Programming for Biology
Protein Evolution / Similarity Searching

What BLAST Does / Why BLAST works

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Sequence Similarity - Conclusions

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

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Establishing homology from statistically significant similarity
Why BLAST works

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

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This talk is not about:

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

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Protein Evolution and Sequence Similarity

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

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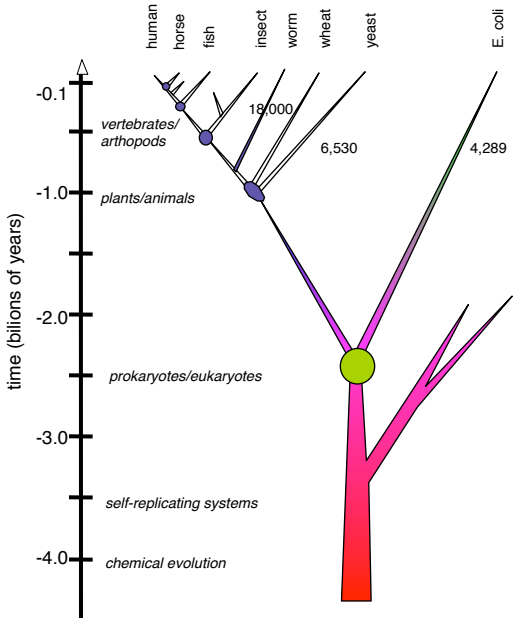
Homologues share a common ancestor



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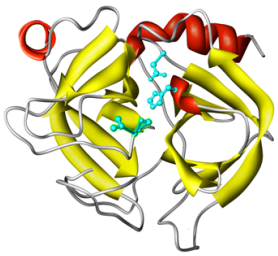
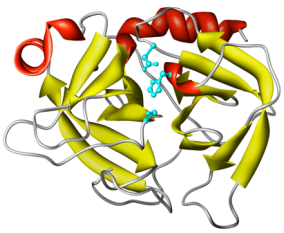
When do we infer homology?

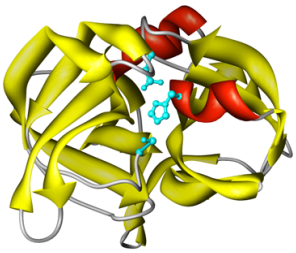


Bovine trypsin (5ptp)
Structure: E()< 10^{-23}; RMSD 0.0 A
Sequence: E()< 10^{-84} 100% 223/223

Homology <=> structural similarity
? sequence similarity



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



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

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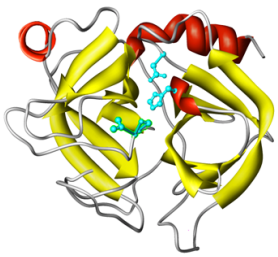
When can we infer non-homology?



Bovine trypsin (5ptp)
Structure: E()<10^{-23} RMSD 0.0 A
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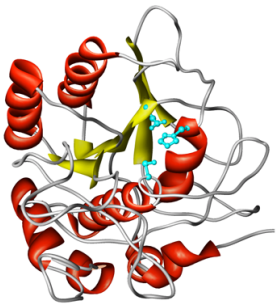
Non-homologous proteins have different structures



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

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

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

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

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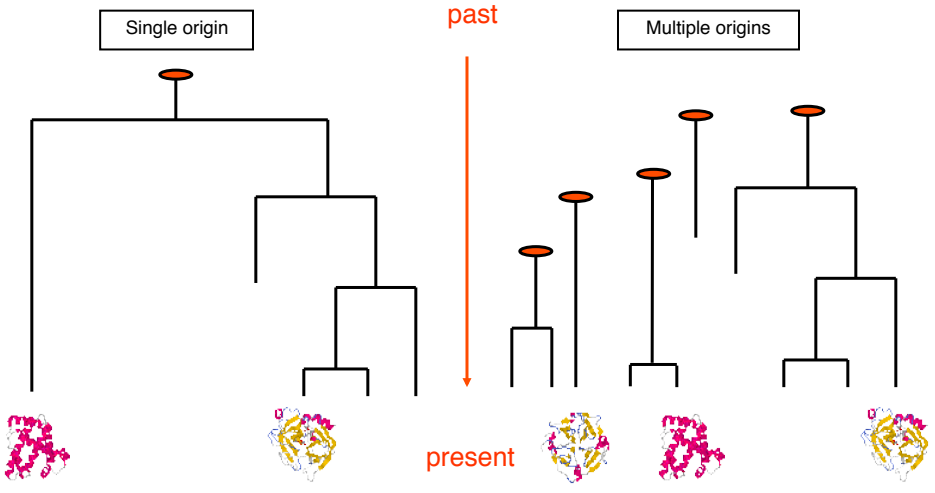
Homology is confusing II: Are All Sequences Homologous?

No Homology without excess similarity



Homology from (sequence/structure) 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 evidence *cannot* be used to infer non-homology.
  - Proteins with different structures are non-homologous
- There are always two alternative hypotheses: homology (common ancestry), or independence – one must weigh the evidence for each hypothesis (independence is the *null* hypothesis).

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What BLAST does:

Similarity $\overset{?}{\Leftrightarrow}$ Homology

Why BLAST works:

Statistical Significance $\overset{?}{\Leftrightarrow}$ Biological Significance

Divergence ? Convergence

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E. coli proteins vs Human – Ancient Protein Domains

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

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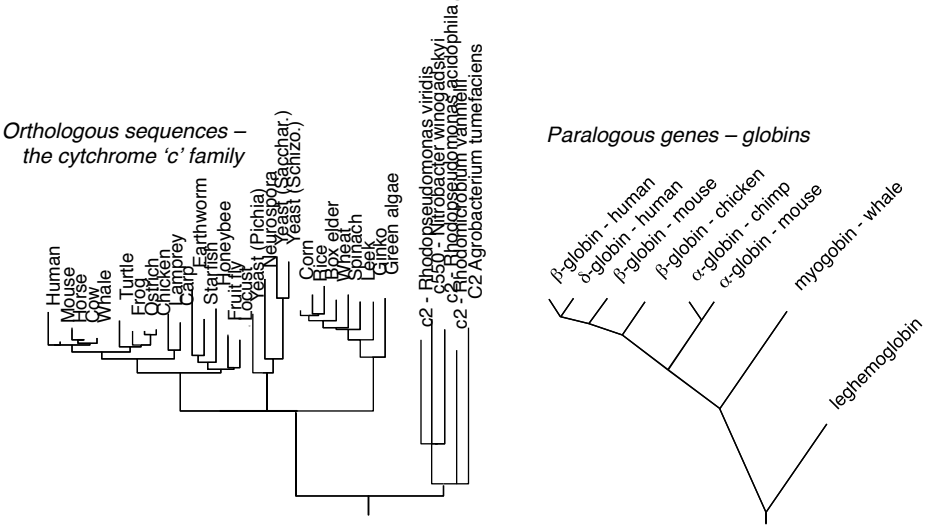
Orthologs and Paralogs – Inferring Function



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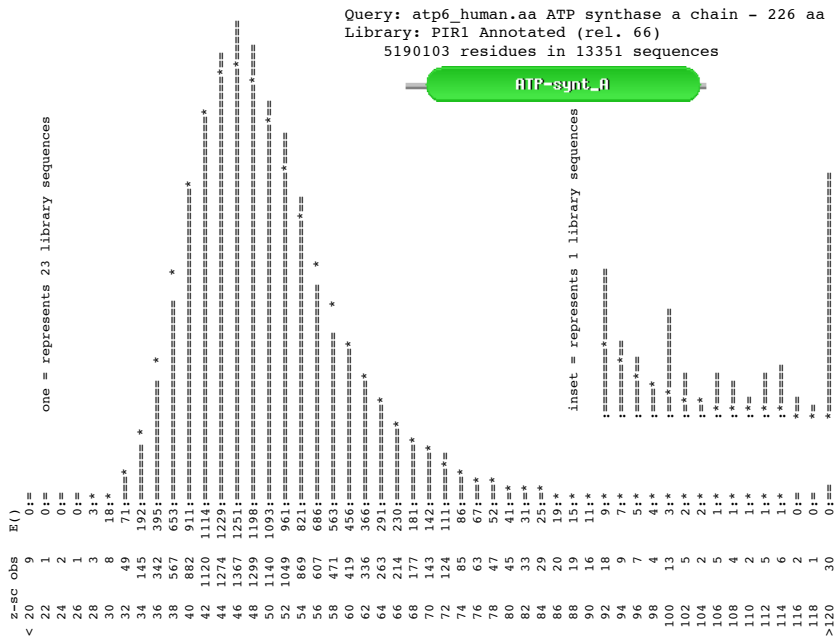

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Inferring Homology from Statistical Significance

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

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

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

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

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

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

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

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


The PAM250 matrix

```
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
```

```
>>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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```
                Query: atp6_human.aa ATP synthase a chain - 226 aa
                Library: 5190103 residues in 13351 sequences
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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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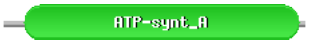
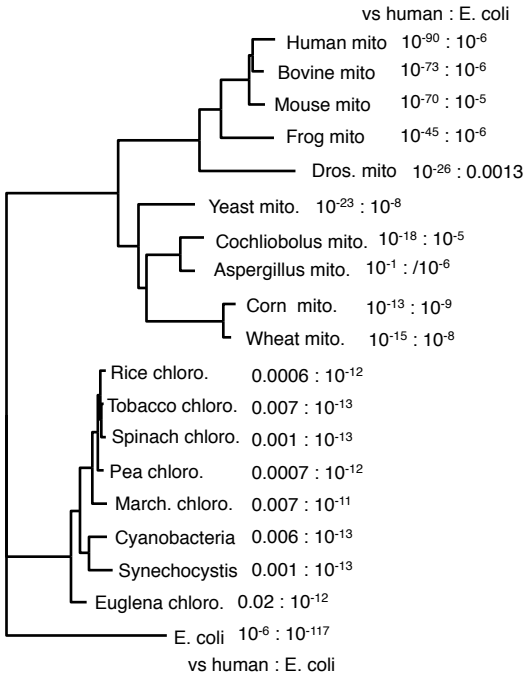
Homology is Transitive (on domains)





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Homology and Domains – Histone deacetylase PCAF

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

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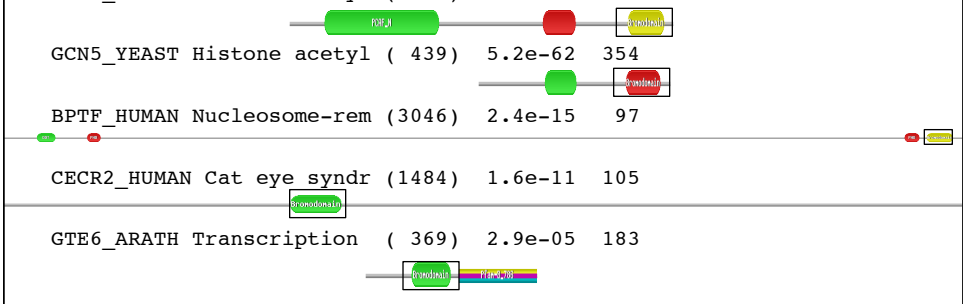
Homology and Domains – Histone deacetylase PCAF

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



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Protein Evolution and Sequence Similarity

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

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

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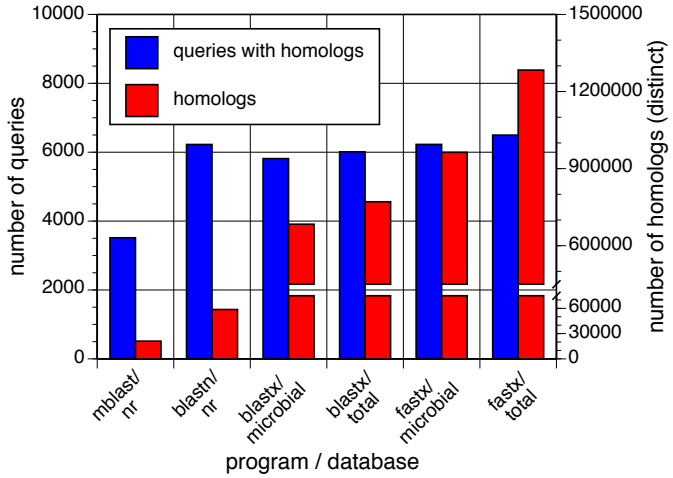
Improving search strategies (windshield splatter metagenomics)



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

Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. How to avoid mistakes (what to look out for)
5. When to do something different (changing scoring matrices)

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1. What question to ask?

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

Questions not to ask:

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

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2. What program to run?

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

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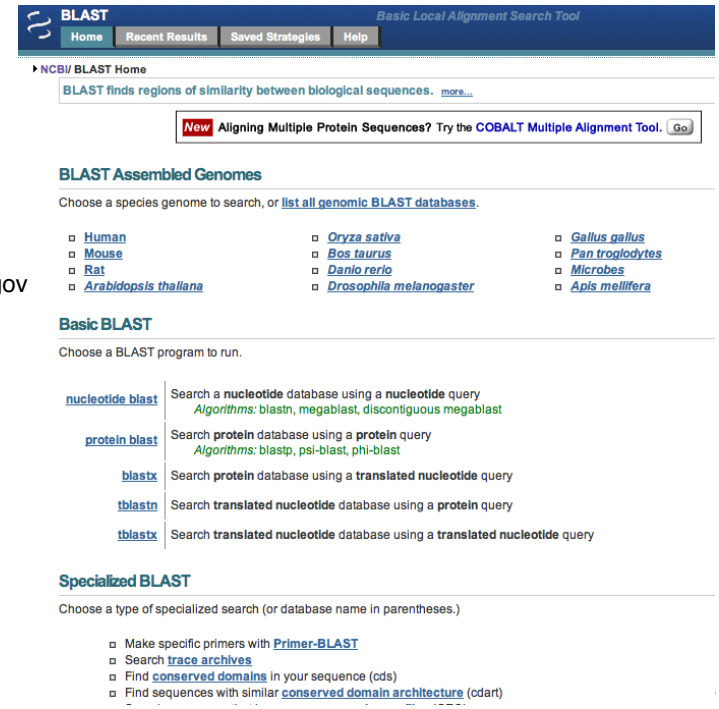
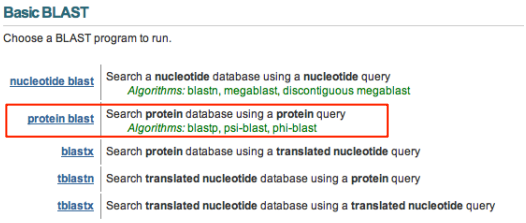
NCBI BLAST Server

blast.ncbi.nlm.nih.gov



NCBI BLAST Server

blast.ncbi.nlm.nih.gov



What is wrong with this picture?

Always compare protein sequences

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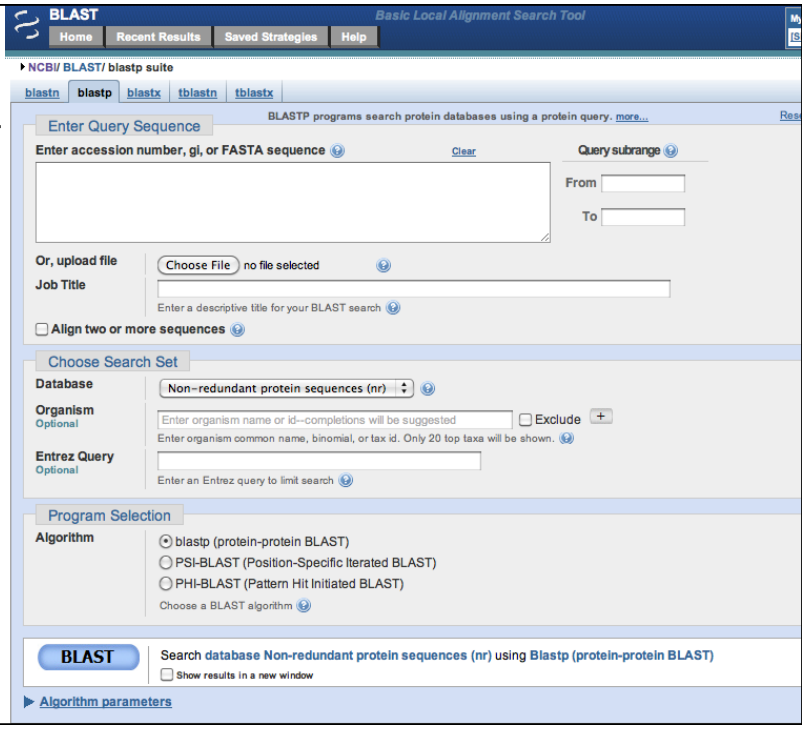
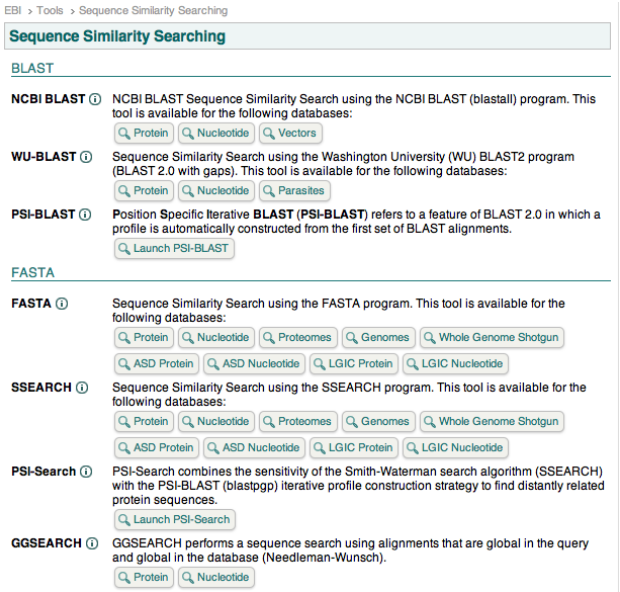
NCBI BLAST Server



Searching at the EBI
www.ebi.ac.uk/Tools/sss/



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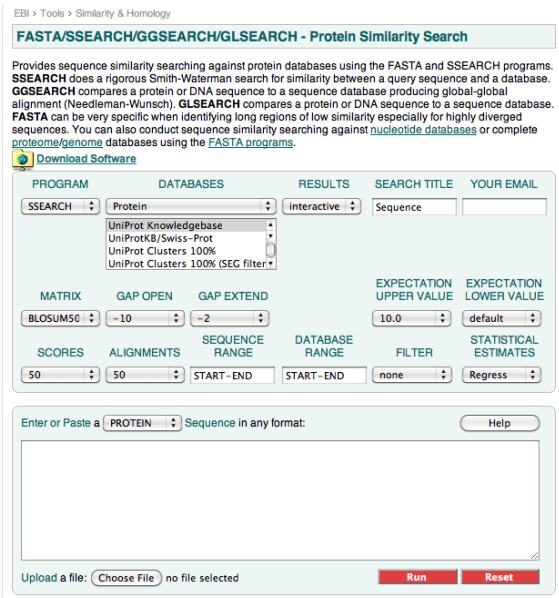
Searching at the EBI – ssearch



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3. What database to search?

- Search the smallest comprehensive database likely to contain your protein
  - vertebrates – human proteins (40,000)
  - fungi – S. cerevisiae (6,000)
  - bacteria – E. coli, gram positive, etc. (<100,000)
- Search a richly annotated protein set (SwissProt, 450,000)
- Always search NR (> 12 million) *LAST*
- Never Search “GenBank” (DNA)

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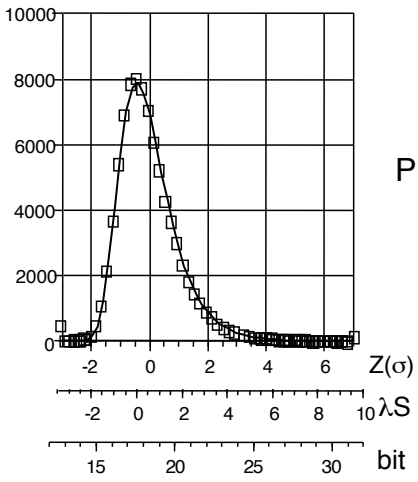
Why smaller databases are better – statistics



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

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

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

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

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

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

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

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

$E(40 \mid D=12E6) = 1.8$

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What is a "bit" score?

- Scoring matrices (PAM250, BLOSUM62, VTML40) contain "log-odds" scores:
  - $s_{i,j}$ (bits) = $\log_2(q_{i,j}/p_i p_j)$
  - $s_{i,j}$ (bits) = 2 -> a residue is 4-times more likely to occur by homology compared with chance (at one residue)
  - $s_{i,j}$ (bits) = -1 -> a residue is 2-times more likely to occur by chance compared with homology (at one residue)
- An alignment score is the maximum sum of $s_{i,j}$ bit scores across the aligned residues. A 40-bit score is 2^{40} more likely to occur by homology.
- How often should a score occur by chance? In a 400 * 400 alignment, there are 160,000 places where the alignment could start by chance, so we expect a score of 40 bits would occur:
  - $400 \times 400 \times 2^{-40} = 1.6 \times 10^5 / 2^{40} (10^{13.3}) = 0.8 \times 10^{-8}$ times
  - Thus, the probability of a 40 bit score in ONE alignment is ~ 10^{-8}
- But we did not ONE alignment, we did 4,000, 40,000, 400,000, or 16 million):
  - $E(p \mid D) = p(40 \text{ bits}) \times \text{database size}$
  - $E(40 \mid 4{,}000) = 10^{-8} \times 4{,}000 = 4 \times 10^{-5}$ (significant)
  - $E(40 \mid 40{,}000) = 10^{-8} \times 4 \times 10^4 = 4 \times 10^{-4}$ (significant)
  - $E(40 \mid 400{,}000) = 10^{-8} \times 4 \times 10^5 = 4 \times 10^{-3}$ (not significant)

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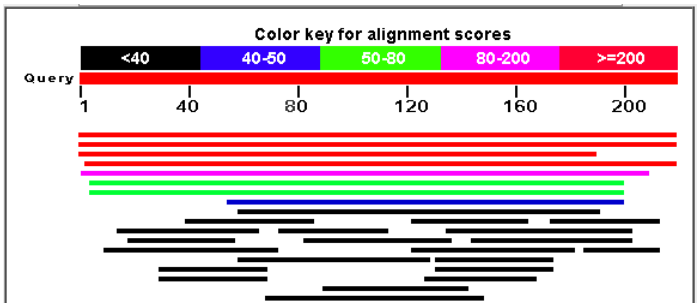
How many “bits” do I need?

E(p I D) = p(40 bits) x database size

E(40 I 4,000) = 10^{-8} x 4,000 = 4 x 10^{-5} (significant)
E(40 I 40,000) = 10^{-8} x 4 x 10^{4} = 4 x 10^{-4} (significant)
E(40 I 400,000) = 10^{-8} x 4 x 10^{5} = 4 x 10^{-3} (not significant)

To get E() ~ 10^{-3} :

genome (10,000) p ~ $10^{-3}/10^{4}$ = 10^{-7}/160,000 = 40 bits
SwissProt (500,000) p ~ $10^{-3}/10^{6}$ = 10^{-9}/160,000 = 47 bits
Uniprot/NR (10^{7}) p ~ $10^{-3}/10^{7}$ = 10^{-10}/160,000 = 50 bits



very significant 10^{-50}
significant 10^{-6}
significant 10^{-3}
not significant

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Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. How to avoid mistakes (what to look out for)
5. When to do something different

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Inferring Homology from Statistical Significance

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

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Smith-Waterman (ssearch)

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

Highest scoring unrelated sequence E() ~ 1.0

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Unrelated ≠ Random (low complexity)

Search with complete grou_drome:

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

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

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

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`pseg` removes low-complexity regions

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

                              1-8    MYPSPVRH
                     paaggpppqgp     9-19
                             20-131  IKFTIADTLERIKEEFNFLQAQYHSIKLEC
                                     EKLSNEKTEMQRHYVMYYEMSYGLNVEMHK
                                     QTEIAKRLNTLINQLLPFLQADHQQQVLQA
                                     VERAKQVTMQELNLIIGQQIHA
                     qqvpggpppqpmg  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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Validating homologs/statistics

- In general, BLASTP statistical estimates are accurate
- The most common errors occur because of low-complexity regions, or biased amino-acid composition
- To confirm statistical accuracy, find the highest scoring non homolog
  - No need to test every hit, test hits that are surprising
  - Confirm homology/non-homology by searching against a different comprehensive database, e.g. SwissProt, or refseq.
  - Non-homologs will find many significant members of other families, but not the family you are testing for
- Statistical estimates can be confirmed with shuffles (see ISMB2000 tutorial, fasta.bioch.virginia.edu/fasta_www2 shuffle link)

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Scoring matrices

- Scoring matrices can set the evolutionary look-back time for a search
  - Lower PAM (PAM10/MDM10 … PAM60) for closer (10% … 50% identity)
  - Higher BLOSUM for higher conservation (BLOSUM50 distant, BLOSUM80 conserved)
- Shallow scoring matrices for short domains/short queries (metagenomics)
  - Matrices have “bits/position” (score/position), 40 aa at 0.7 bits/position (BLOSUM62) means 28 bit max score (50 bits significant)
- Deep scoring matrices allow alignments to continue, possibly outside the homologous region

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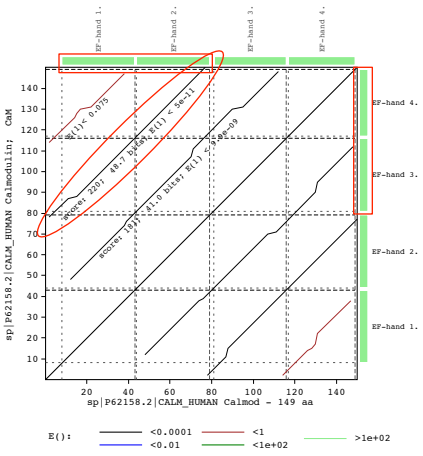
Finding Domains – Local alignments: calmodulin

```
 qRegion: 8-43:84-116 : score=102; bits=25.6; Q=33.5 :  DOMAIN_N: EF-hand 1.
 qRegion: 44-76:117-149 : score=101; bits=25.4; Q=32.9 :  DOMAIN_N: EF-hand 2.
 Waterman-Eggert score: 220;  48.7 bits; E(1) <  5e-11
46.1% identity (73.7% similar) in 76 aa overlap (1-76:77-149)
             [10        20        30        40  ][    50        60        70
sp|P62 MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARK
       : :  .::.:   .::: .::::::.: :.. ::  :: .::.. :. :...:: :.: ::.: ... ::. ::. :
sp|P62 MKDTDSEEEI---REAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK
         80          90        100       110       120       130       140
-------------------------------------------------------------------------------
 qRegion: 11-43:47-80 : score=61; bits=17.5; Q=9.6 :  DOMAIN_N: EF-hand 1.
 qRegion: 44-79:81-119 : score=62; bits=17.7; Q=10.1 :  DOMAIN_N: EF-hand 2.
 qRegion: 81-111:121-147 : score=59; bits=17.1; Q=8.5 :  DOMAIN_N: EF-hand 3.
 Waterman-Eggert score: 181;  41.0 bits; E(1) <  9.9e-09
34.3% identity (64.8% similar) in 105 aa overlap (11-111:47-147)
              20        30        40  ][    50        60            70        ]0[
sp|P62 AEFKEAFSLFDKDGDGTITTKELGTVM-RSLGQNPTEAELQDMINEVDADGNGTIDFPEF---LTMMARKMKDTDSEEEI
       ::... ..  : ::.:::   :. :.: :.. .. .: :... .   : :::: :.  :.   .: ...:. : . .: :
sp|P62 AELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMI
         50        60        70        80        90       100       110       120
         90       100       110
sp|P62 REAFRVFDKDGNGYISAAELRHVMT
       :::    : ::.: ..  :. ..::
sp|P62 REA----DIDGDGQVNYEEFVQMMT
          130       140
-------------------------------------------------------------------------------
qRegion: 8-37:120-146 : score=45; bits=14.4; Q=1.9 :  DOMAIN_N: EF-hand 1.
 Waterman-Eggert score: 64;  18.1 bits; E(1) <  0.075
34.2% identity (71.1% similar) in 38 aa overlap (1-37:113-146)
             [10        20        30
sp|P62 MADQLTEEQIAEF-KEAFSLFDKDGDGTITTKELGTVM
       ....::.:.. :. .::    : :::: .. .:.  .:
sp|P62 LGEKLTDEEVDEMIREA----DIDGDGQVNYEEFVQMM
          120       130       140
```


Repeated domains with local alignments

```
 EF-hand 1:  8-43: 84-116:s=102; bits=25.6; Q=33.5
 EF-hand 2: 44-76:117-149:s=101; bits=25.4; Q=32.9

Waterman-Eggert score: 220; 48.7 bits; E(1)< 5e-11
46.1% identity in 76 aa overlap (1-76:77-149)
            [10        20        30        40
sp|P62 MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSL
       : :  .::.:   .::: .::::::.: :.. ::  :: .:
sp|P62 MKDTDSEEEI---REAFRVFDKDGNGYISAAELRHVMTNL
         80          90        100       110
         ][    50        60        70
sp|P62 GQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARK
       :.. :. :...:: :.: ::.: ... ::. ::. :
sp|P62 GEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK
         120       130       140
```



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

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

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

$l_2\ S_{ij} = \lg_2 (q_{ij}/p_i p_j)$ $\quad l_e\ S_{ij} = \ln(q_{ij}/p_i p_j)$ $\quad p_R p_N = 0.002193$

$l_2\ S_{R:N(40)} = \lg_2 (0.000435/0.00219) = -2.333$

$l_2 = 1/3$; $S_{R:N(40)} = -2.333/l_2 = -7$

$l\ S_{R:N(250)} = \lg 2\ (0.002193/0.002193) = 0$

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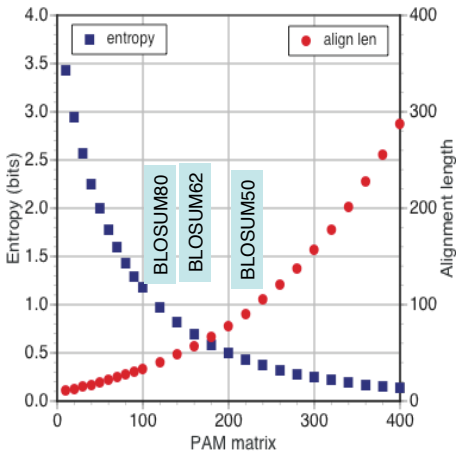
PAM matrices and alignment length



Short domains require “shallow” scoring matrices

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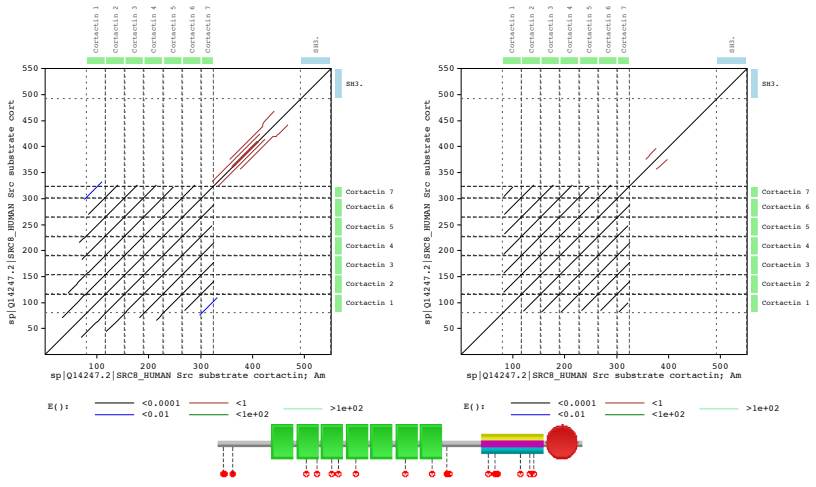
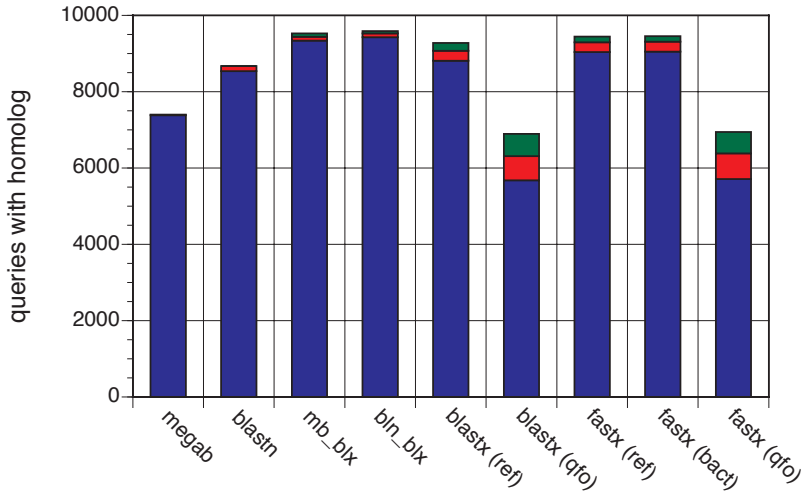
Scoring matrices affect alignment boundaries

BLOSUM62 -11/-1

VTML80 -14/-2



Metagenomics, Human Microbiome Long Reads



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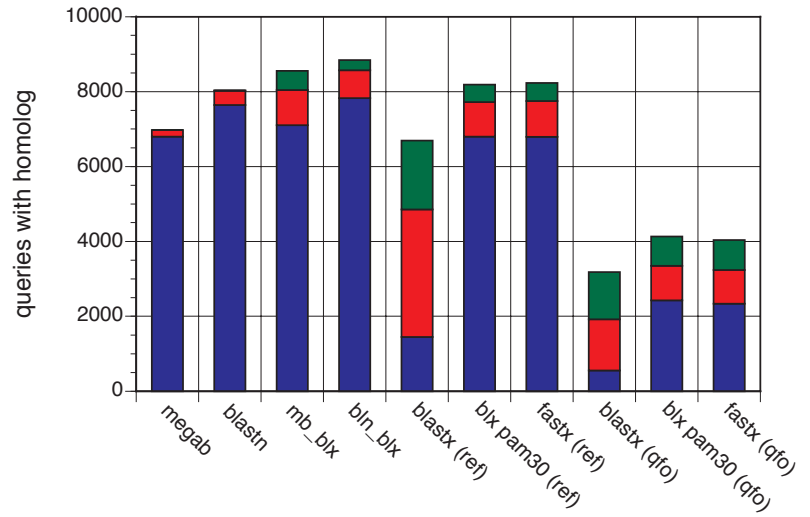
Metagenomics, Human Microbiome Short Reads



For short reads (ave 90 nt), BLOSUM62 misses most homologs

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Scoring Matrices - Summary

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

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Similarity Searching II

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

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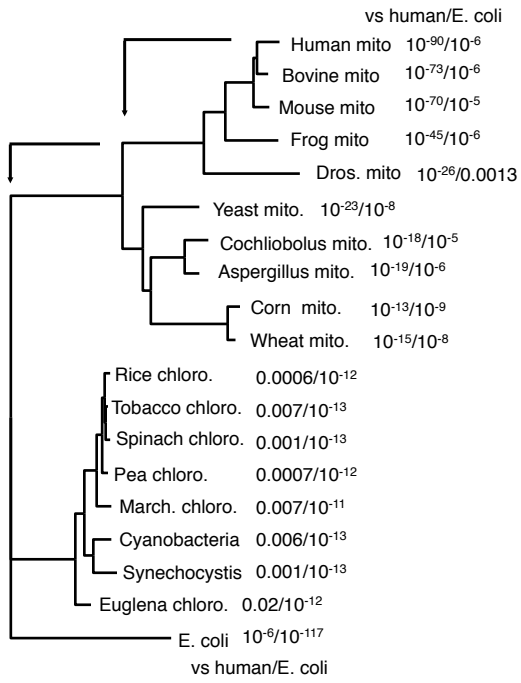

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ATP synthase - matrices, gaps, algorithms

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

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Metazoan ATP Synthases

```
CLUSTAL W (1.81) multiple sequence alignment

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

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

ATP6_BOVIN  SLFIQPMALAVRLTANITAGHLLIHLIGGATLALMSISTTTALITFTILILLTILEFAVAMIQAYVFTLLVSLYLHDNT
ATP6_MOUSE  SLFIQPMALAVRLTANITAGHLLMHLIGGATLVLMNISPPTATITFIILLLLTILEFAVALIQAYVFTLLVSLYLHDNT
ATP6_HUMAN  SLLIQPMALAVRLTANITAGHLLMHLIGSATLAMSTINLPSTLIIFTILILLTILEIAVALIQAYVFTLLVSLYLHDNT
ATP6_XENLA  SLFIRPLALGVRLTANLTAGHLLIQLIATAAFVLLSIMPTVAILTSIVLFLLTLLEIAVAMIQAYVFVLLLSLYLQENV
ATP6_DROYA  SNIIRPGTLAVRLTANMIAGHLLLTLLGNTGPSMSYLLVTFLLVAQIALLVL---ESAVTMIQSYVFAVLSTLYSSEVN
            * :*:* :*.******: *****: *:. :   :  :  .   :    *::*   * **::**:***.:* :**  :
```

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PSI-BLAST ATP6_HUMAN - 4 iterations

Results from round:	(1)		(2)		(3)		(4)	
Sequences producing significant alignments:	Score (bits)	E Value	Score (bits)	E Value	Score (bits)	E Value	Score (bits)	E Value
ATP6_HUMAN ATP synthase a chain (ATPase protein 6)	296	3e-81	257	1e-69	241	2e-62	222	5e-59
ATP6_BOVIN ATP synthase a chain (ATPase protein 6)	253	2e-68	257	2e-69	239	8e-65	230	2e-61
ATP6_MOUSE ATP synthase a chain (ATPase protein 6)	245	5e-66	247	3e-66	234	4e-64	225	6e-60
ATP6_XENLA ATP synthase a chain (ATPase protein 6)	142	9e-35	227	1e-60	189	3e-49	177	2e-45
ATP6_DROYA ATP synthase a chain (ATPase protein 6)	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				

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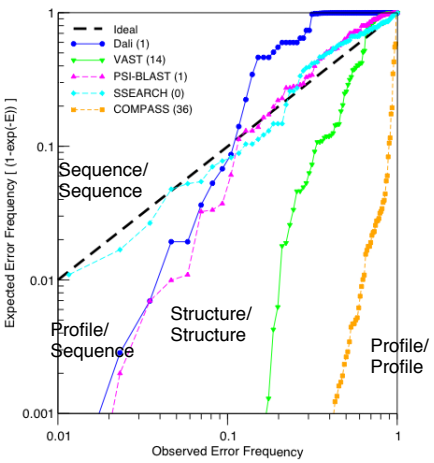
Position-Specific Scores
ATP Synthase, 4 iterations

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

Accuracy of statistical estimates



- SSEARCH (Smith-Waterman) provides very accurate statistical estimates
- PSI-BLAST can provide estimates that off by 10–100-fold

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Why does PSI-BLAST fail?

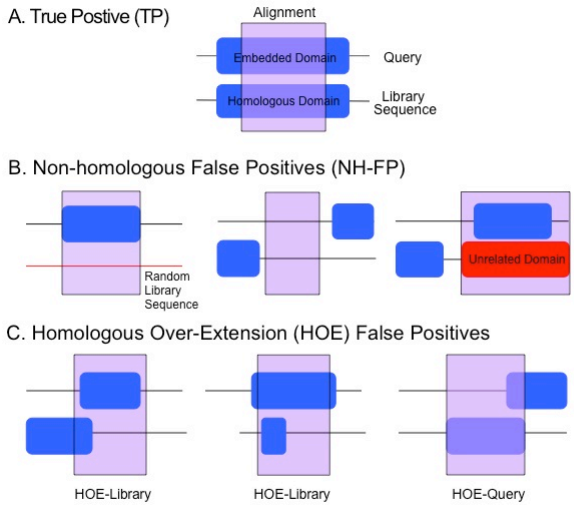

Figure 1

Sensitive searches with PSI-BLAST

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

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Sequence Similarity - Conclusions

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

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Discussion questions

1. What is the difference between similarity and homology? When does high identity not imply homology? What conclusions can be drawn from homology?
2. What is the difference between homology and common ancestry?
3. When the *M. janaschii* genome was first sequenced, Venter and his colleagues stated that almost 60% of the open reading frames (proteins or genes) were novel to this organism. (For eubacterial like *E. coli* or *H. influenzae*, a similar number would be 20 - 40%.) On what would they base such a statement? Is it likely to be correct?
4. Name two reasons why protein sequence comparison is more effective (longer evolutionary look-back time) than DNA sequences?
5. 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)?
6. 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?
7. How can a sequence be homologous if you search a small database (e.g. human, 40,000 sequences), but not share significant similarity if you search a complete database (>4 million sequences)?
8. What scoring matrix should be used to identify protein orthologs that have diverged over the past 100 My (e.g. human/mouse)?
9. What scoring matrix should be used when comparing Illumina 90 nt reads against a protein database?

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