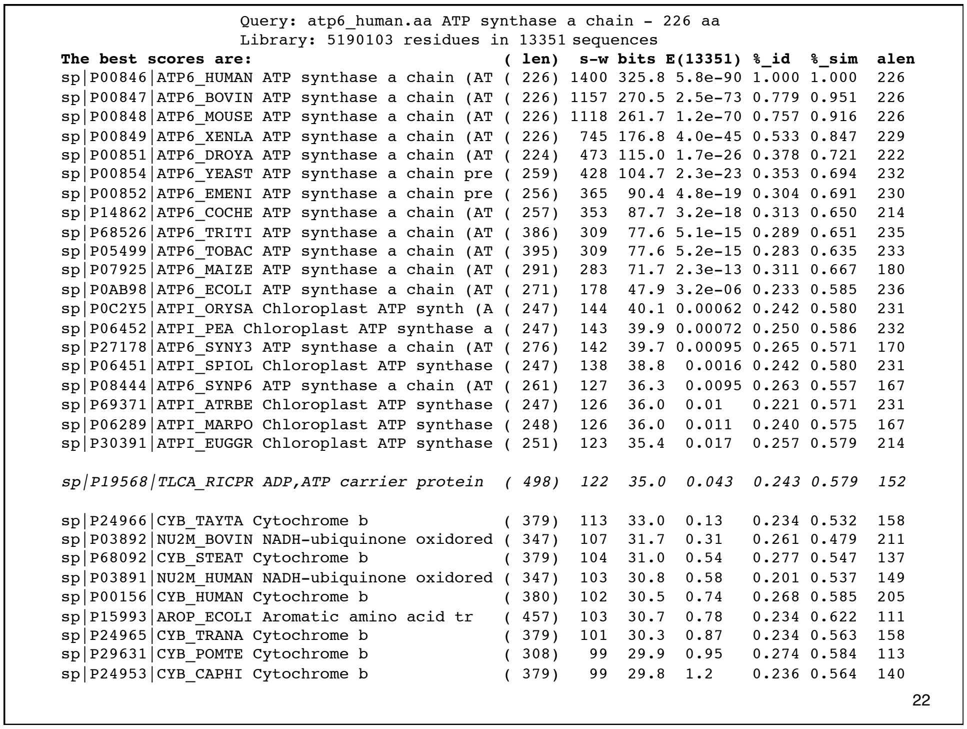
Pam250

	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



```
>>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
```

21



```
                   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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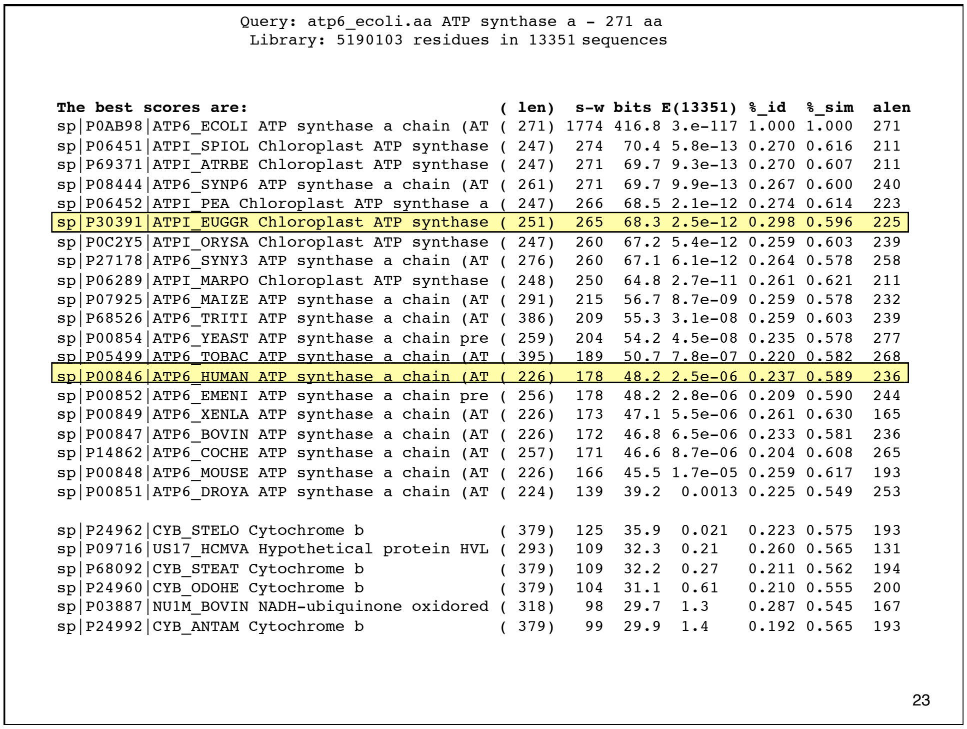

```
                  Query: atp6_ecoli.aa ATP synthase a - 271 aa
                   Library: 5190103 residues in 13351 sequences


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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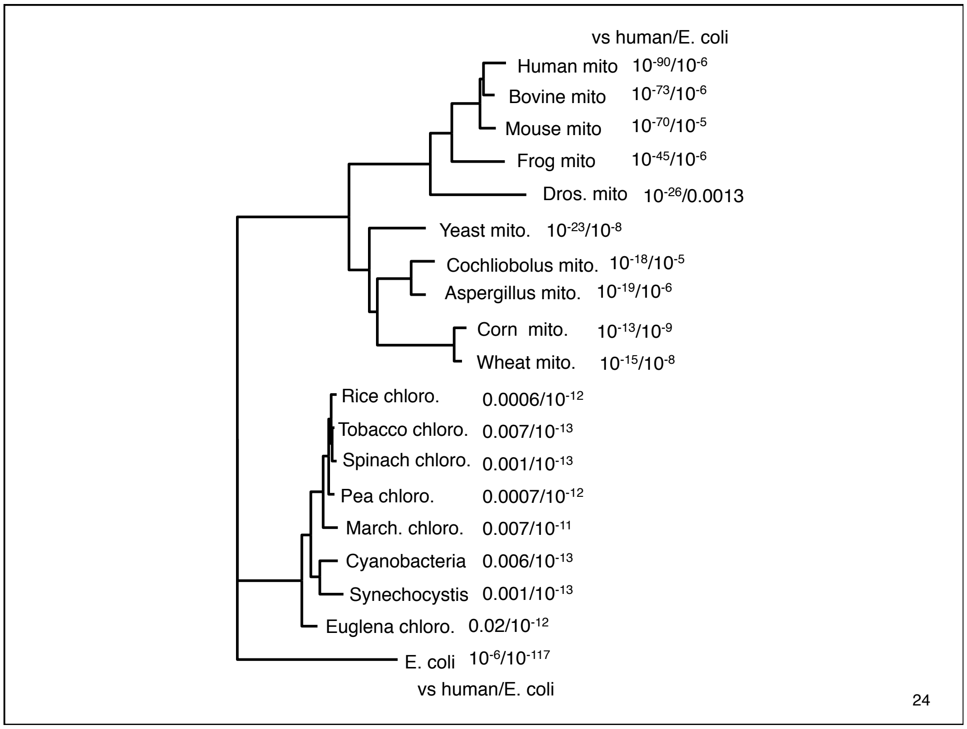
vs human/E. coli
Human mito 10^-90/10^-6
Bovine mito 10^-73/10^-6
Mouse mito 10^-70/10^-5
Frog mito 10^-45/10^-6
Dros. mito 10^-26/0.0013
Yeast mito. 10^-23/10^-8
Cochliobolus mito. 10^-18/10^-5
Aspergillus mito. 10^-19/10^-6
Corn mito. 10^-13/10^-9
Wheat mito. 10^-15/10^-8
Rice chloro. 0.0006/10^-12
Tobacco chloro. 0.007/10^-13
Spinach chloro. 0.001/10^-13
Pea chloro. 0.0007/10^-12
March. chloro. 0.007/10^-11
Cyanobacteria 0.006/10^-13
Synechocystis 0.001/10^-13
Euglena chloro. 0.02/10^-12
E. coli 10^-6/10^-117
vs human/E. coli

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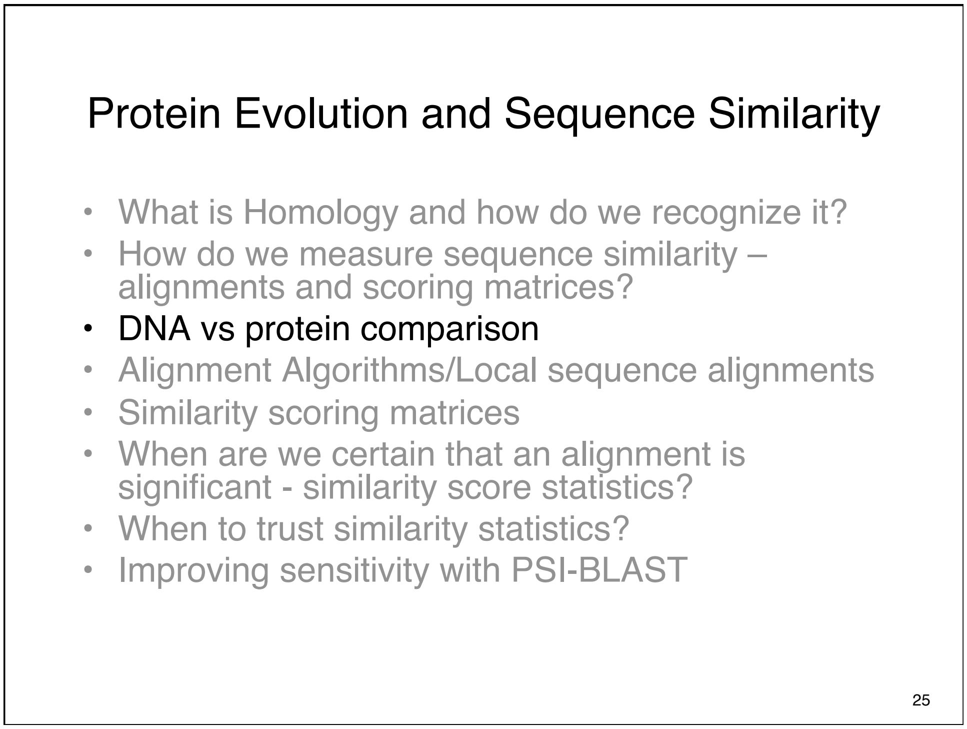

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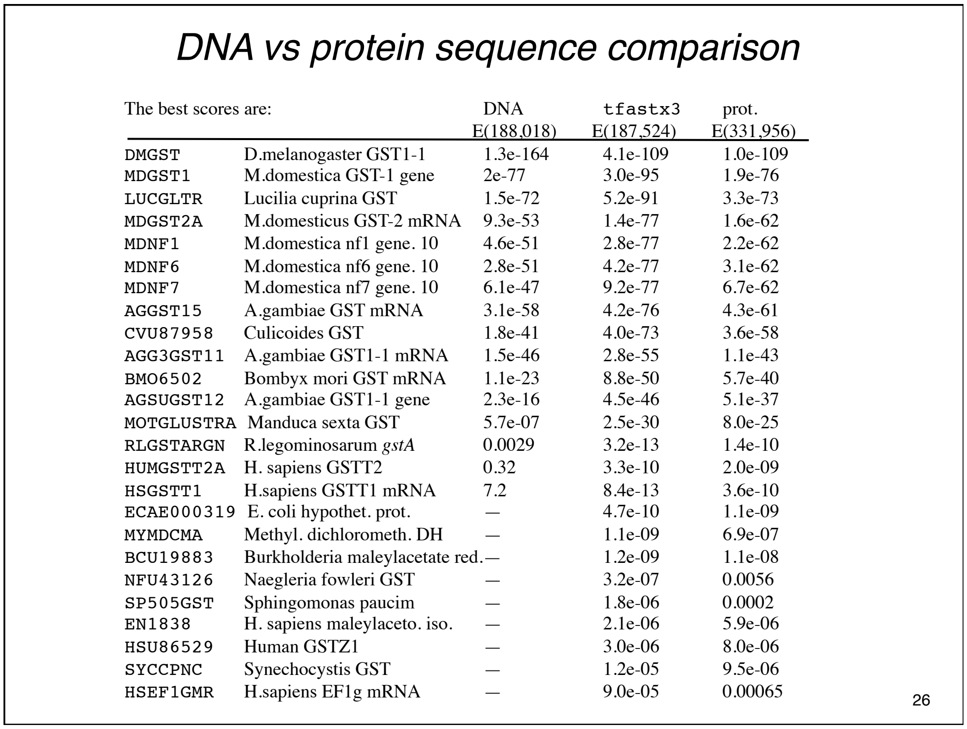


DNA vs protein sequence comparison

The best scores are:		DNA	`tfastx3`	prot.
		E(188,018)	E(187,524)	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

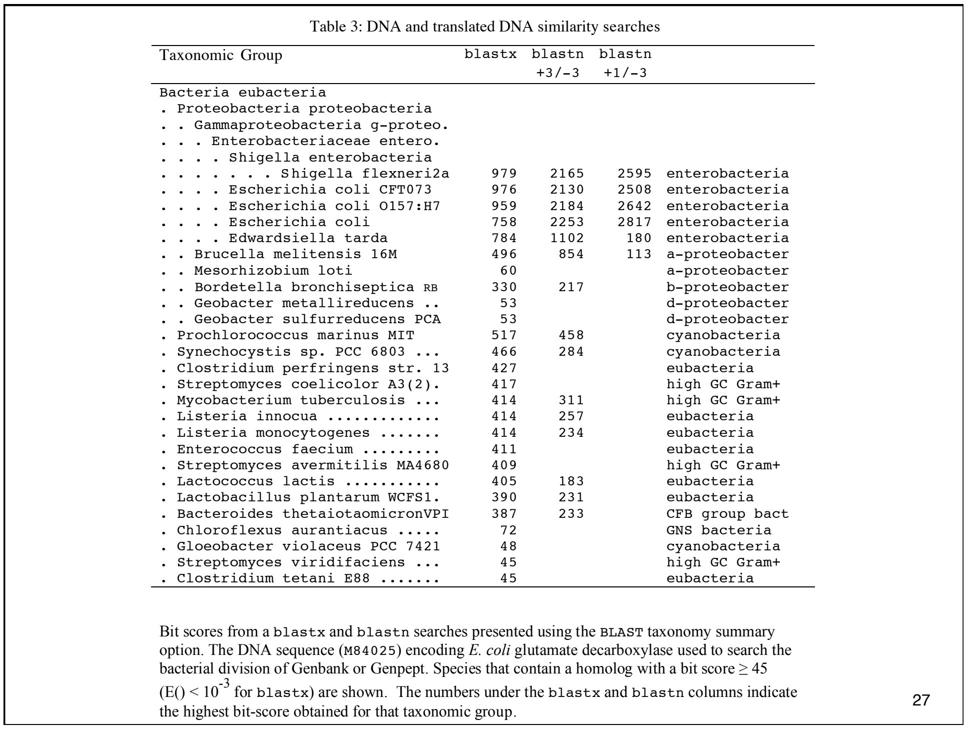
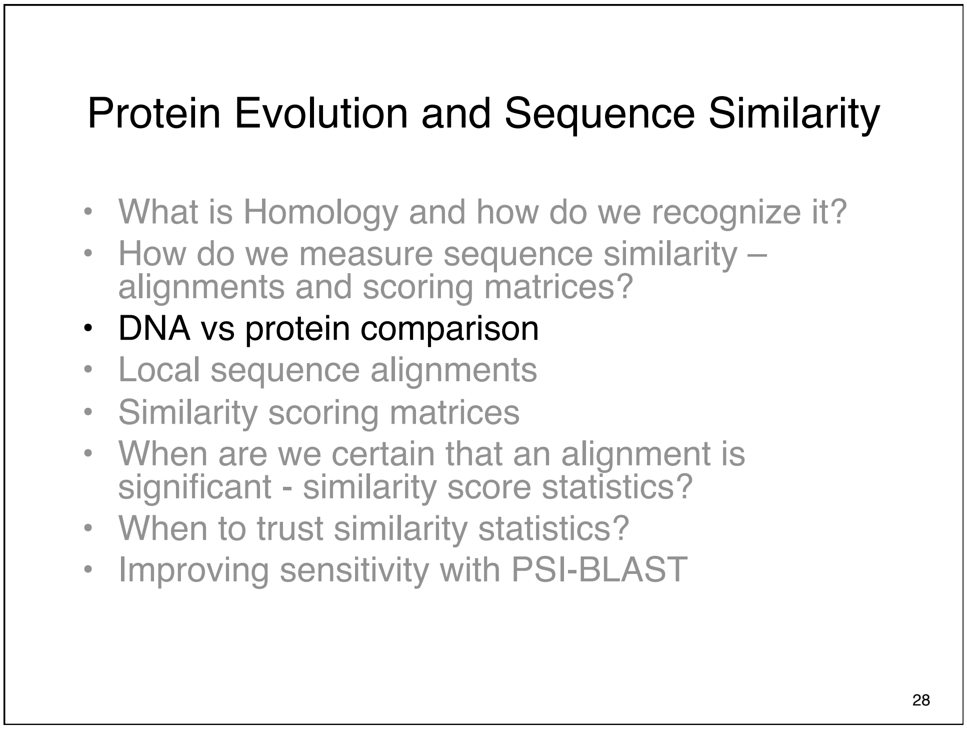


Table 3: DNA and translated DNA similarity searches

Taxonomic Group	blastx	blastn	blastn	
		+3/-3	+1/-3	
Bacteria eubacteria				
. Proteobacteria proteobacteria				
. . Gammaproteobacteria g-proteo.				
. . . Enterobacteriaceae entero.				
. . . . Shigella enterobacteria				
. . . . Shigella flexneri2a	979	2165	2595	enterobacteria
. . . . Escherichia coli CFT073	976	2130	2508	enterobacteria
. . . . Escherichia coli O157:H7	959	2184	2642	enterobacteria
. . . . Escherichia coli	758	2253	2817	enterobacteria
. . . . Edwardsiella tarda	784	1102	180	enterobacteria
. . Brucella melitensis 16M	496	854	113	a-proteobacter
. . Mesorhizobium loti	60			a-proteobacter
. . Bordetella bronchiseptica RB	330	217		b-proteobacter
. . Geobacter metallireducens ..	53			d-proteobacter
. . Geobacter sulfurreducens PCA	53			d-proteobacter
. Prochlorococcus marinus MIT	517	458		cyanobacteria
. Synechocystis sp. PCC 6803 ...	466	284		cyanobacteria
. Clostridium perfringens str. 13	427			eubacteria
. Streptomyces coelicolor A3(2).	417			high GC Gram+
. Mycobacterium tuberculosis ...	414	311		high GC Gram+
. Listeria innocua	414	257		eubacteria
. Listeria monocytogenes	414	234		eubacteria
. Enterococcus faecium	411			eubacteria
. Streptomyces avermitilis MA4680	409			high GC Gram+
. Lactococcus lactis	405	183		eubacteria
. Lactobacillus plantarum WCFS1.	390	231		eubacteria
. Bacteroides thetaiotaomicronVPI	387	233		CFB group bact
. Chloroflexus aurantiacus	72			GNS bacteria
. Gloeobacter violaceus PCC 7421	48			cyanobacteria
. Streptomyces viridifaciens ...	45			high GC Gram+
. Clostridium tetani E88	45			eubacteria

Bit scores from a blastx and blastn searches presented using the BLAST taxonomy summary option. The DNA sequence (M84025) encoding *E. coli* glutamate decarboxylase used to search the bacterial division of Genbank or Genpept. Species that contain a homolog with a bit score ≥ 45 ($E() < 10^{-3}$ for blastx) are shown. The numbers under the blastx and blastn columns indicate the highest bit-score obtained for that taxonomic group.



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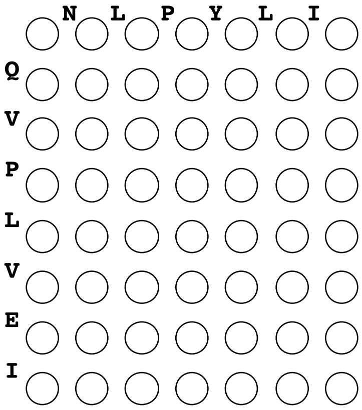
Smith-Waterman



1. score every cell:

$S_{x,y} = max\{$

$S_{x-1,y-1} + \text{match}_{xy}$

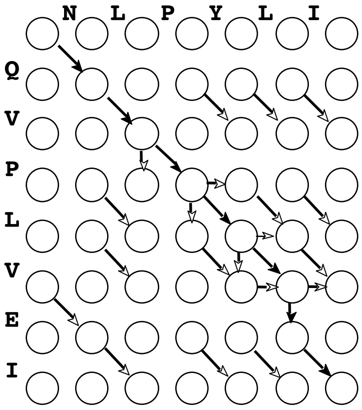
$S_{x,y-1} - \text{gappen}$

$S_{x-1,y} - \text{gappen}$

0

$\}$

Smith-Waterman



1. score every cell:

$S_{x,y} = max\{$

$S_{x-1,y-1} + \text{match}_{xy}$

$S_{x,y-1} - \text{gappen}$

$S_{x-1,y} - \text{gappen}$

0

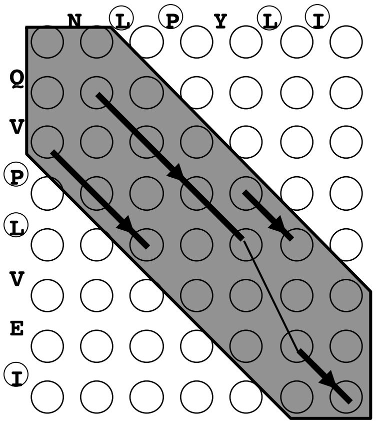
$\}$

2. follow “traceback”

```
NLPYL-I
..: . :
QVPLVEI
```

Outcome: one continuous,optimal gapped alignment

FASTA



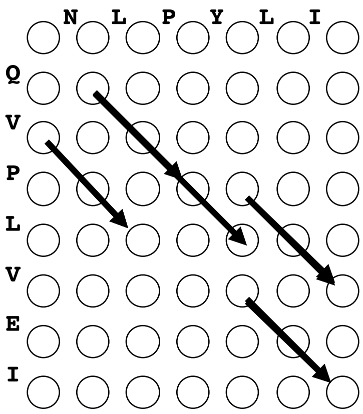
1. Identify identical matches
   (length = *ktup*)
2. Extend along diagonal
   (local maximum)
3. Join diagonal segments (DP)
   (maintain linearity)
   (optimal sum score)

4. Banded Smith-Waterman

```
NLPYL-I
..: . :
QVPLVEI
```

Outcome: one continuous, near-optimal gapped alignment

BLAST



1. neighborhood word hits
   (word length)
2. extend from diagonal ends
   (X-drop threshold)
3. report HSP linkages
   (maintain linearity)
   (probability)

```
NL   NLP  LI
.:   ..:  .:
PL   QVP  EI
```

Outcome: multiple HSPs, multiple linkages; only partially aligned

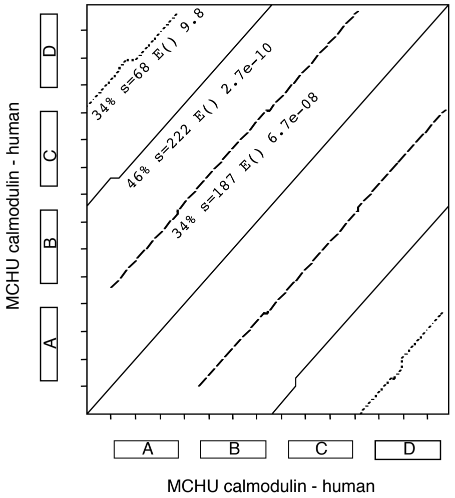
Local alignments - calmodulin

```
 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



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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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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

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

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

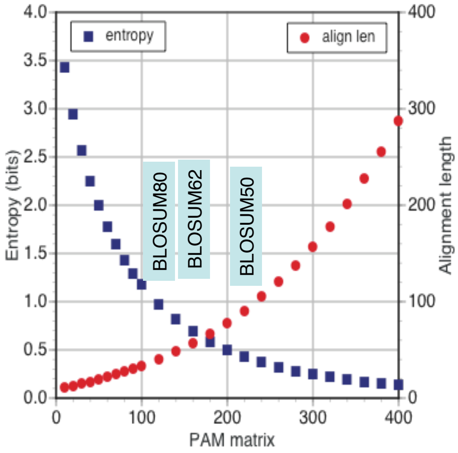
$\lambda_2\ S_{ij} = \lg_2\ (q_{ij}/p_ip_j)$ $\lambda_e\ S_{ij} = \ln(q_{ij}/p_ip_j)$ $p_Rp_N = 0.002193$

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

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

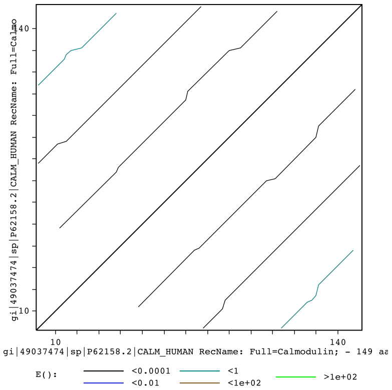
$\lambda\ S_{R:N(250)} = \lg2\ (0.002193/0.002193) = 0$

PAM matrices and alignment length

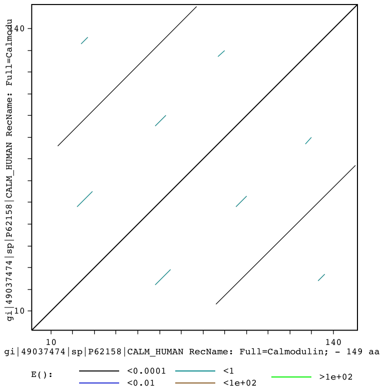


Scoring matrices set look back time

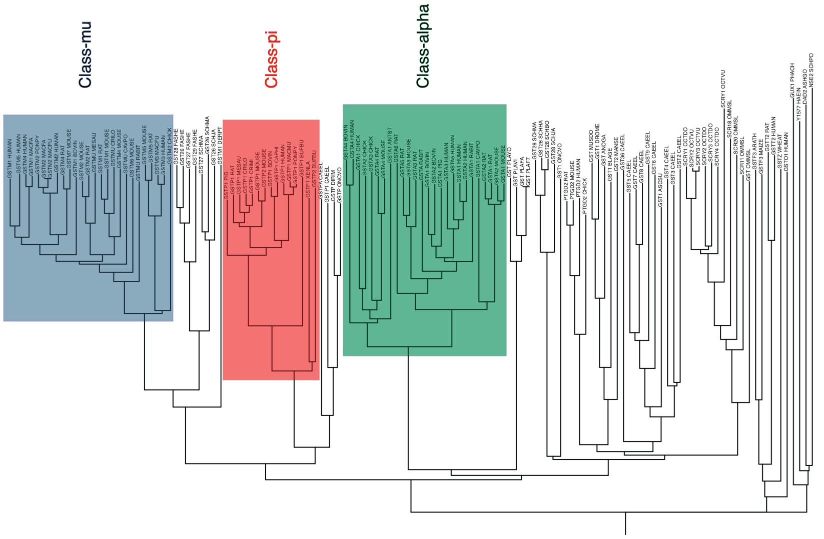
BLOSUM50 -10/-2



MD20 -26/-4



Glutathione Transferases (gstm1_human)



40

Class-mu

Class-pi

Class-alpha

```
                BLOSUM50-10/-2 BLOSUM62-11/-    MD40 -21/-     MD10 -23/-4
               E(320363) f_id E(320363) f_id E(320363) f_id E(320363) f_id

 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

        GSTP1_PIG     2.9e-20 0.327  1.2e-2  0.327  0.00034  0.409
        GSTP1_XENLA   5.2e-19 0.333  6.0e-2  0.330  0.12     0.464
        GSTP2_MOUSE   8.0e-17 0.294  1.3e-2  0.294  1.1      0.395
        GSTP1_CAEEL   1.1e-16 0.324  4.3e-2  0.319  1.1      0.706
        GSTP1_HUMAN   3.0e-16 0.284  2.2e-2  0.284  0.29     0.467
        GSTP1_BUFBU   1.2e-14 0.285  7.2e-1  0.272  9.7      0.588
        GSTPA_CAEEL   1.1e-13 0.298  2.8e-1  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

                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
```

Scoring matrices influence alignment lengths

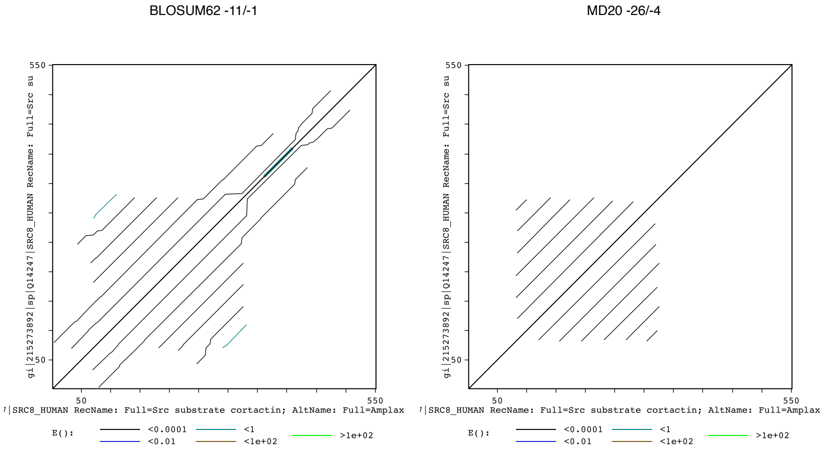
A. Search with MJ0050

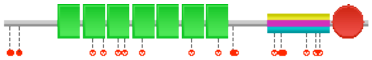
	BLOSUM50 -10/-2				BLOSUM62 -7/-1				BLOSUM62 -11/-1			
The best scores are:	s-w	E()	%_id	alen	s-w	E()	%_id	alen	s-w	E()	%_id	alen
NP_416010 glutamate decarb.	250	e-11	24.9	401	216	e-7	25.3	415	137	e-8	22.9	332
NP_417379 glycine decarb.	169	e-05	22.1	420	163	0.001	23.3	430	88	0.004	22.1	331
NP_417025 aminotransferase	122	0.02	23.6	254	119	0.12	24.5	257	76	0.04	23.7	118
NP_414772 aminoacyl-his.	110	0.15	23.4	188	108	0.74	23.2	311	57	6.9	23.4	188
NP_415139 alkyl hydroperoxide	99	1.1	26.9	156	104	1.5	24.5	233	62	2.0	28.9	97

B. Search with MJ1633

	BLOSUM50 -10/-2				BLOSUM62 -7/-1				BLOSUM62 -11/-1			
The best scores are:	s-w	E()	%_id	alen	s-w	E()	%_id	alen	s-w	E()	%_id	alen
NP_417809 KefB	196	e-06	28.2	177	162	0.02	27.3	176	143	e-8	34.4	96
NP_414589 K+ antiporter	175	e-04	25.4	142	141	0.2	24.7	166	131	e-7	25.4	142
NP_415011 transport protein	133	0.03	23.2	142	113	4.4	23.2	142	89	0.005	23.2	142
NP_417748 TrkA	128	0.04	23.7	135	114	2.9	22.2	176	99	e-3	21.8	133
NP_416807 NAD(P) binding	103	0.98	26.1	92					70	0.29	26.1	92

Scoring matrices influence alignment lengths





Similarity Scoring Matrices - Summary

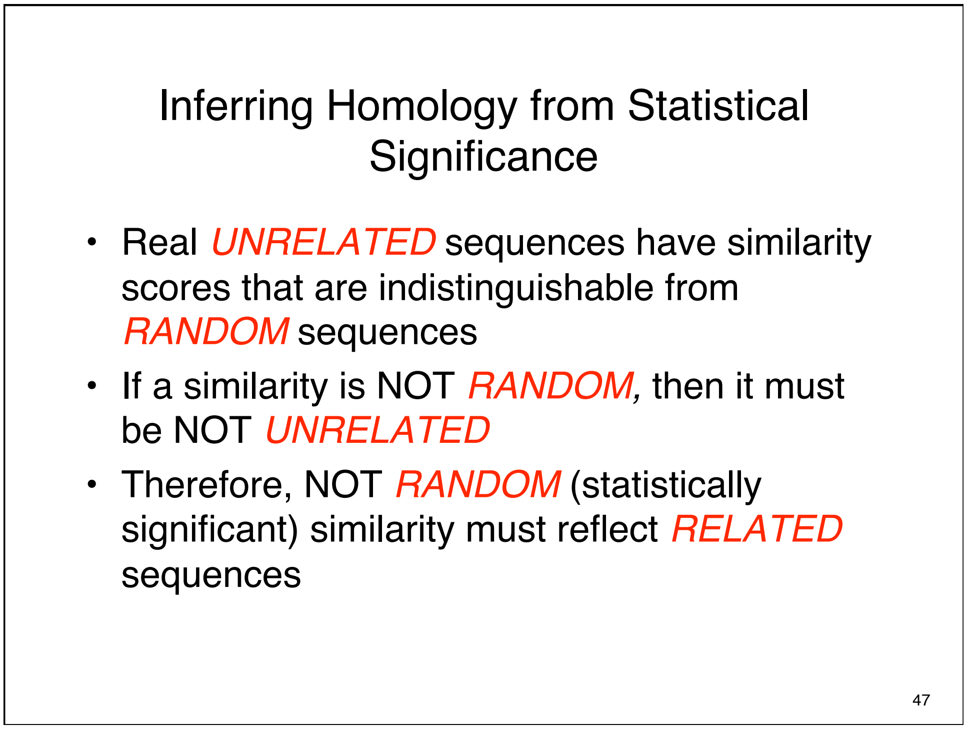
- Similarity scoring matrices are “log-odds” matrices, reporting the “odds” that an alignment reflects homology rather than chance
- One can predict evolutionary changes using a simple random model, which can generate mutation frequencies at any evolutionary distance
- The optimal scoring matrix has an evolutionary distance that matches that of the alignment. Matrices that are shallower than the true distance produce short alignments, while matrices that are deeper produce long alignments.
- Shallower scoring matrices have more information content, or “bits/residue”, and thus can be used to find shorter domains
- Scoring matrices set evolutionary look back times

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

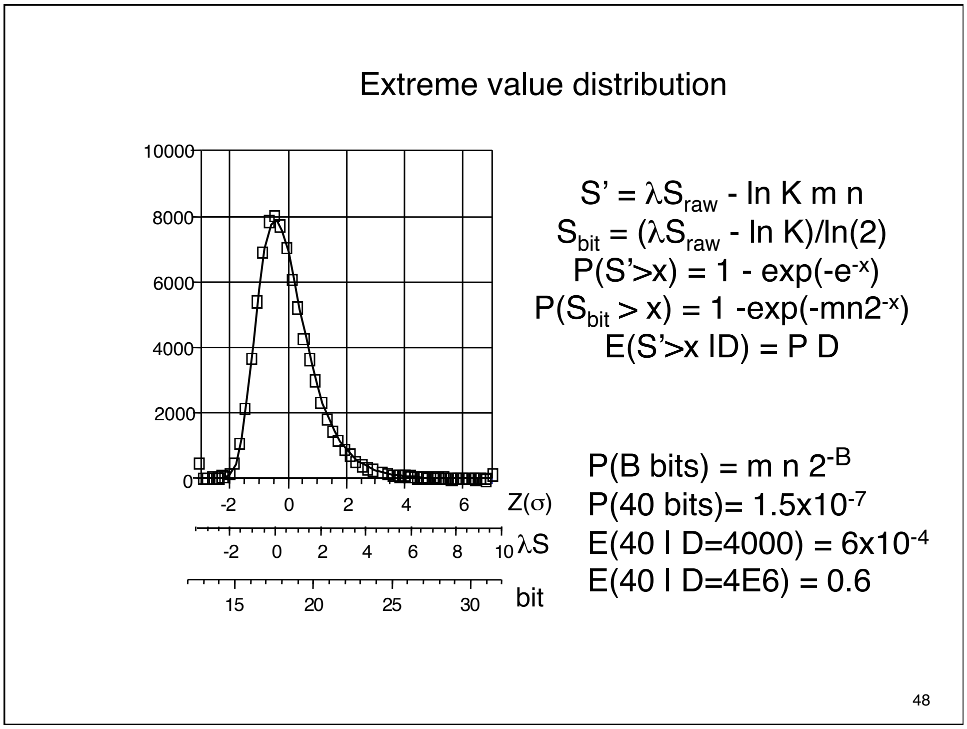
Protein Evolution and Sequence Similarity

- 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



Inferring Homology from Statistical Significance

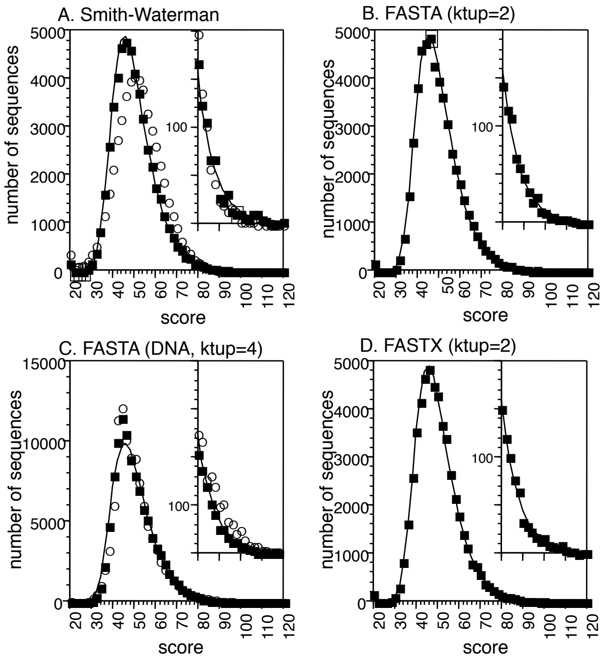
- Real *UNRELATED* sequences have similarity scores that are indistinguishable from *RANDOM* sequences
- If a similarity is NOT *RANDOM,* then it must be NOT *UNRELATED*
- Therefore, NOT *RANDOM* (statistically significant) similarity must reflect *RELATED* sequences



Extreme value distribution

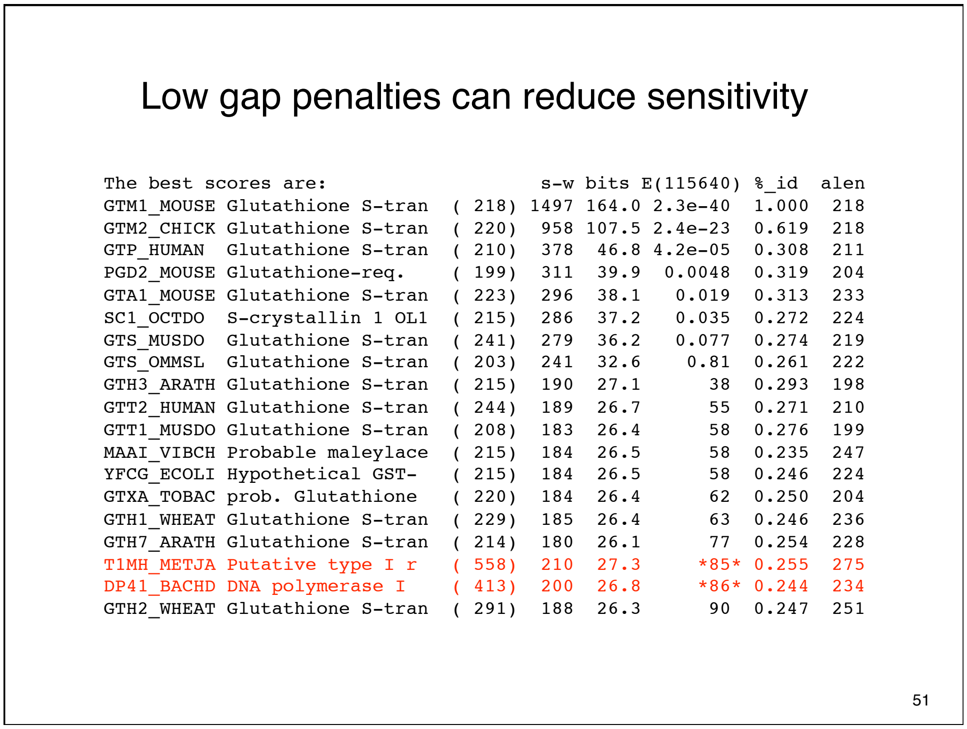
$$S' = \lambda S_{raw} - \ln K\ m\ n$$
$$S_{bit} = (\lambda S_{raw} - \ln K)/\ln(2)$$
$$P(S'>x) = 1 - \exp(-e^{-x})$$
$$P(S_{bit} > x) = 1 - \exp(-mn2^{-x})$$
$$E(S'>x\ \mathrm{I}\ D) = P\ D$$

$$P(B\ \text{bits}) = m\ n\ 2^{-B}$$
$$P(40\ \text{bits}) = 1.5\text{x}10^{-7}$$
$$E(40\ \mathrm{I}\ D{=}4000) = 6\text{x}10^{-4}$$
$$E(40\ \mathrm{I}\ D{=}4E6) = 0.6$$



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
VP2_AHSV3  outer capsid prot   (1057)  108  31.5   *8.9*  0.205  200
GTH5_ARATH Glutathione S-trans ( 218)   96  29.2     9.2  0.258   66
DCMA_METSP dichloromethane DM  ( 288)   98  29.5     9.3  0.195  200
GTXA_ARATH Glutathione S-trans ( 224)   96  29.1     9.5  0.248  125
SLT_HAEIN  Putative soluble l  ( 593)  103  30.5   *9.9*  0.227  185
```



Low gap penalties can reduce sensitivity

```
The best scores are:                       s-w bits E(115640) %_id  alen
GTM1_MOUSE Glutathione S-tran  ( 218) 1497 164.0 2.3e-40  1.000  218
GTM2_CHICK Glutathione S-tran  ( 220)  958 107.5 2.4e-23  0.619  218
GTP_HUMAN  Glutathione S-tran  ( 210)  378  46.8 4.2e-05  0.308  211
PGD2_MOUSE Glutathione-req.    ( 199)  311  39.9  0.0048  0.319  204
GTA1_MOUSE Glutathione S-tran  ( 223)  296  38.1   0.019  0.313  233
SC1_OCTDO  S-crystallin 1 OL1  ( 215)  286  37.2   0.035  0.272  224
GTS_MUSDO  Glutathione S-tran  ( 241)  279  36.2   0.077  0.274  219
GTS_OMMSL  Glutathione S-tran  ( 203)  241  32.6    0.81  0.261  222
GTH3_ARATH Glutathione S-tran  ( 215)  190  27.1      38  0.293  198
GTT2_HUMAN Glutathione S-tran  ( 244)  189  26.7      55  0.271  210
GTT1_MUSDO Glutathione S-tran  ( 208)  183  26.4      58  0.276  199
MAAI_VIBCH Probable maleylace  ( 215)  184  26.5      58  0.235  247
YFCG_ECOLI Hypothetical GST-   ( 215)  184  26.5      58  0.246  224
GTXA_TOBAC prob. Glutathione   ( 220)  184  26.4      62  0.250  204
GTH1_WHEAT Glutathione S-tran  ( 229)  185  26.4      63  0.246  236
GTH7_ARATH Glutathione S-tran  ( 214)  180  26.1      77  0.254  228
T1MH_METJA Putative type I r   ( 558)  210  27.3    *85* 0.255  275
DP41_BACHD DNA polymerase I    ( 413)  200  26.8    *86* 0.244  234
GTH2_WHEAT Glutathione S-tran  ( 291)  188  26.3      90  0.247  251
```

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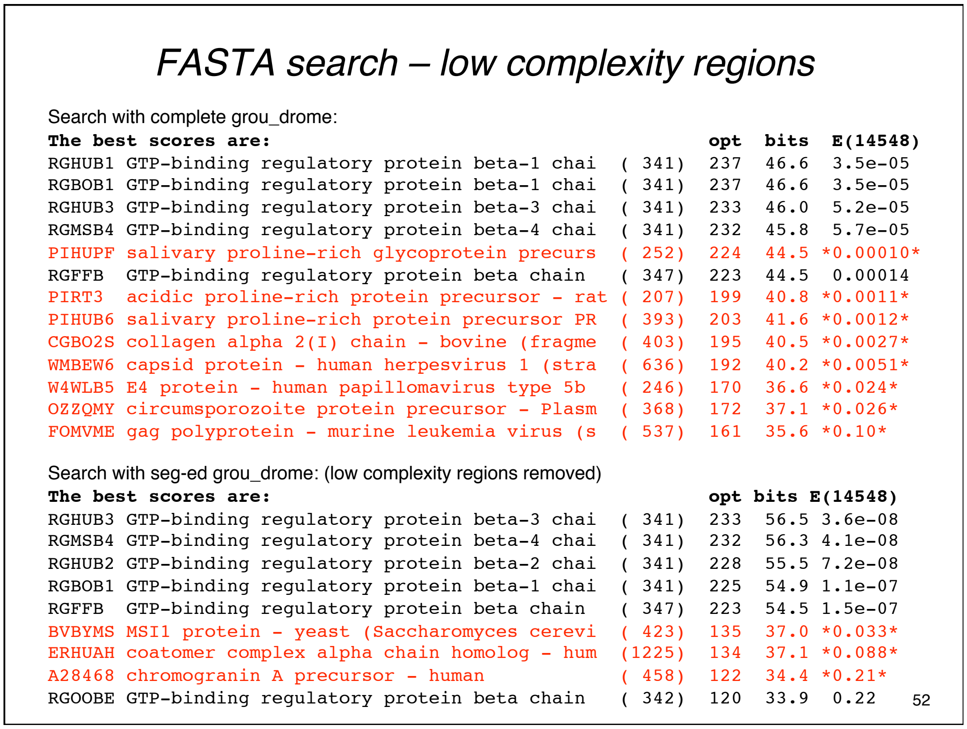

FASTA search – low complexity regions

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*
W4WLB5 E4 protein - human papillomavirus type 5b   ( 246)  170  36.6 *0.024*
OZZQMY circumsporozoite protein precursor - Plasm  ( 368)  172  37.1 *0.026*
FOMVME gag polyprotein - murine leukemia virus (s  ( 537)  161  35.6 *0.10*
```

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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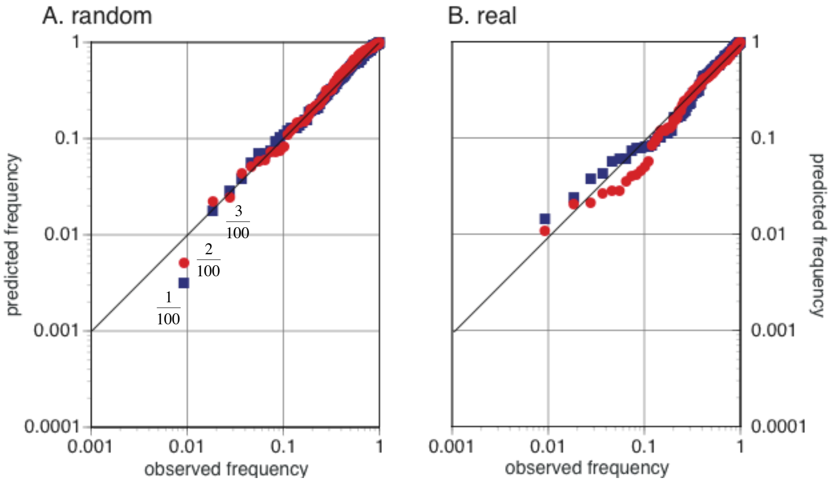
Protein Sequence Comparison Statistics are Accurate



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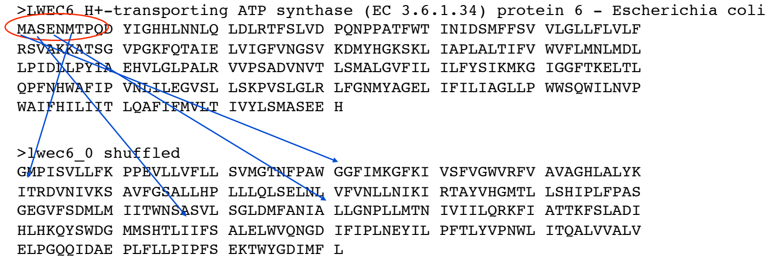
Statistical estimates from random shuffles

- 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

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
```

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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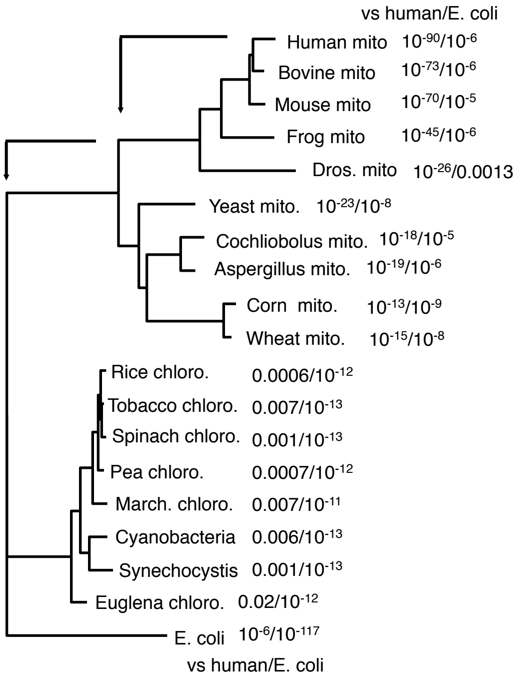
vs human/E. coli
Human mito $10^{-90}/10^{-6}$
Bovine mito $10^{-73}/10^{-6}$
Mouse mito $10^{-70}/10^{-5}$
Frog mito $10^{-45}/10^{-6}$
Dros. mito $10^{-26}/0.0013$
Yeast mito. $10^{-23}/10^{-8}$
Cochliobolus mito. $10^{-18}/10^{-5}$
Aspergillus mito. $10^{-19}/10^{-6}$
Corn mito. $10^{-13}/10^{-9}$
Wheat mito. $10^{-15}/10^{-8}$
Rice chloro. $0.0006/10^{-12}$
Tobacco chloro. $0.007/10^{-13}$
Spinach chloro. $0.001/10^{-13}$
Pea chloro. $0.0007/10^{-12}$
March. chloro. $0.007/10^{-11}$
Cyanobacteria $0.006/10^{-13}$
Synechocystis $0.001/10^{-13}$
Euglena chloro. $0.02/10^{-12}$
E. coli $10^{-6}/10^{-117}$
vs human/E. coli

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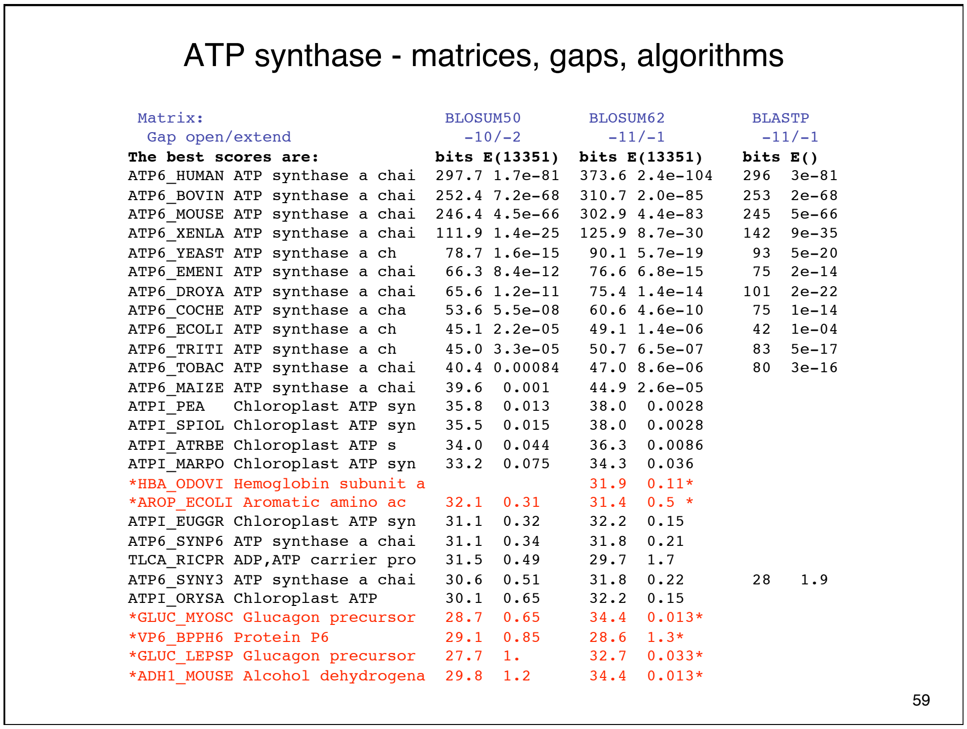
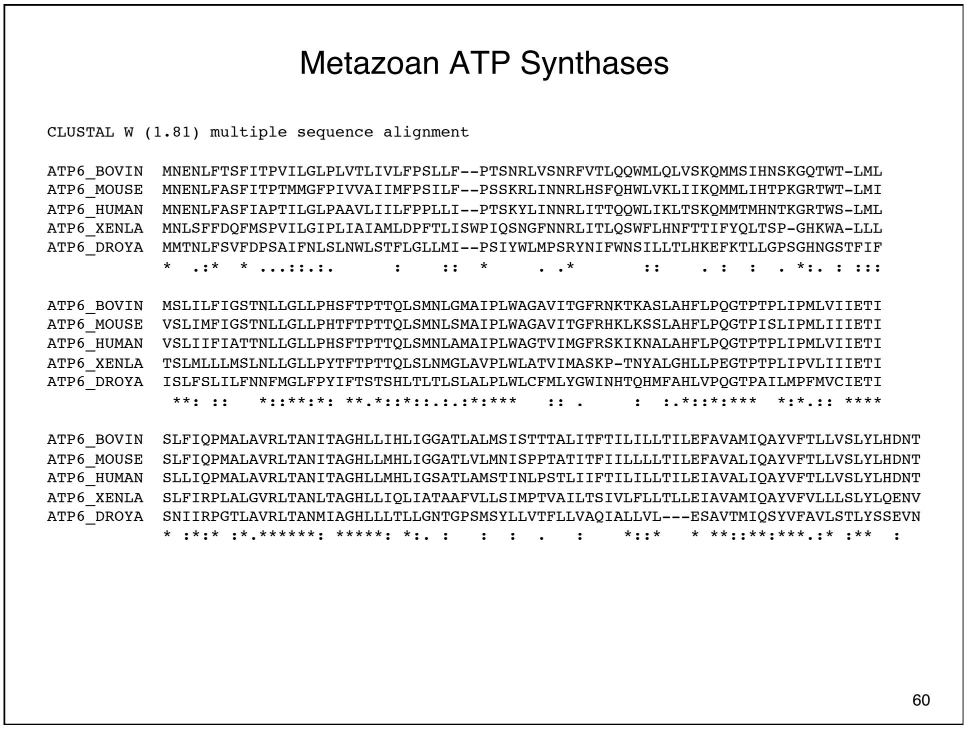


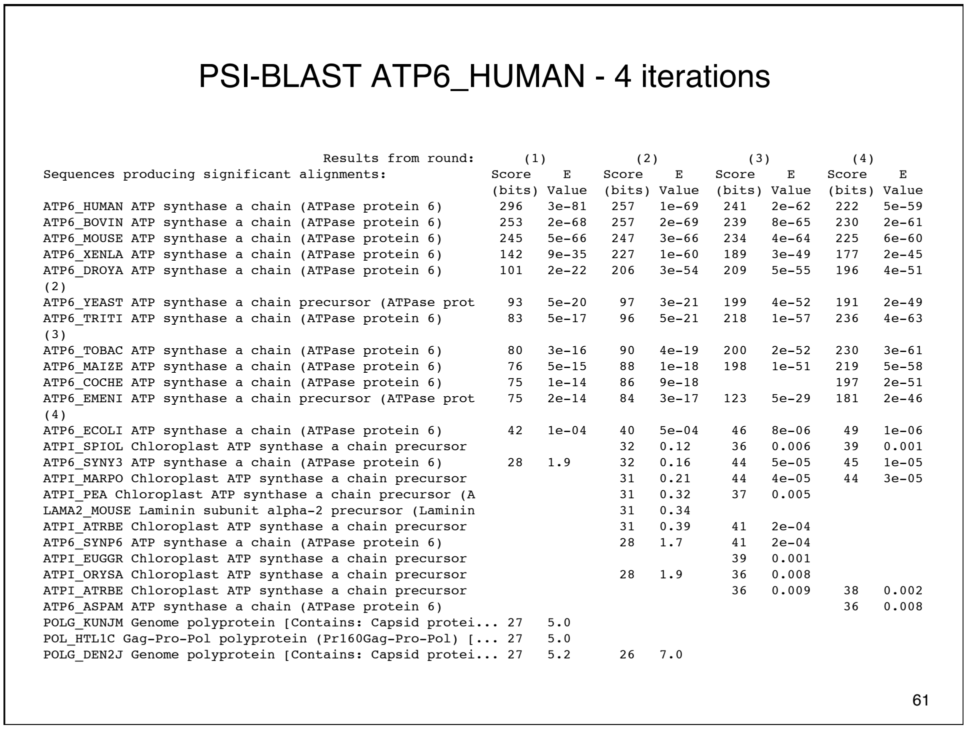
ATP synthase - matrices, gaps, algorithms

```
 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*
```



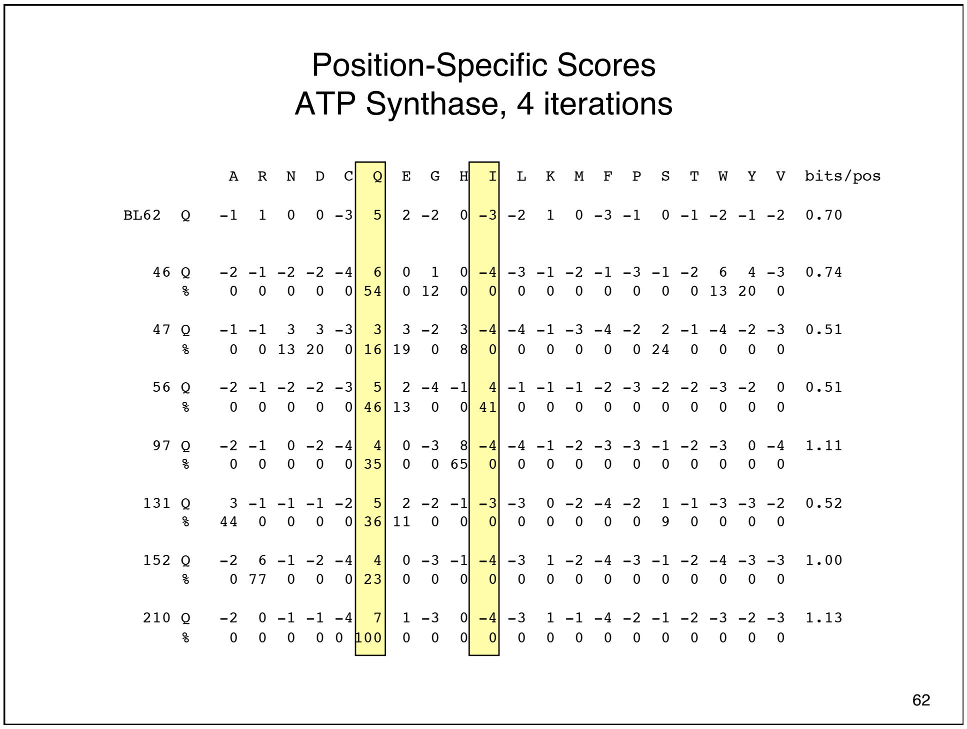
Metazoan ATP Synthases

```
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
            * :*:* :*.******: *****: *:. :   :   :  .   :    *::*   * **::**:***.:* :**  :
```



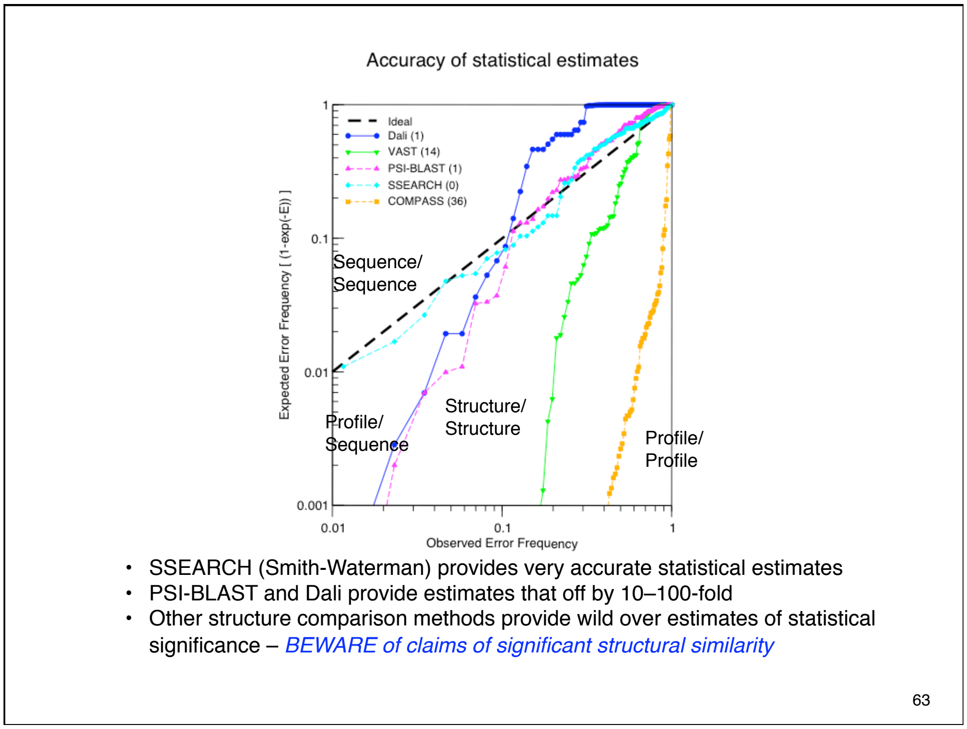
PSI-BLAST ATP6_HUMAN - 4 iterations

```
                                 Results from round:      (1)          (2)          (3)          (4)
Sequences producing significant alignments:             Score    E    Score    E    Score    E    Score    E
                                                        (bits) Value  (bits) Value  (bits) Value  (bits) 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)       101   2e-22   206   3e-54   209   5e-55   196   4e-51
(2)
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)        83   5e-17    96   5e-21   218   1e-57   236   4e-63
(3)
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    75   2e-14    84   3e-17   123   5e-29   181   2e-46
(4)
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
```



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  0100  0  0  0  0  0  0  0  0  0  0  0  0  0  0
```



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*

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 lead to “clans”

Sequence Similarity - Conclusions

- 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

Discussion (exam) 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 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)?
3. 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?
4. What scoring matrix should be used to identify protein orthologs that have diverged over the past 100 My (e.g. human/mouse)?
5. 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 bacteria 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?