Programming for Biology
Protein Evolution / Similarity Searching

What BLAST Does / Why BLAST works

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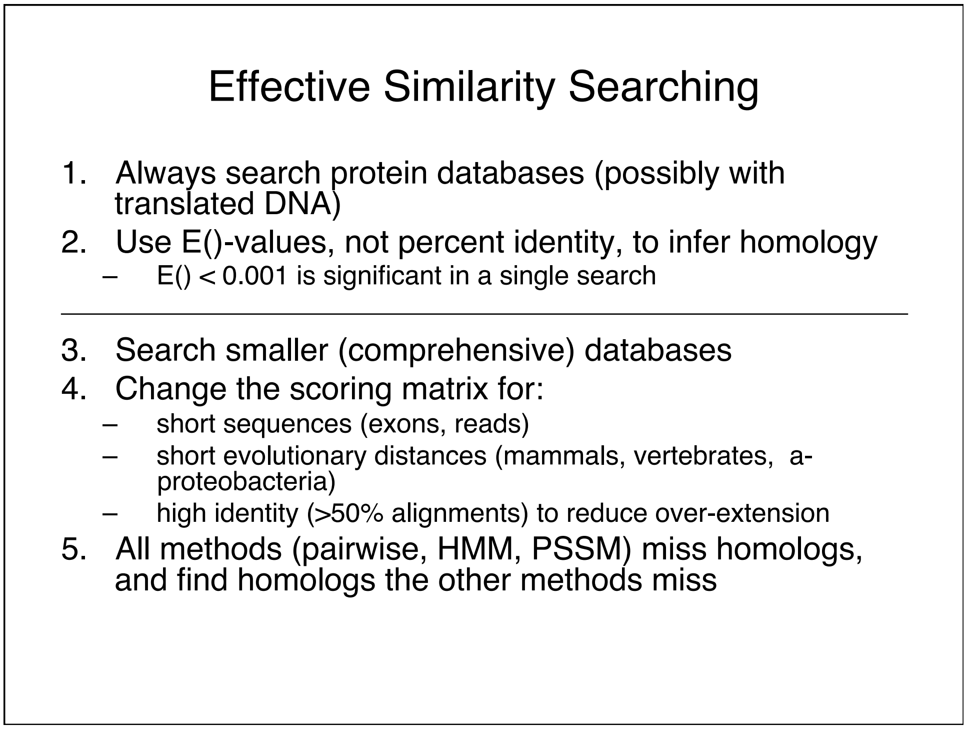
Protein Evolution/ Similarity Searching

- 9:00 – Homology and Expectation value
- 10:30 – Similarity searching workshop I
- 1:30 – Practical Similarity Searching, improving sensititivty
- 3:00 – Workshop II – Parsing search results



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

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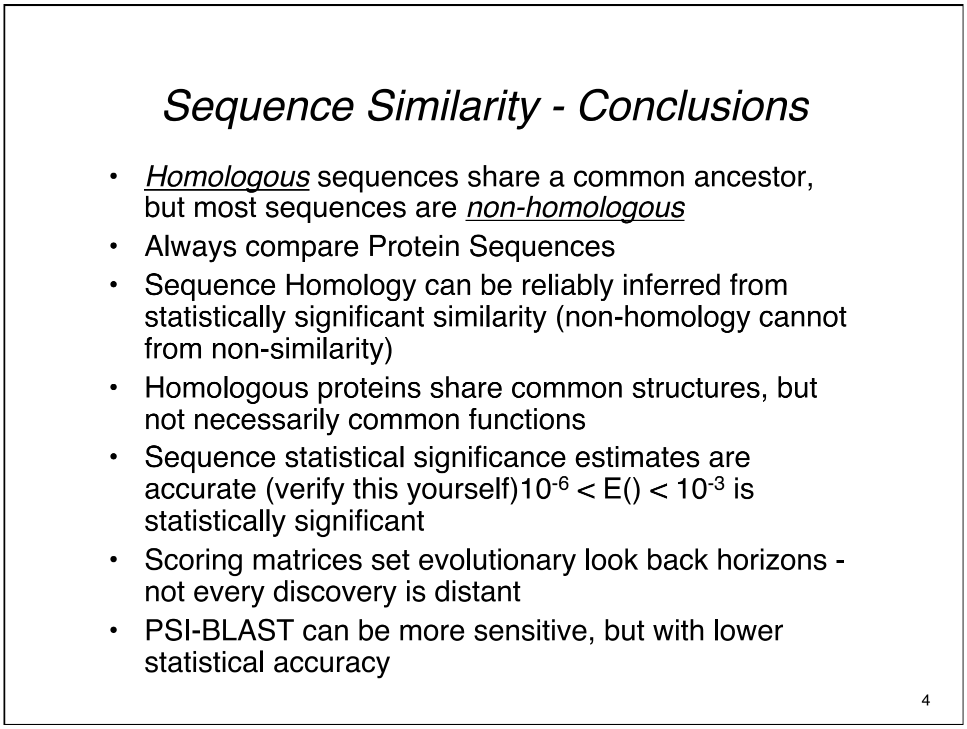
3. Search smaller (comprehensive) databases
4. Change the scoring matrix for:
   – short sequences (exons, reads)
   – short evolutionary distances (mammals, vertebrates, a-proteobacteria)
   – high identity (>50% alignments) to reduce over-extension
5. All methods (pairwise, HMM, PSSM) miss homologs,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



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

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

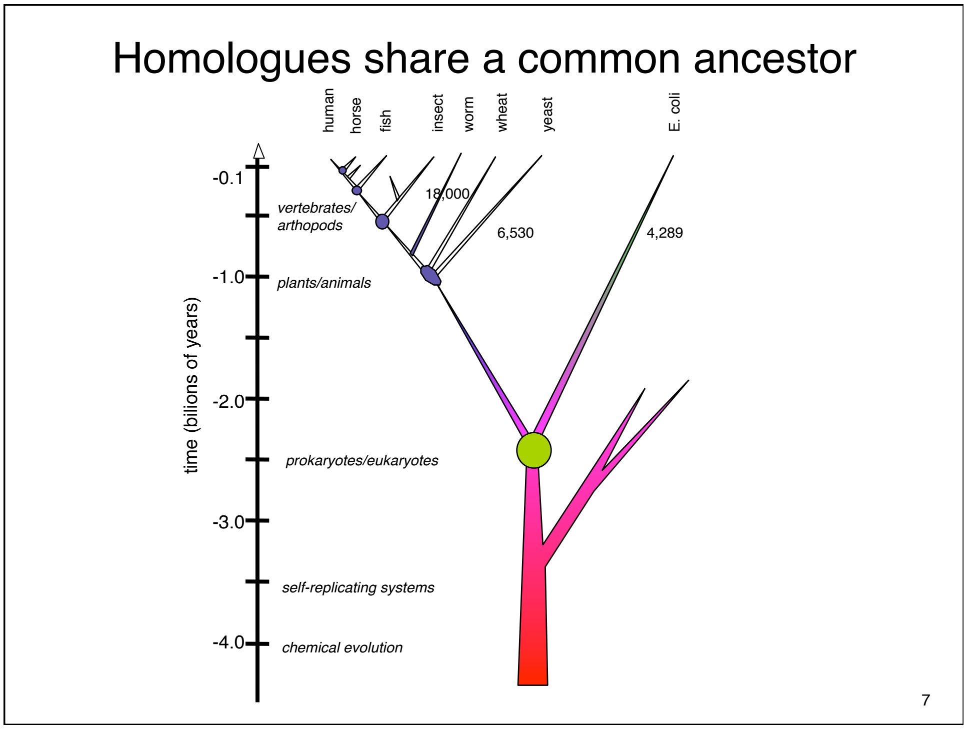
Protein Evolution and Sequence Similarity

Similarity Searching I

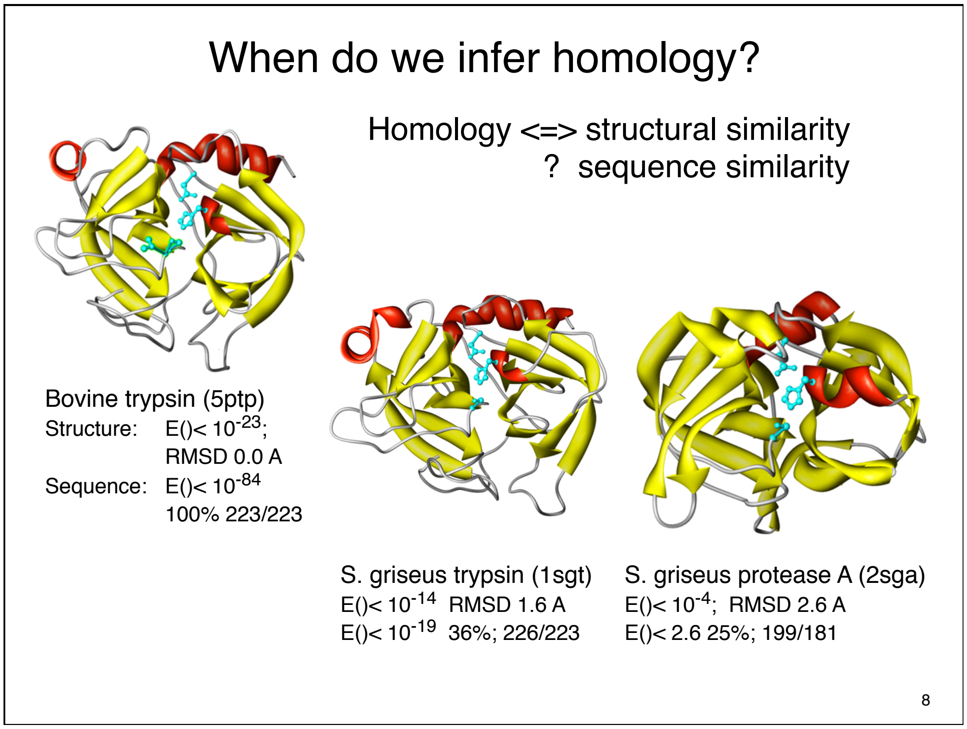
- What is Homology and how do we recognize it?
- How do we measure sequence similarity – alignments and scoring matrices?
- DNA vs protein comparison

Similarity Searching II

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



Homologues share a common ancestor



When do we infer homology?

Homology <=> structural similarity
? sequence similarity

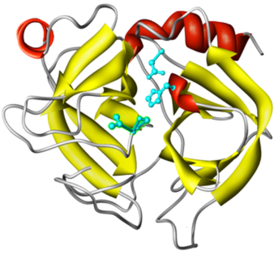
Bovine trypsin (5ptp)
Structure: $E() < 10^{-23}$; RMSD 0.0 A
Sequence: $E() < 10^{-84}$ 100% 223/223

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

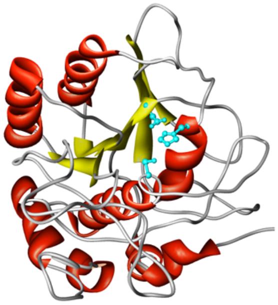
S. griseus protease A (2sga)
$E() < 10^{-4}$; RMSD 2.6 A
$E() < 2.6$ 25%; 199/181

When can we infer non-homology?

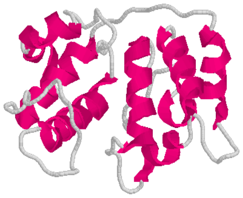
Non-homologous proteins have different structures



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



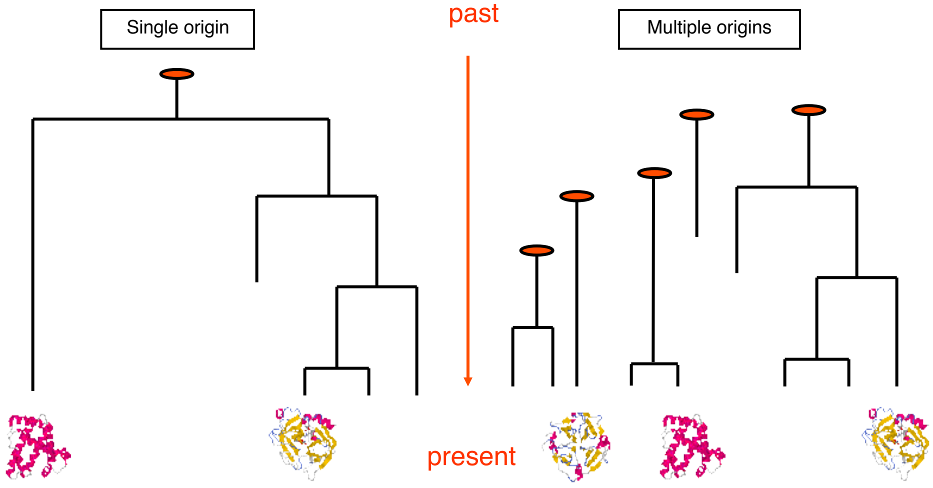
Cytochrome c4 (1etp)
E() > 100
E()<5.5; 23% 171/190

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

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

Homology is confusing II: Are All Sequences Homologous?

No Homology without excess similarity



Homology from sequence similarity

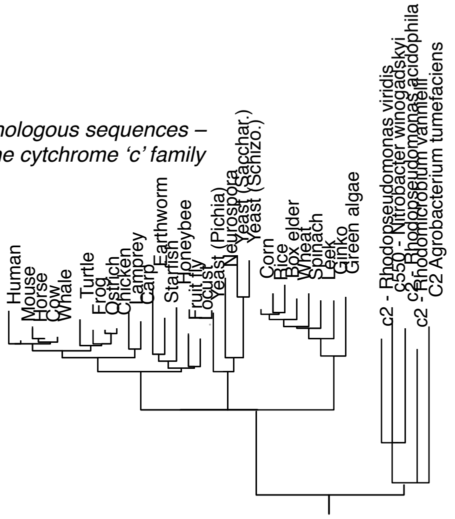
- Sequences are inferred to share a common ancestor based on statistically significant *excess* similarity. Any evidence of *excess* similarity can be used to infer homology
- Lack of sequence evidence *cannot* be used to infer non-homology.
  - Proteins with different structures are non-homologous
-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

E. coli proteins vs Human – Ancient Protein Domains

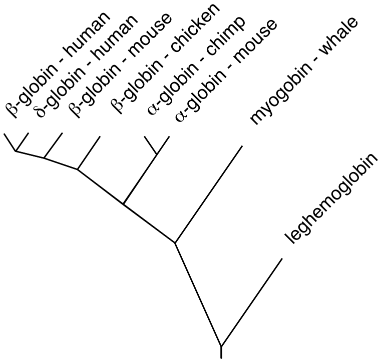
```
+----------+------+------+--------------------------+--------------------------+------------+
| expect   | %_id | alen | E coli descr             | Human descr              | sp_name    |
+----------+------+------+--------------------------+--------------------------+------------+
| 2.7e-206 | 53.8 |  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



Protein Evolution and Sequence Similarity

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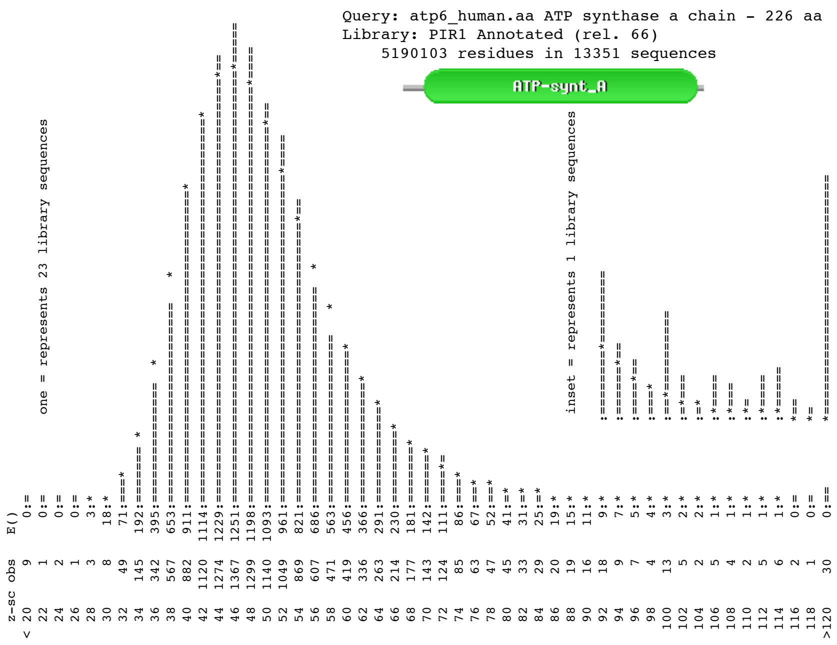

```
Query: atp6_human.aa ATP synthase a chain - 226 aa
Library: PIR1 Annotated (rel. 66)
     5190103 residues in 13351 sequences
```

```
 z-sc obs  E()
< 20    9    0:=          one = represents 23 library sequences
  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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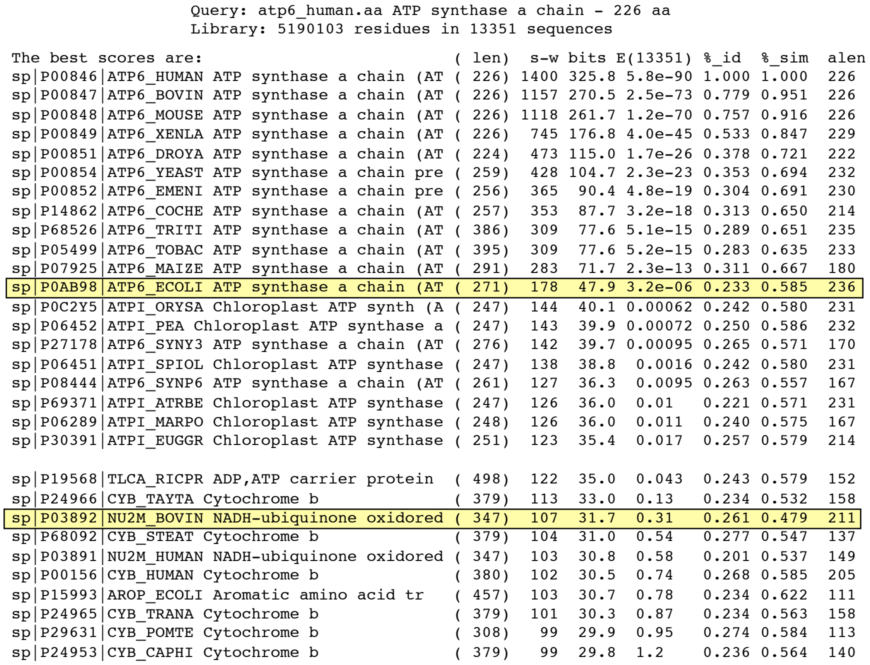
Inferring Homology from Statistical Significance

- 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



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

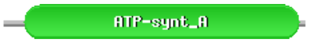
The best scores are:                                    ( len)  s-w bits E(13351) %_id  %_sim  alen
sp|P00846|ATP6_HUMAN ATP synthase a chain (AT ( 226) 1400 325.8 5.8e-90 1.000 1.000  226
sp|P00847|ATP6_BOVIN ATP synthase a chain (AT ( 226) 1157 270.5 2.5e-73 0.779 0.951  226
sp|P00848|ATP6_MOUSE ATP 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
```

Alberts is wrong about sequence similarity (three times in three claims)

> "With such a large number of proteins in the database, the search programs find *many nonsignificant matches*, resulting in a background noise level that makes it very difficult to pick out all but the closest relatives. Generally speaking, *one requires a 30% identity* in sequence to consider that two proteins match. However, we know the function of many short signature sequences ("fingerprints"), and *these are widely used to find more distant relationships*."
>
> – Alberts, Molecular Biology of the Cell (5th ed) p. 139

- Sequences producing statistically significant alignments ALWAYS share a common structure
- Many significant alignments share < 30% identity (<25% identity is routine, and <20% identity can be significant)
- In the absence of significant similarity, "fingerprints" should never be trusted.



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

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

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

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

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

The PAM250 matrix

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

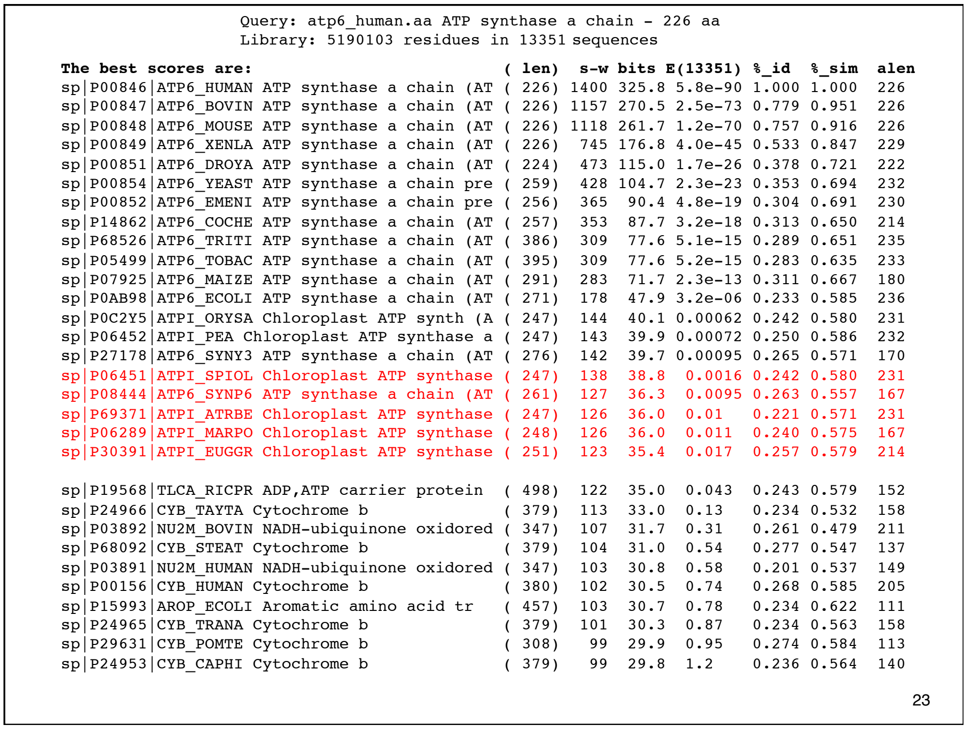
```
>>sp|P30391|ATPI_EUGGR Chloroplast ATP synthase a chain precursor   (251 aa)
 s-w opt: 123  Z-score: 151.3  bits: 35.4 E(): 0.017
Smith-Waterman score: 123; 25.7% identity (57.9% similar) in 214 aa overlap (21-222:50-243)

                                    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
```



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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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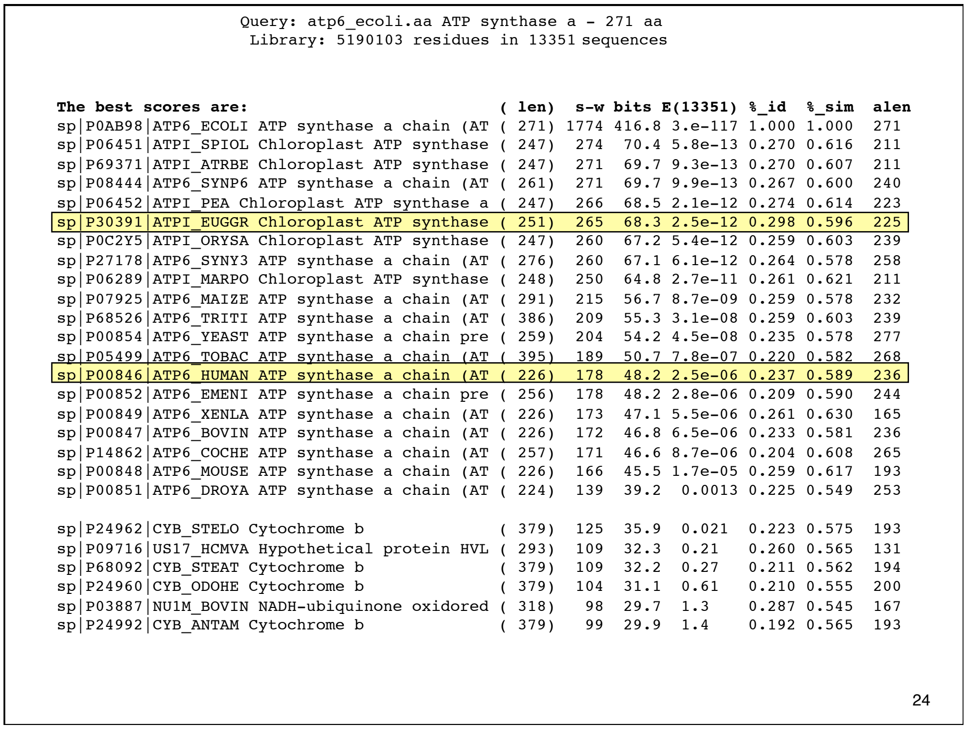

```
                    Query: atp6_ecoli.aa ATP synthase a - 271 aa
                     Library: 5190103 residues in 13351 sequences


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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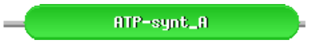
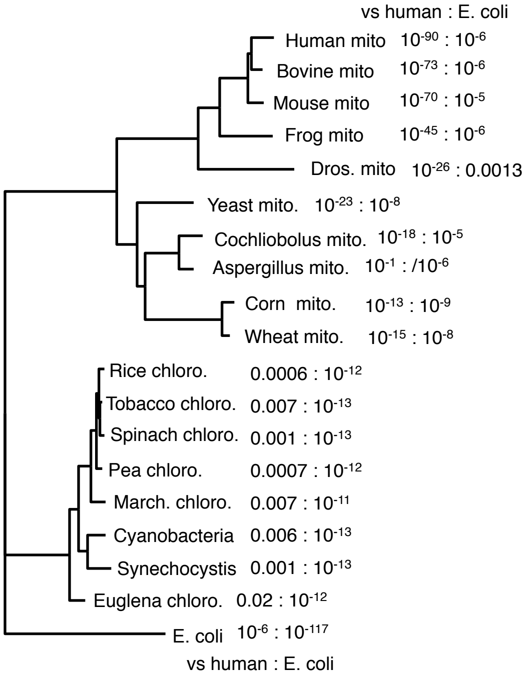
Homology is Transitive (on domains)

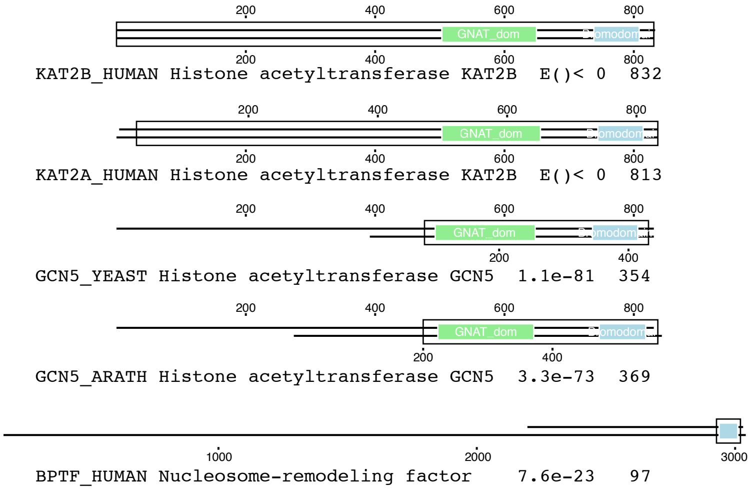



vs human : E. coli
Human mito 10^{-90} : 10^{-6}
Bovine mito 10^{-73} : 10^{-6}
Mouse mito 10^{-70} : 10^{-5}
Frog mito 10^{-45} : 10^{-6}
Dros. mito 10^{-26} : 0.0013
Yeast mito. 10^{-23} : 10^{-8}
Cochliobolus mito. 10^{-18} : 10^{-5}
Aspergillus mito. 10^{-1} : /10^{-6}
Corn mito. 10^{-13} : 10^{-9}
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

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
```

Homology and Domains – Histone deacetylase KAT2B



```
KAT2B_HUMAN Histone acetyltransferase KAT2B  E()< 0  832
KAT2A_HUMAN Histone acetyltransferase KAT2B  E()< 0  813
GCN5_YEAST Histone acetyltransferase GCN5  1.1e-81  354
GCN5_ARATH Histone acetyltransferase GCN5  3.3e-73  369
BPTF_HUMAN Nucleosome-remodeling factor    7.6e-23   97
```

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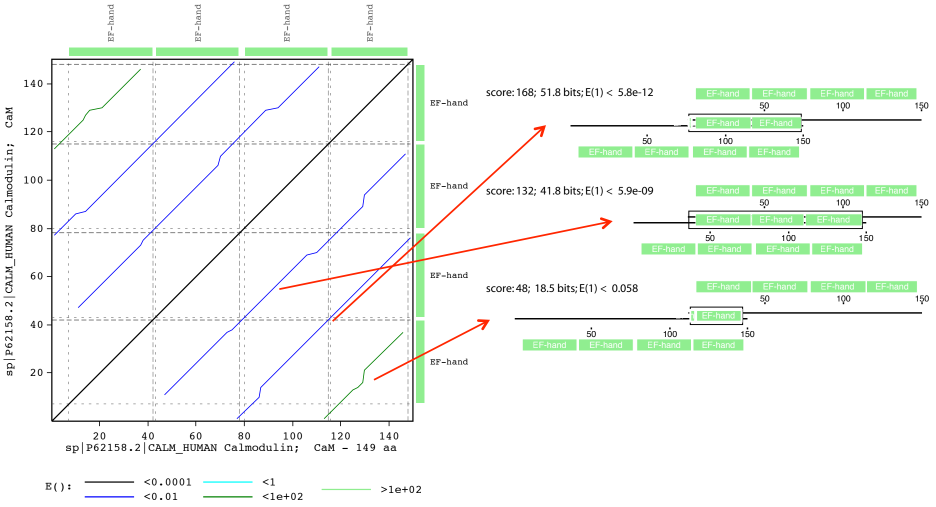
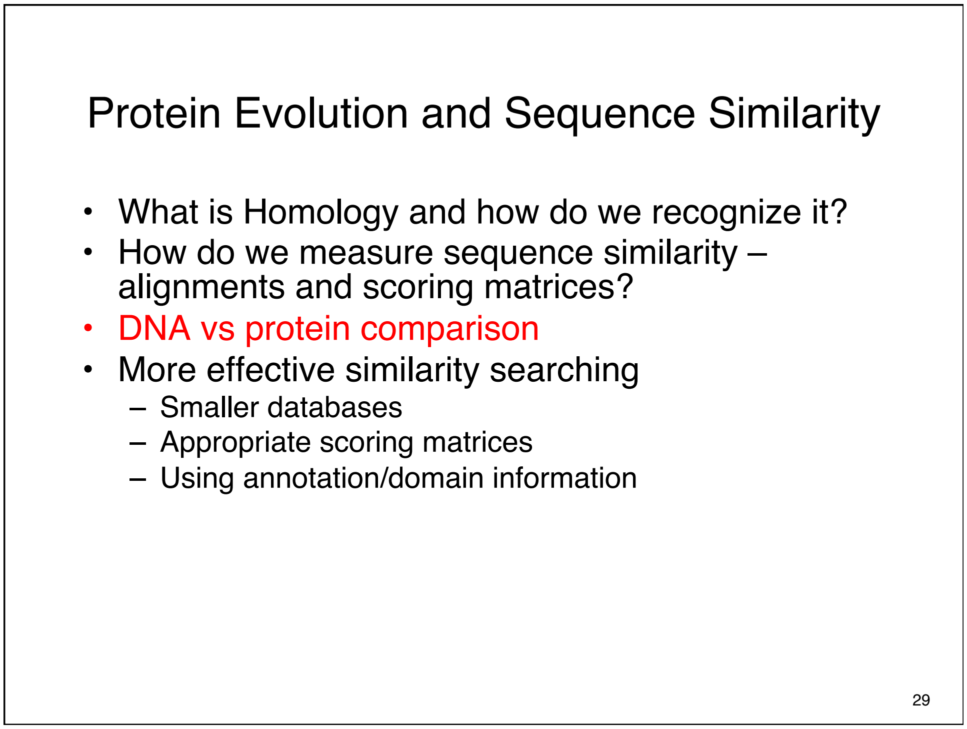
LALIGN – Identifying mobile domains: mobile (duplicated) domains in local alignments



score: 168; 51.8 bits; E(1) < 5.8e-12

score: 132; 41.8 bits; E(1) < 5.9e-09

score: 48; 18.5 bits; E(1) < 0.058



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
  - Using annotation/domain information

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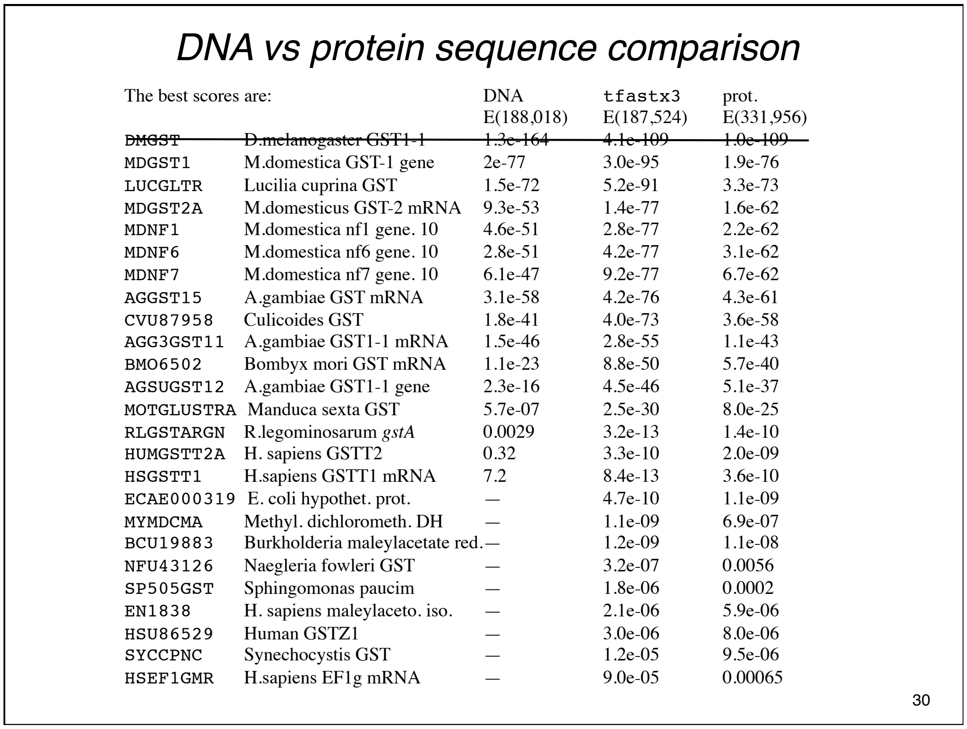

DNA vs protein sequence comparison

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. dichorometh. 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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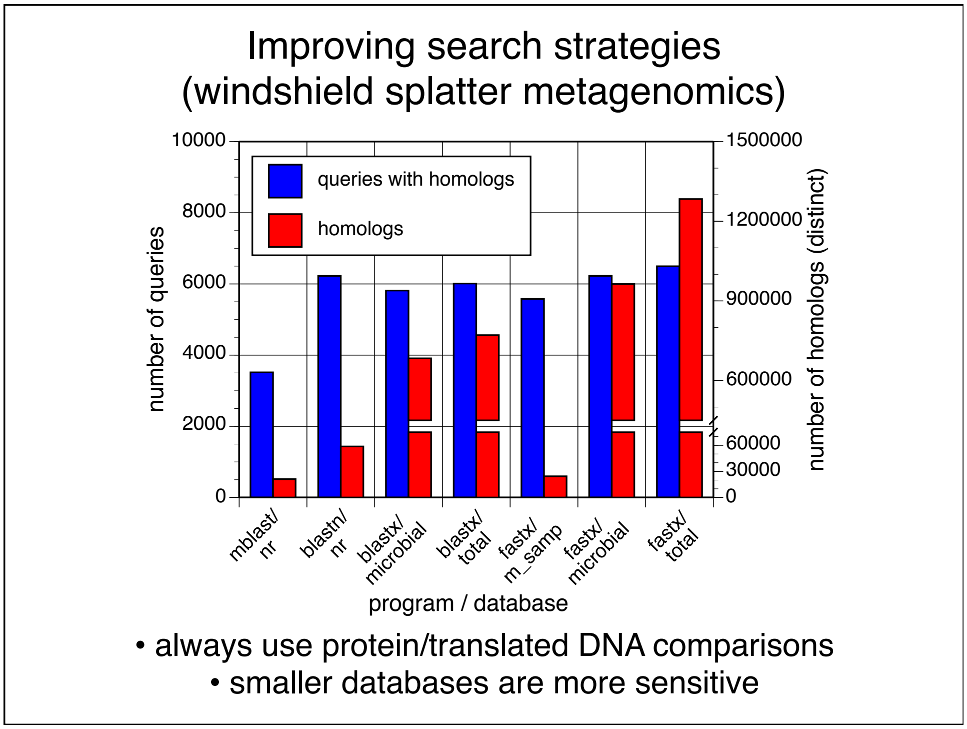
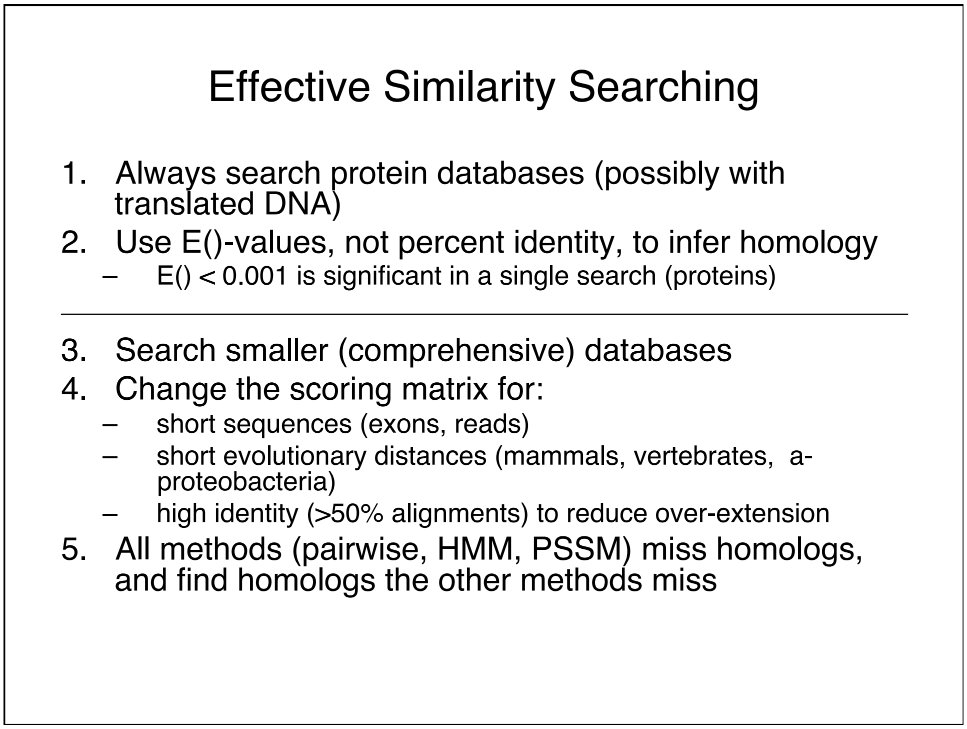


Improving search strategies (windshield splatter metagenomics)

- always use protein/translated DNA comparisons
- smaller databases are more sensitive



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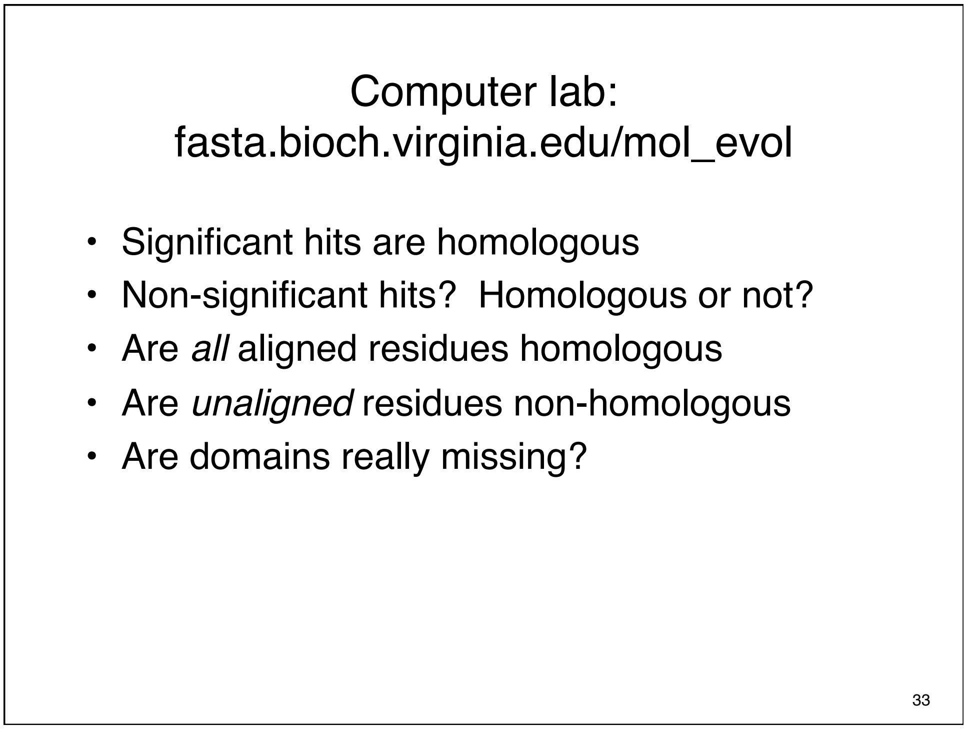
3. Search smaller (comprehensive) databases
4. Change the scoring matrix for:
   – short sequences (exons, reads)
   – short evolutionary distances (mammals, vertebrates, a-proteobacteria)
   – high identity (>50% alignments) to reduce over-extension
5. All methods (pairwise, HMM, PSSM) miss homologs,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



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?