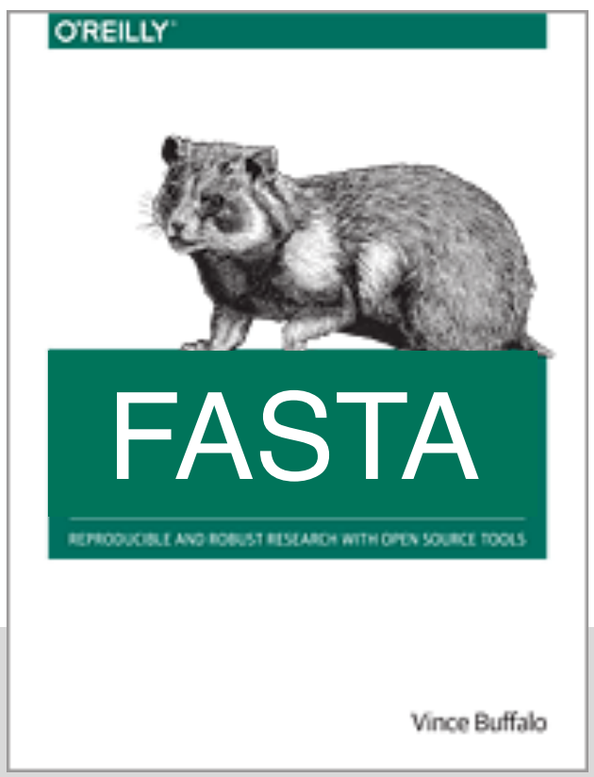
Sequence Homology and Analysis

Understanding How FASTA and BLAST work to optimize your sequence similarity searches.



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Take Home Messages

1. *Homologous* sequences share a common ancestor, but most sequences are *non-homologous*
2. Compare protein sequence for distant comparison and DNA for close comparisons
3. Sequence Homology can be reliably inferred from statistically significant similarity (non-homology cannot from non-similarity)
4. Homologous proteins share common structures, but not necessarily common functions
5. Sequence statistical significance estimates are accurate (verify this yourself)
6. Smaller databases increase search sensitivity
7. Statistical accuracy can be evaluated by examining the “highest scoring unrelated sequence” or by random shuffles

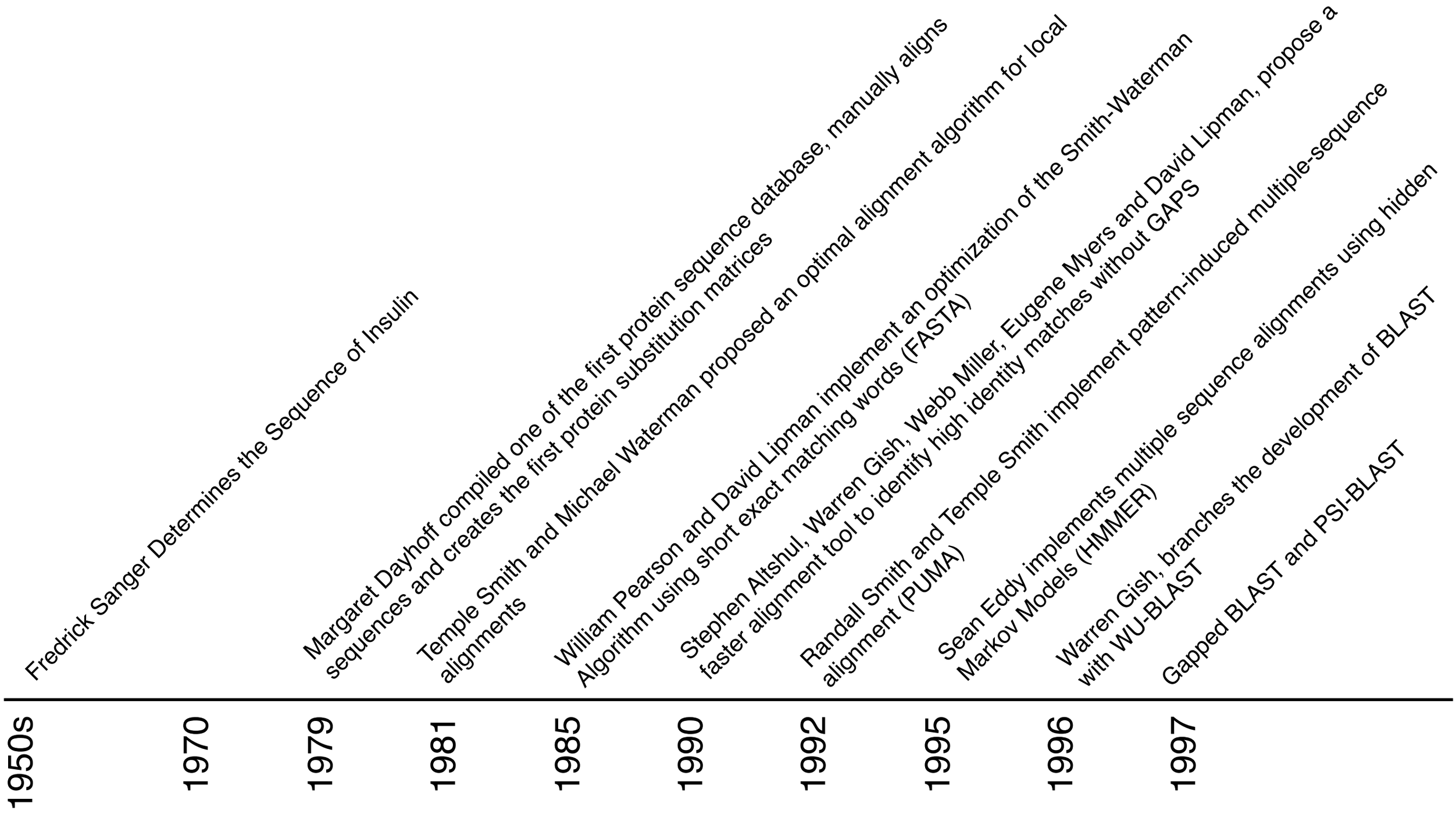
What is Understanding Homology Important

- Most gene databases information is based on sequence similarity searching with a few exceptions
    - In the absence of high-throughput biochemistry experimentation, homology has filled in the gaps on functional and pathway assignments
- Most of these predictions are correct however distinguishing orthologs (deviation from speciation) and paralogs (deviation from gene duplication) is difficult
- E-values are more reliable than percent identity

What is Homology?

How do we recognize it?

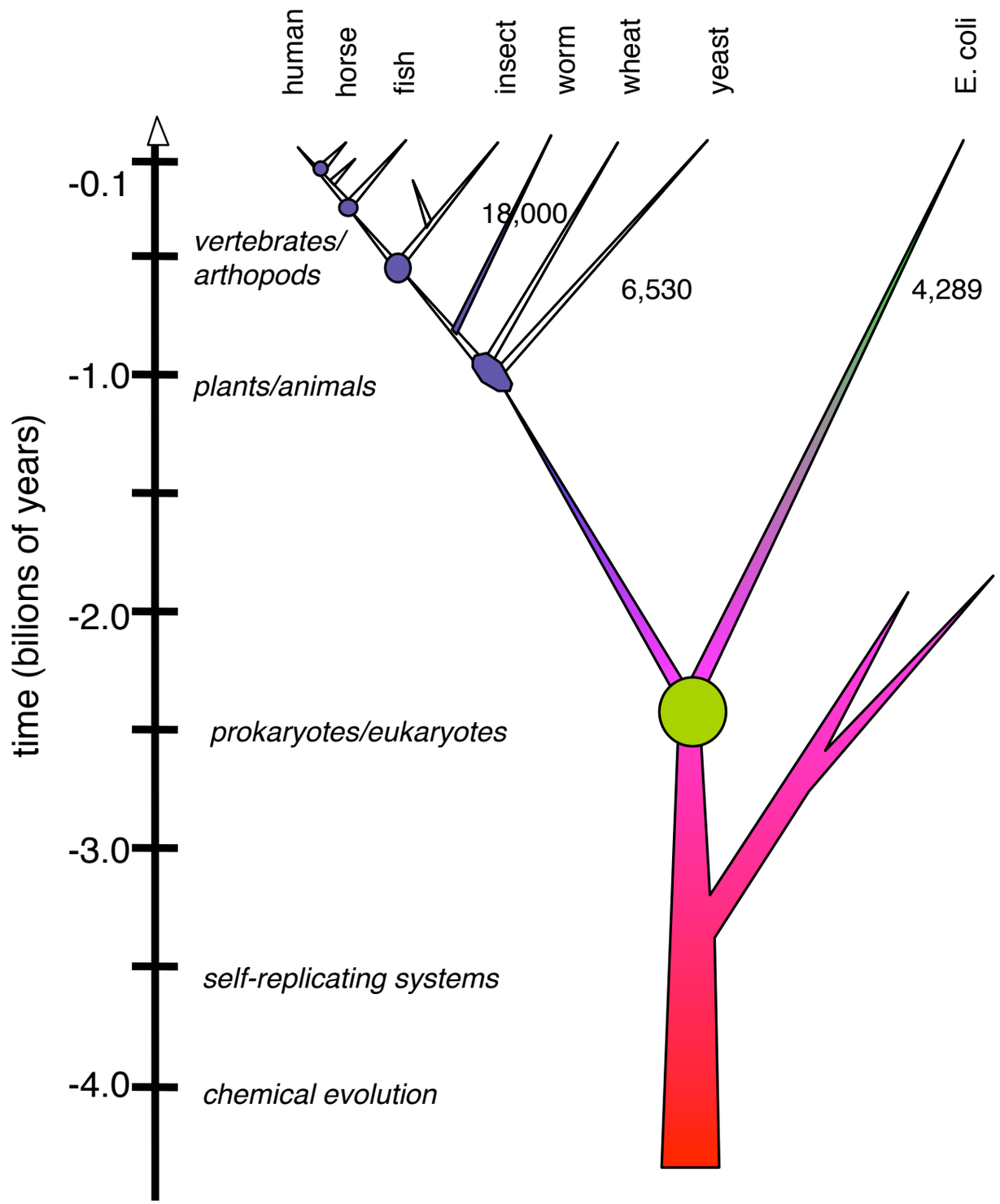
History of Sequence Similarity



Establishing homology from statistically significant similarity

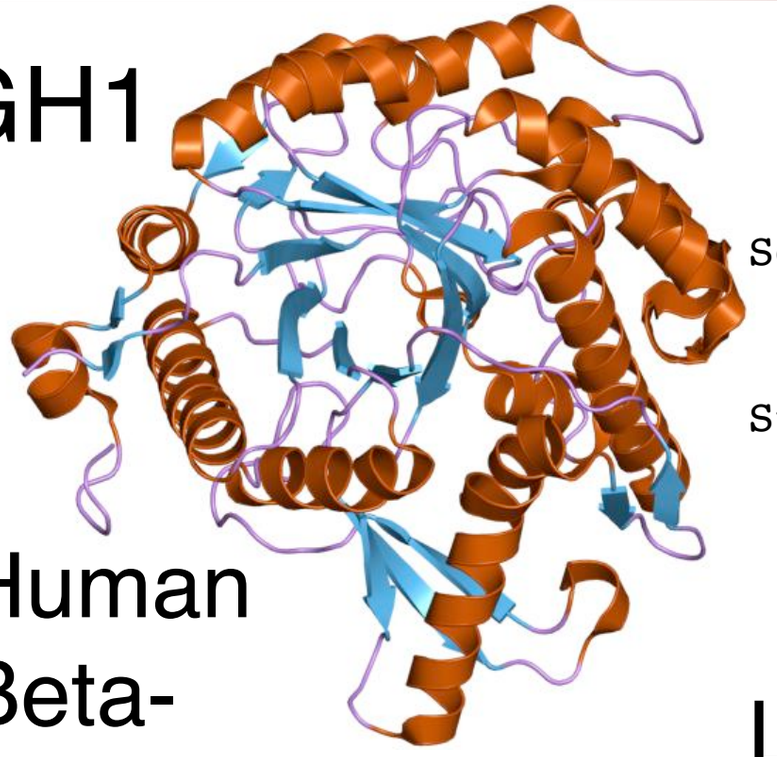
- For most proteins, homologs are easily found over long evolutionary distances (500 My – 2 By) using standard approaches (BLAST, FASTA)
- Difficult for distant relationships or very short domains
- Most default search parameters are optimized for distant relationships and work well

Homologous Sequences Share a Common Ancestor



When do we infer homology?

GH1



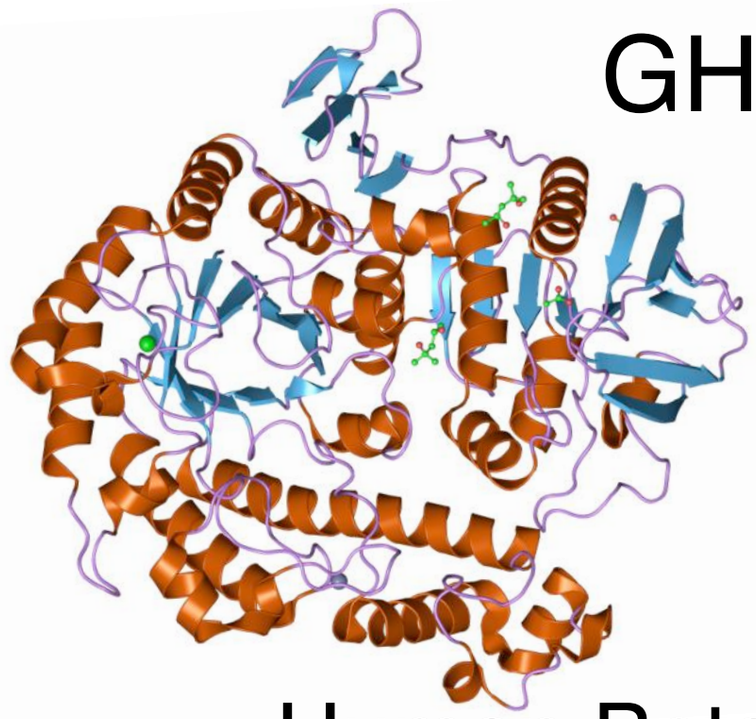
Human Beta-glucosidase

```
Sequence: Score =  205 bits
      Expect = 3e-57
      %ID  = 30%
Structure:RMSD = 2.63
      Score = 1044
      P-Value = 0
```

```
Sequence: Score  = 16.2
      Expect = 1.3
      %ID    = 26%
Structure:RMSD  = 3.80
      Score  = 364.8
      P-Value = 1.97e-02
```

Lactococcus lactis Beta-galactosidase

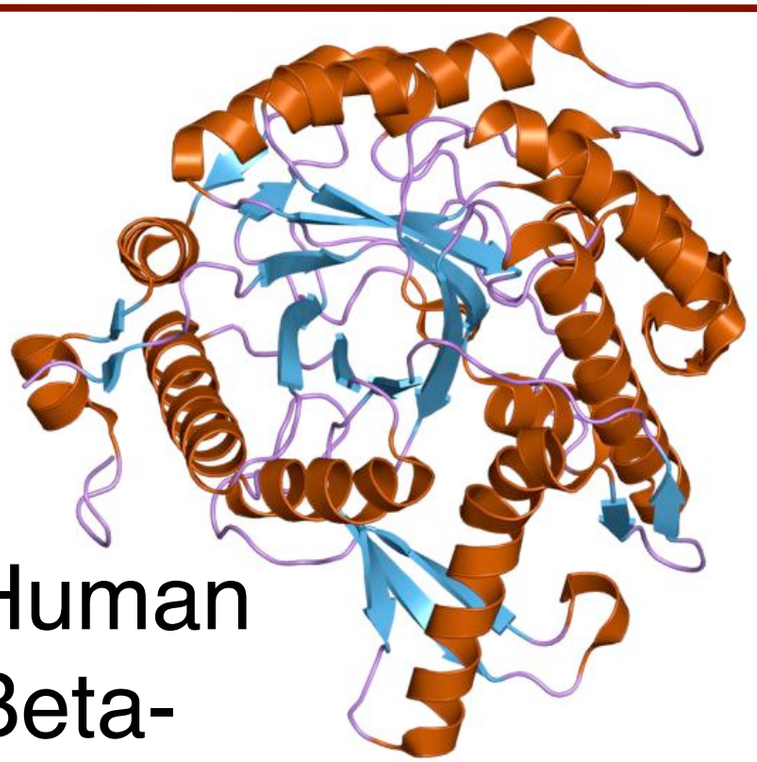
GH42



Human Beta-galactosidase

```
Sequence: Score = 22.3
      Expect = 0.051
      %ID = 25%
Structure: RMSD = 393.5
       Score = 393.5
       P-Value = 1.65e-07
```

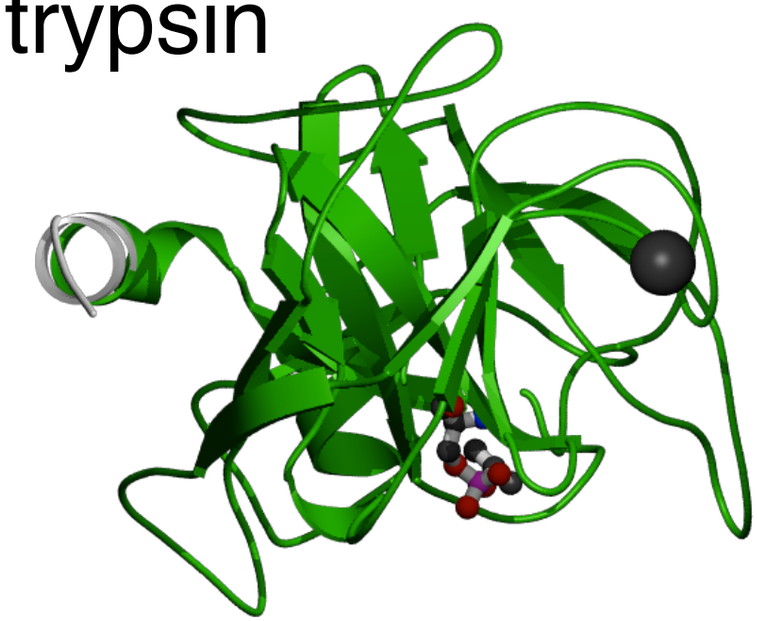
When do we infer non-homology?

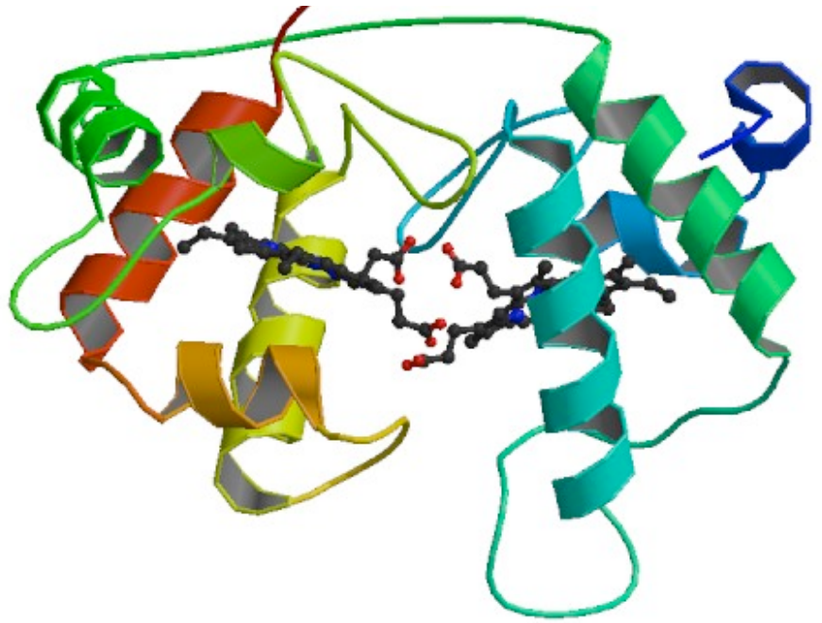


Human Beta-glucosidase

Sequence: Score = 15.8 bits,
Expect = 1.5
%ID = 45%
Structure P-value: 9.87e-01
Score: 48.73
RMSD: 3.36
%Id: 2.9%

Bovine trypsin





Sequence: Score = 13.5 bits (23)
Expect = 6.4
%ID = 36%
Structure:P-value: 7.57e-01
Score: 122.45
RMSD: 4.74
%Id: 4.3%

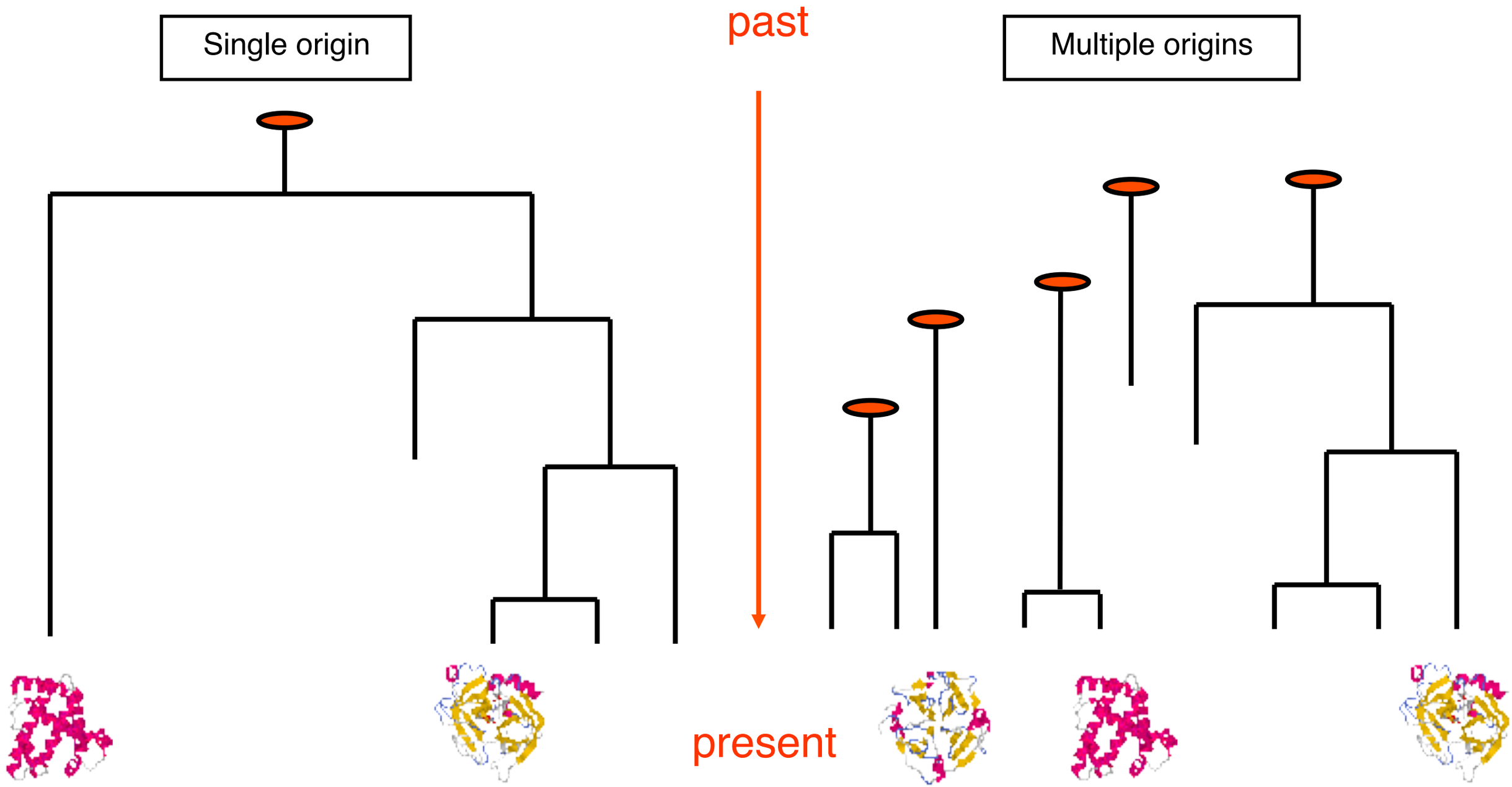
CYTOCHROME C4

Non-homologous Proteins have different structures

Homology is Confusing:
Ways we have seen it defined

- Protein/Genes/DNA that share a common ancestor
- Specific positions/columns in a multiple sequence alignment that have a 1:1 relationship over evolutionary history
    - Is it possible to be 50% homologous?
- Specific mophological/functional characteristics that share a recent divergence (clade)
    - Are all wings homologous (bat, butterfly, eagle)?

Homology is Confusing:
Are all sequences homologous?



Homology Using Sequence/Structural Comparisons

- Homology is shared ancestry
- Convergence are independent events resulting in the same outcome.
- Sequences are inferred to share a common ancestor based on statistically significant excess similarity
- Any evidence of this excess similarity can be used to infer homology (sequence or structure)
- Lack of evidence cannot be used to infer non-homology
- One must weight the evidence for each hypothesis (Convergence or Homology)

What BLAST Does

?

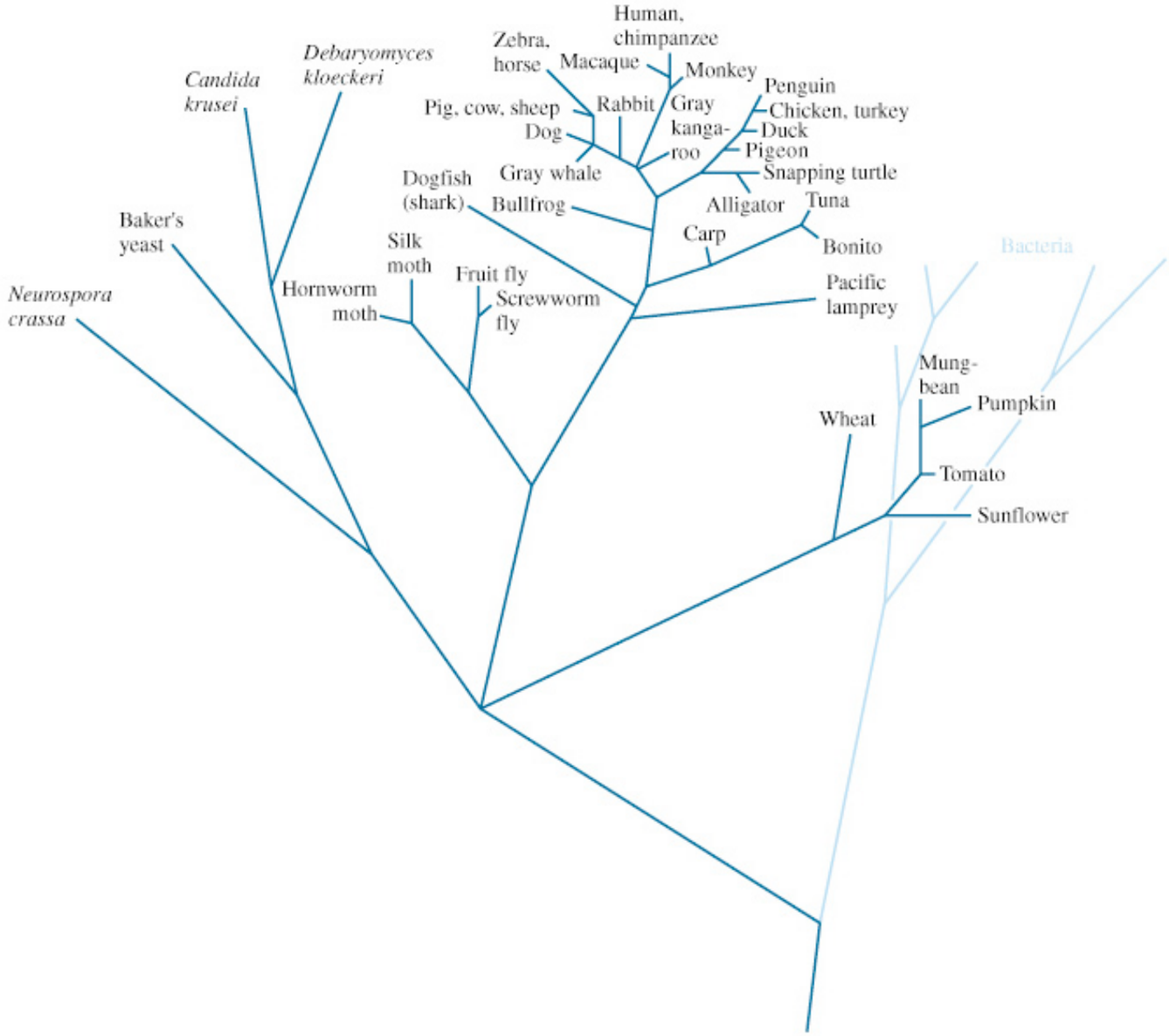
Similarity <=> Homology

?

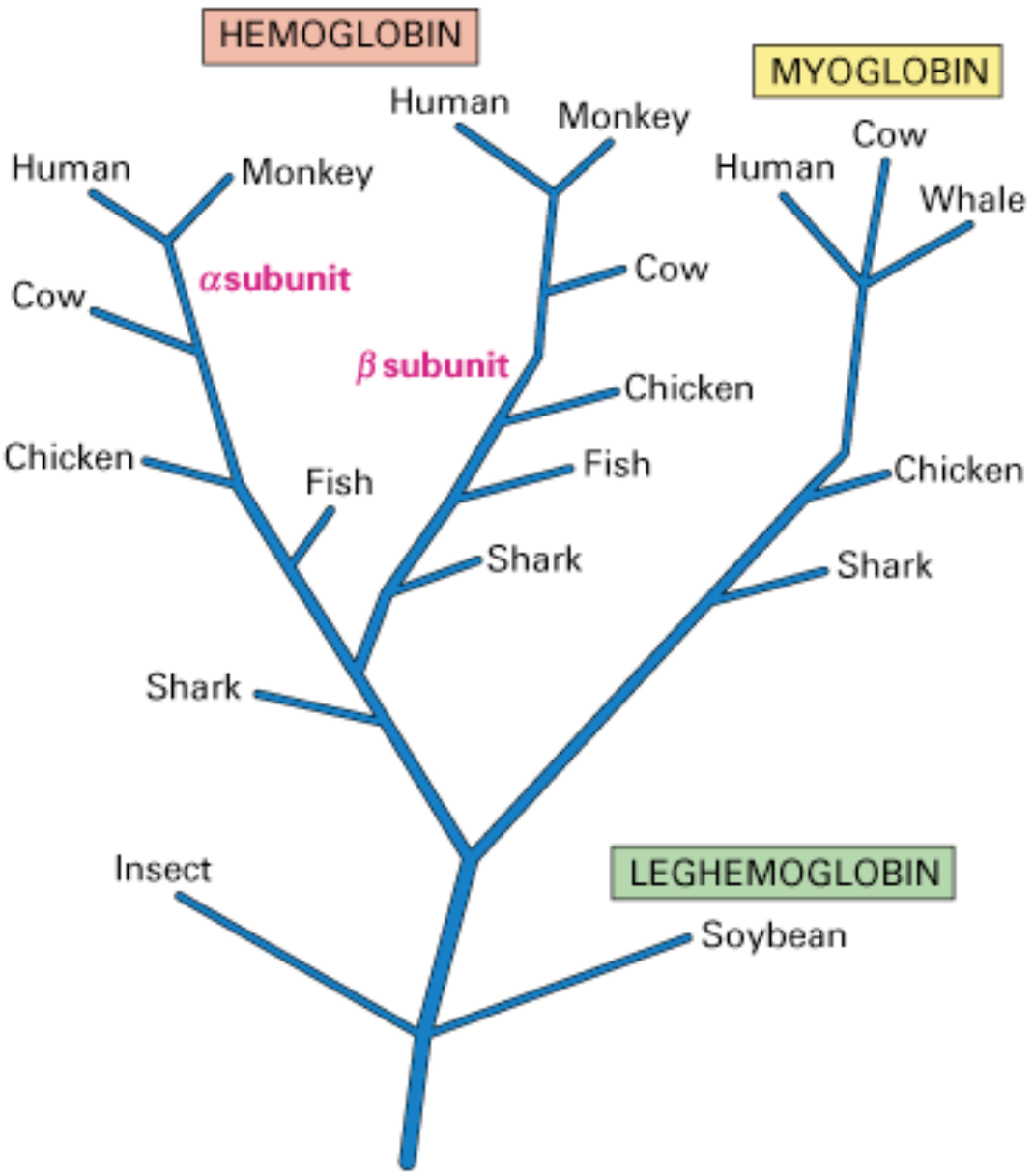
Statistical Significance <=> Biological Significance

Divergence OR Convergence

Orthologs vs Paralogs Inferring Function

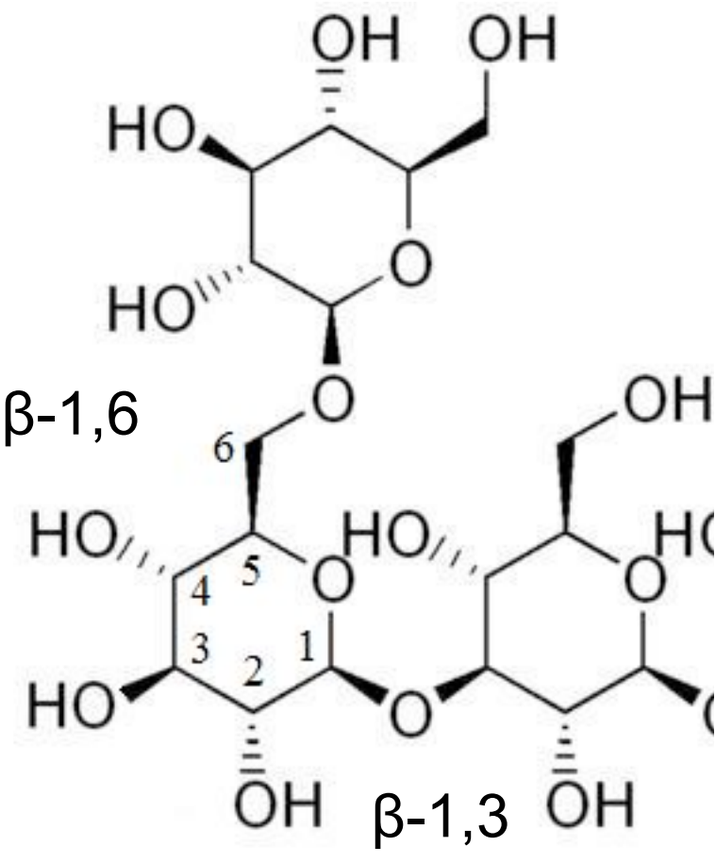


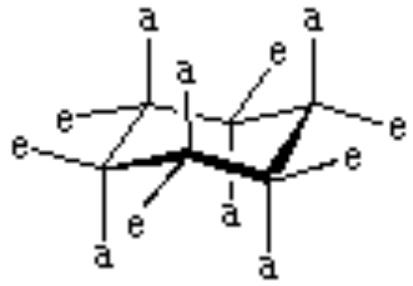
Cytochrome C Family

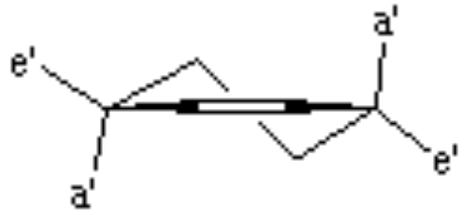


Globin Family

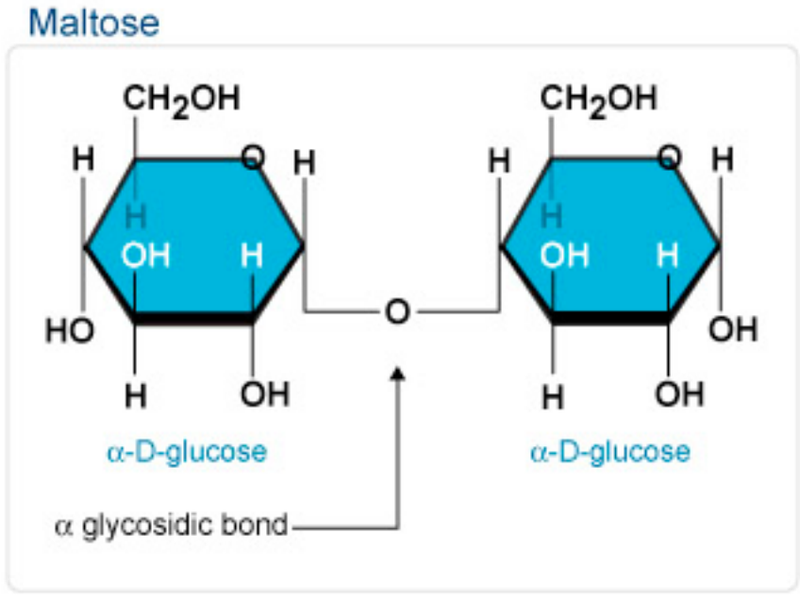
Orthologs vs Paralogs Inferring Function

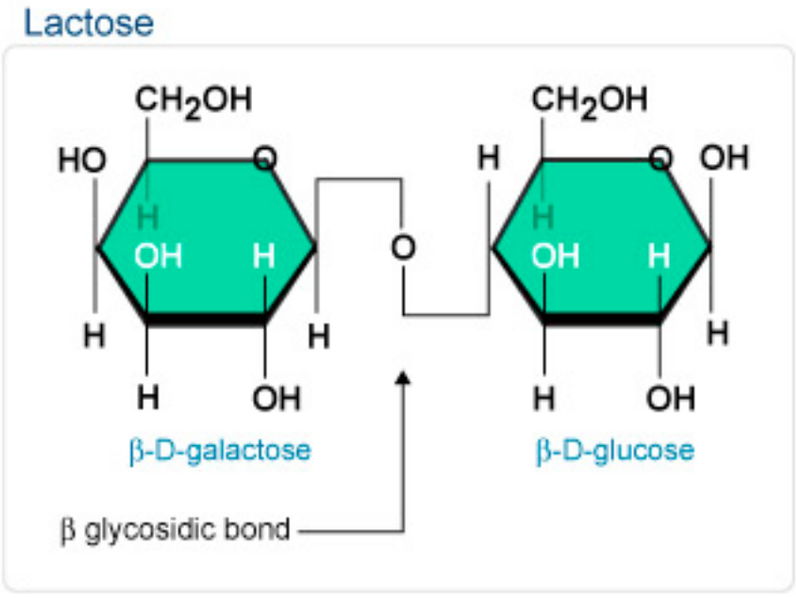






a = axial
e = equatorial
a' = pseudo-axial
e' = pseudo-equatorial



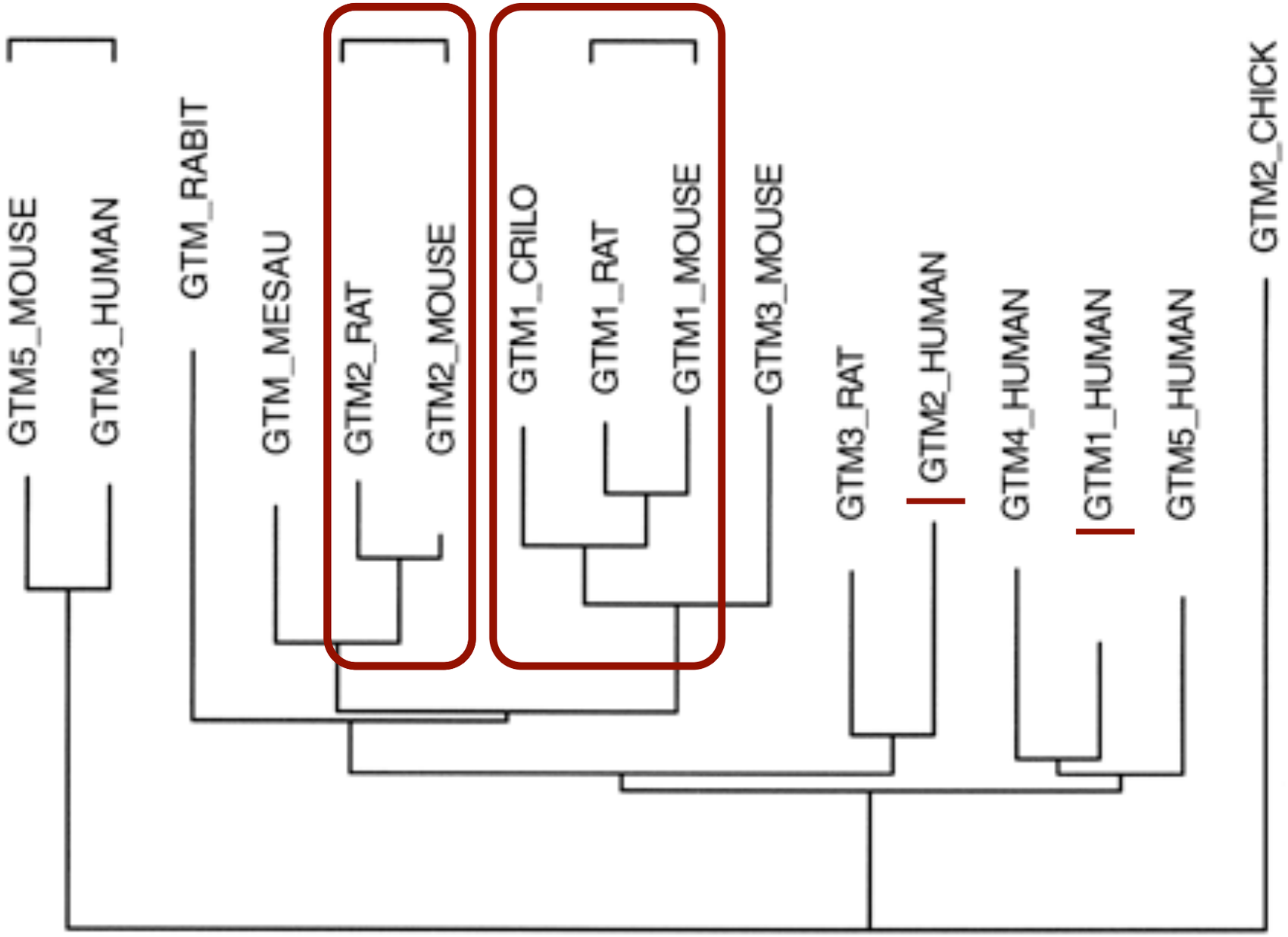


a

e

Homologs Often Maintain Similar Chemical Functions

Orthology can be difficult to infer



doi: 10.1101/gr.9.4.373 *Genome Res. 1999. 9: 373-382*

Orthology can be difficult to infer

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

How do we measure sequence similarity by alignment and scoring matrices?

How to Make an Alignment

```
PMILGYWNVRGL
:     .
PPYTIVYFPVRG
```

```
PMILGYWNVRGL
:  .  :. :::
PPYTIVYFPVRG
```

```
PM-ILGYWNVRGL
:  :. :. :::
PPYTIV-YFPVRG
```

	P	M	I	L	G	Y	W	N	V	R	G	L
P	:											
Y		.		:	.							
T						.	.					
I		:	.				.				.	
V	.	.	.				:				.	
Y	.			:	.							
F	.	.	.		.	.					.	
P	:											
V	.	.	.				:				.	
R								:				
G									:			

Local Alignment

```
AAPMILGYWNVRGLBB
:  .  :. :::
DDPPYTIVYFPVRGCC
```

Global Alignments

Global Alignment

```
-PMILGYWNVRGL
 :  .  :. :::
PPYTIVYFPVRG-
```

Basis:

$F_{0j} = d * j$

$F_{i0} = d * i$

Recursion, based on the principle of optimality:

$$F_{ij} = \max(F_{i-1,j-1} + S(A_i, B_j),\ F_{i,j-1} + d,\ F_{i-1,j} + d)$$

The pseudo-code for the algorithm to compute the F matrix therefore looks like this:

```
for i=0 to length(A)
  F(i,0) ← d*i
for j=0 to length(B)
  F(0,j) ← d*j
for i=1 to length(A)
  for j=1 to length(B)
  {
    Match ← F(i-1,j-1) + S(A_i, B_j)
    Delete ← F(i-1, j) + d
    Insert ← F(i, j-1) + d
    F(i,j) ← max(Match, Insert, Delete)
  }
```

Local Alignments

Local Alignment

```
 AAPMILGYWNVRGLBB
   :  .  :. :::
DDPPYTIVYFPVRGCC
```

A matrix H is built as follows:

$$H(i,0) = 0,\ 0 \leq i \leq m$$

$$H(0,j) = 0,\ 0 \leq j \leq n$$

if $a_i = b_j$ then $w(a_i,b_j) = w(\text{match})$ or if $a_i != b_j$ then $w(a_i,b_j) = w(\text{mismatch})$

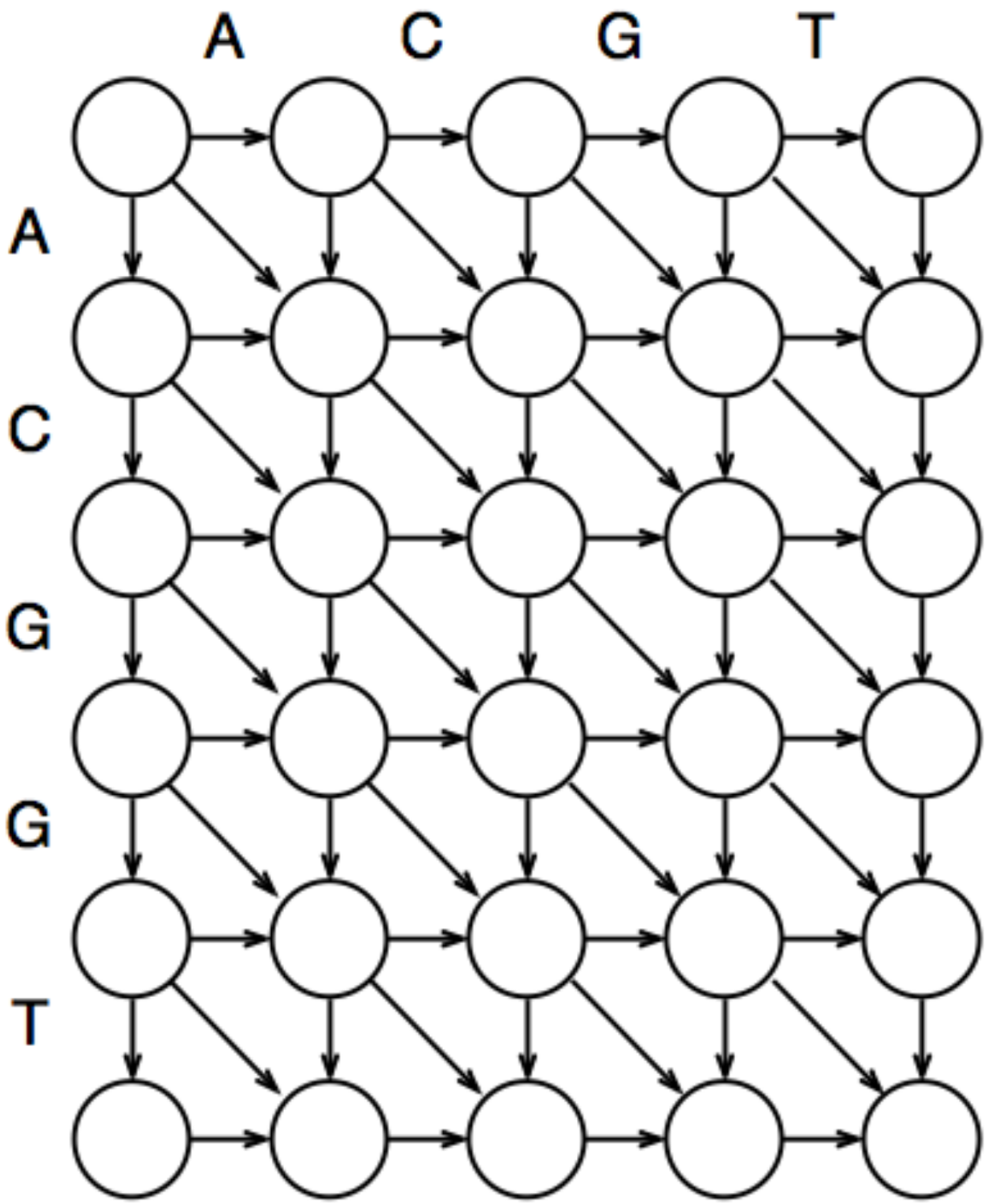
$$H(i,j) = \max \left\{ \begin{array}{cc} 0 & \\ H(i-1,j-1) + w(a_i,b_j) & \text{Match/Mismatch} \\ H(i-1,j) + w(a_i,-) & \text{Deletion} \\ H(i,j-1) + w(-,b_j) & \text{Insertion} \end{array} \right\},\ 1 \leq i \leq m, 1 \leq j \leq n$$

Where:

- a,b = Strings over the Alphabet Σ
- m = length(a)
- n = length(b)
- $H(i,j)$ - is the maximum Similarity-Score between a suffix of a[1...i] and a suffix of b[1...j]
- $w(c,d),\ c,d \in \Sigma \cup \{'-'\}$, '-' is the gap-scoring scheme

Local Alignments

Match: 1
Mismatch: -1
Gap: -2



Local Alignments

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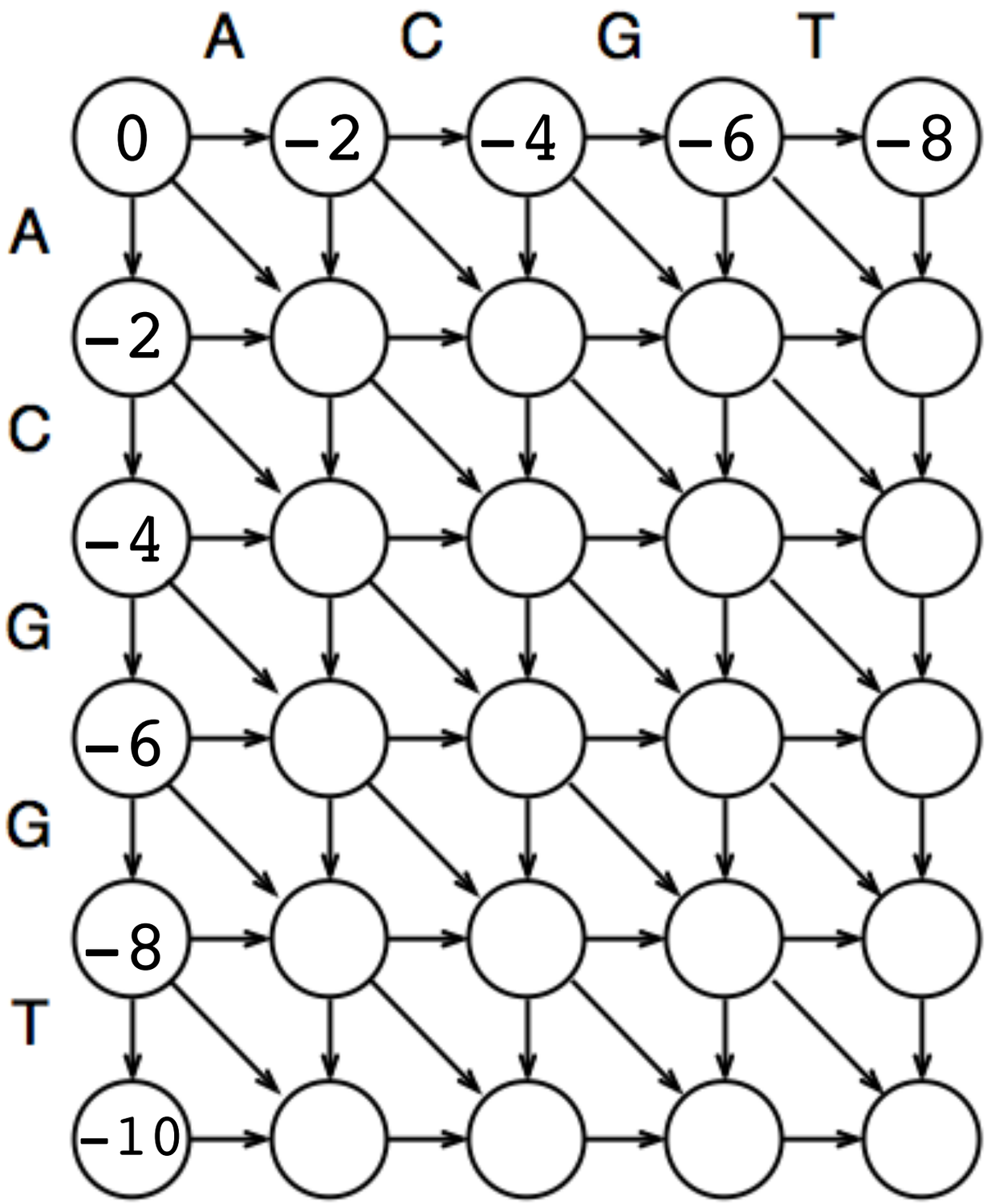

Local Alignments

Match: 1
Mismatch: -1
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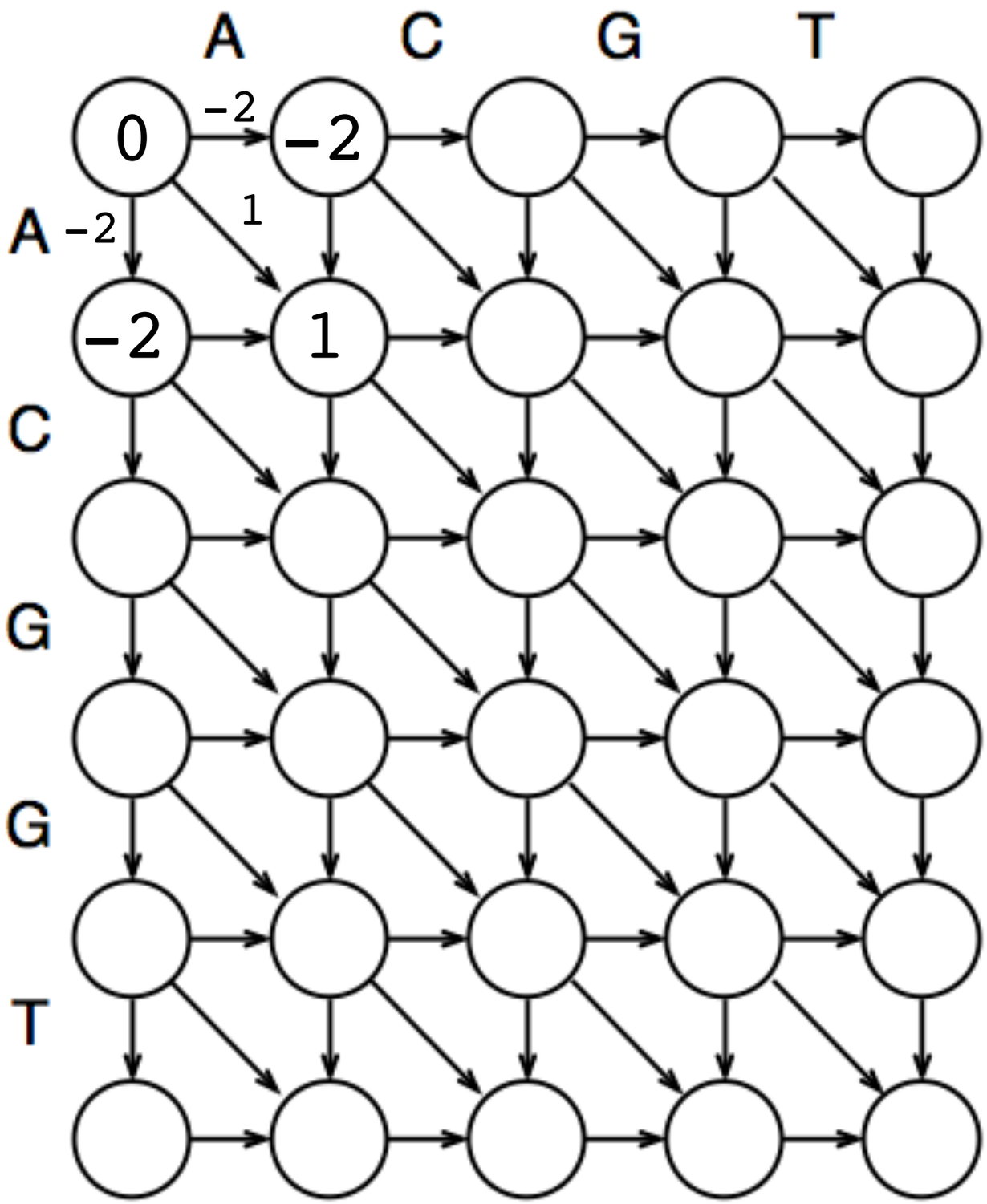

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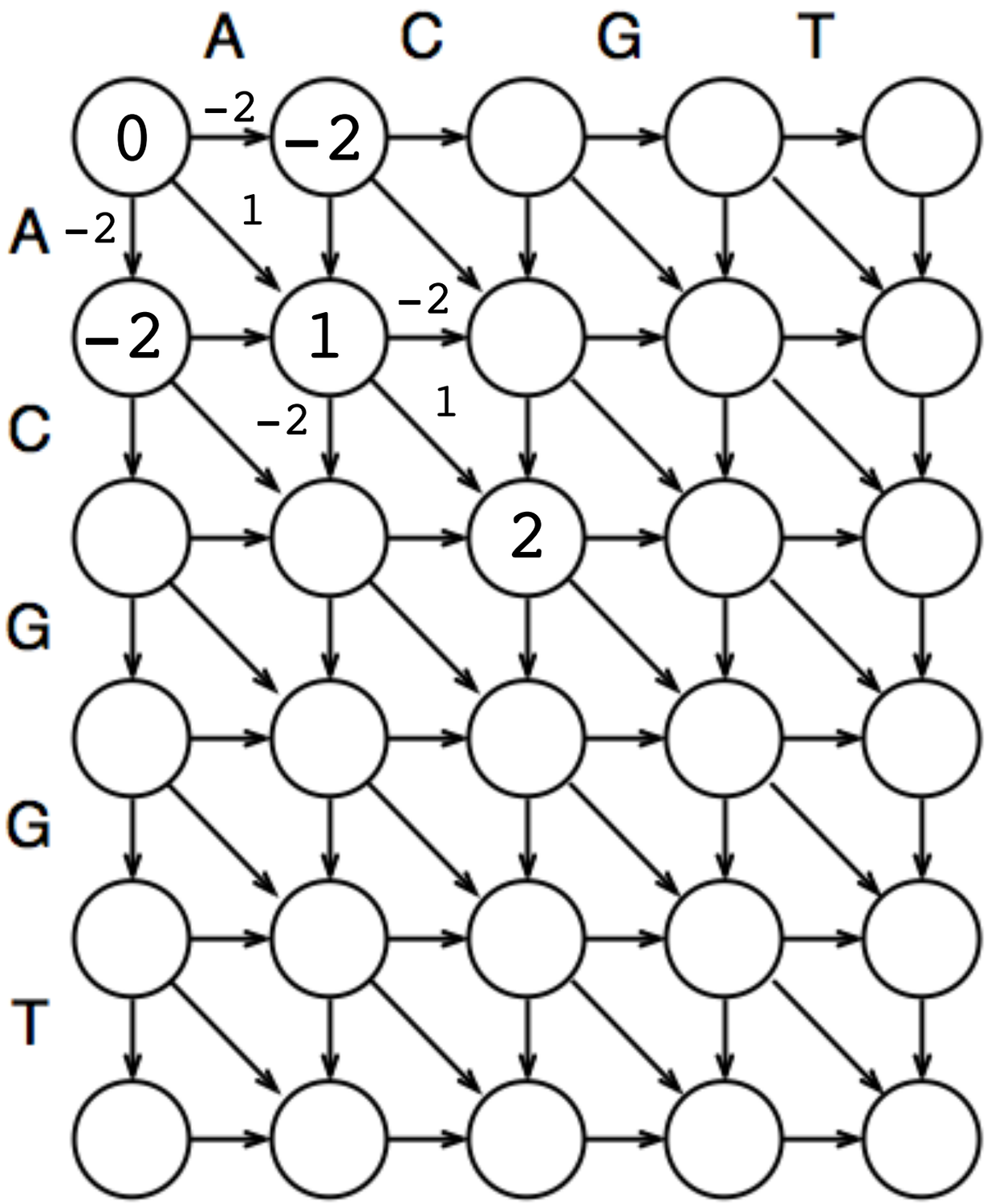

Local Alignments

Match: 1
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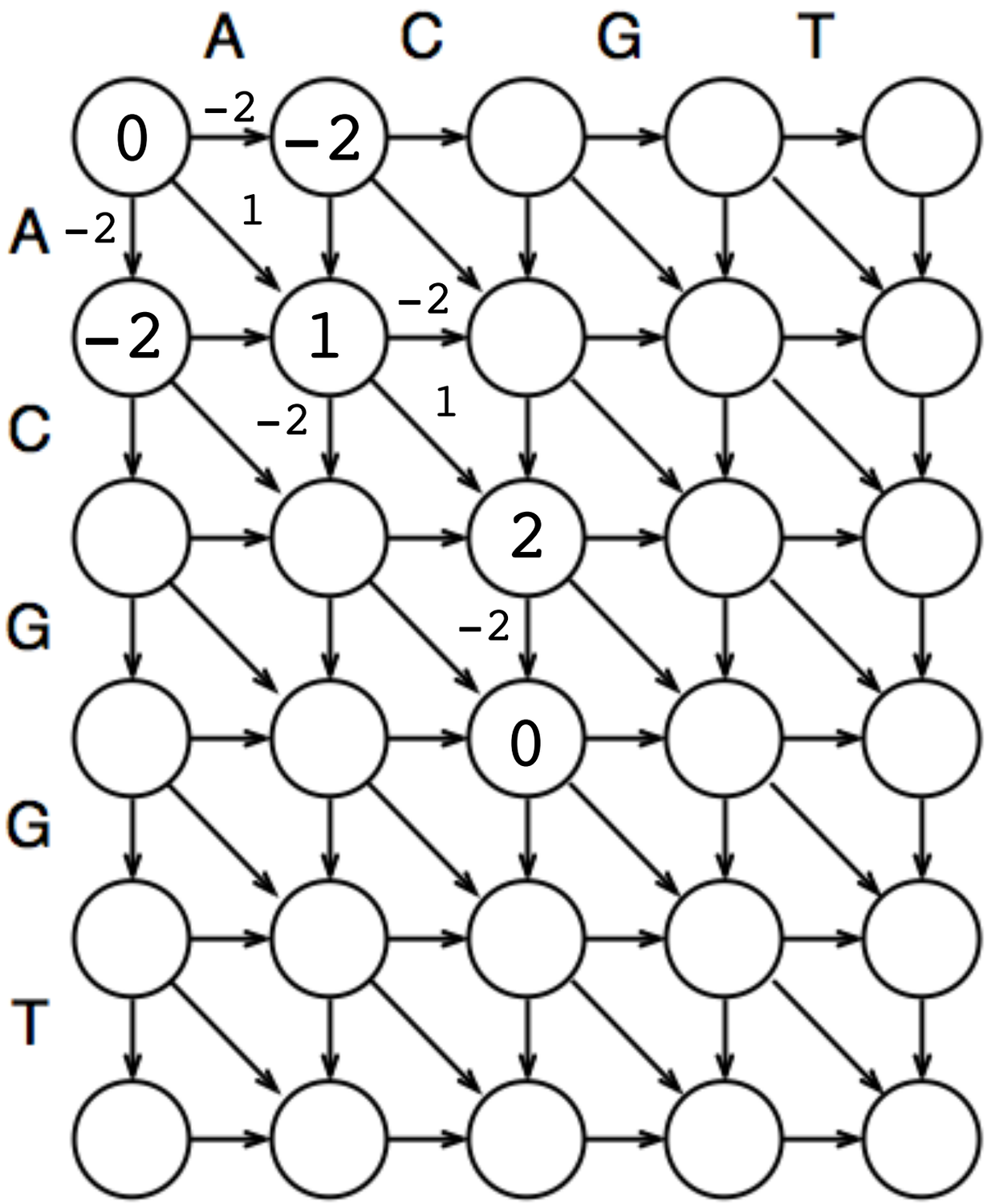

Local Alignments

Match: 1
Mismatch: -1
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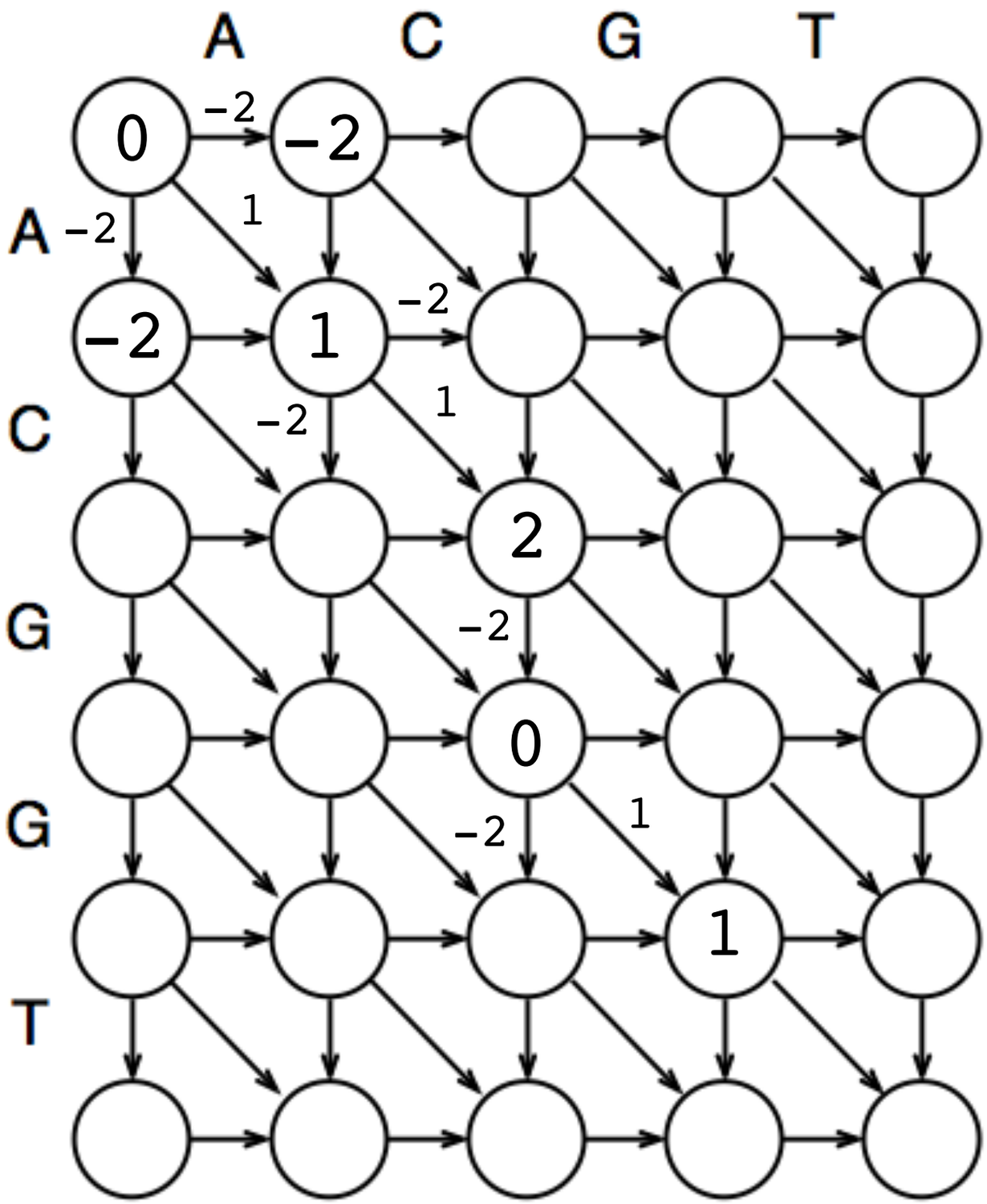
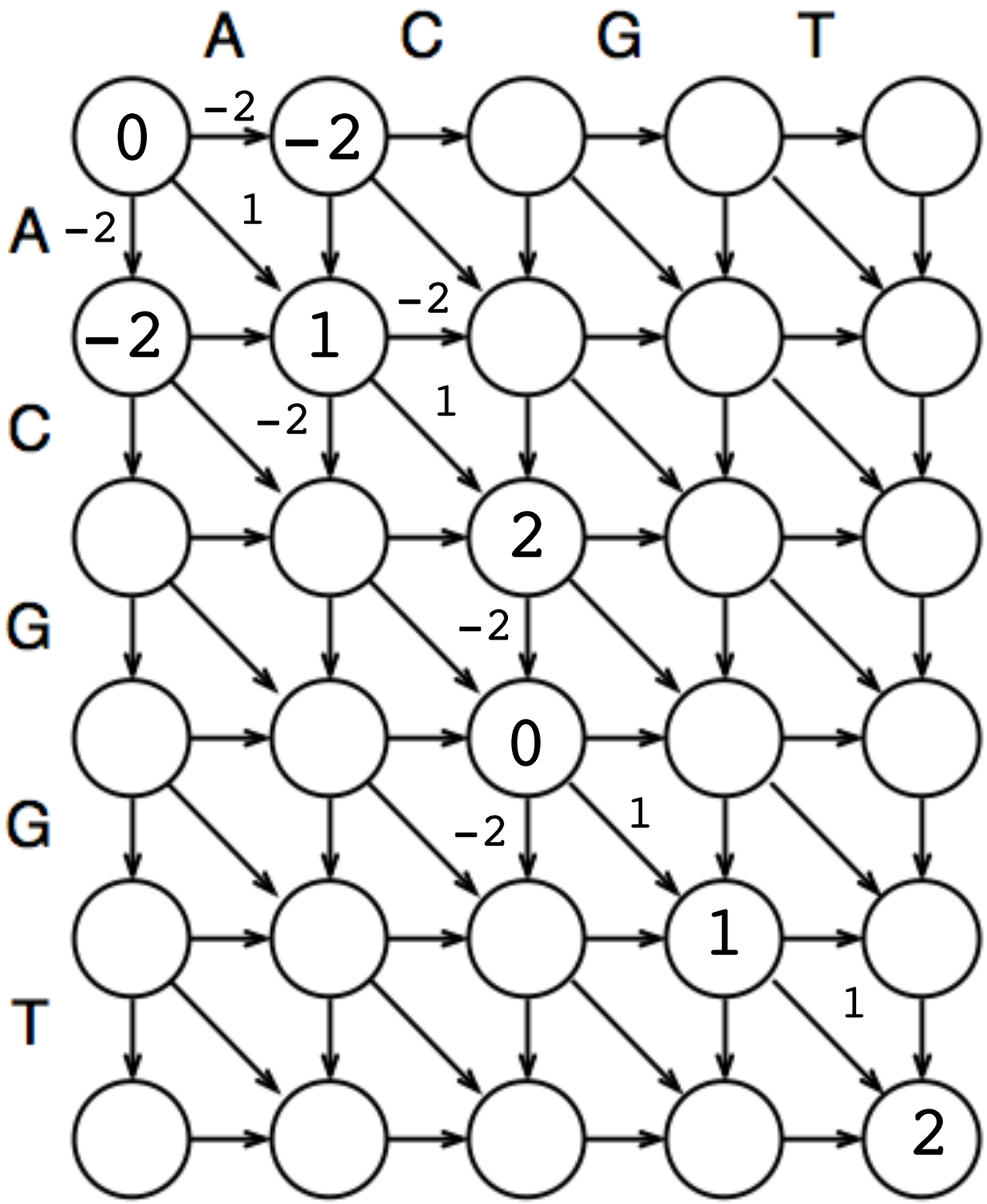


Local Alignments

Match: 1
Mismatch: -1
Gap: -2

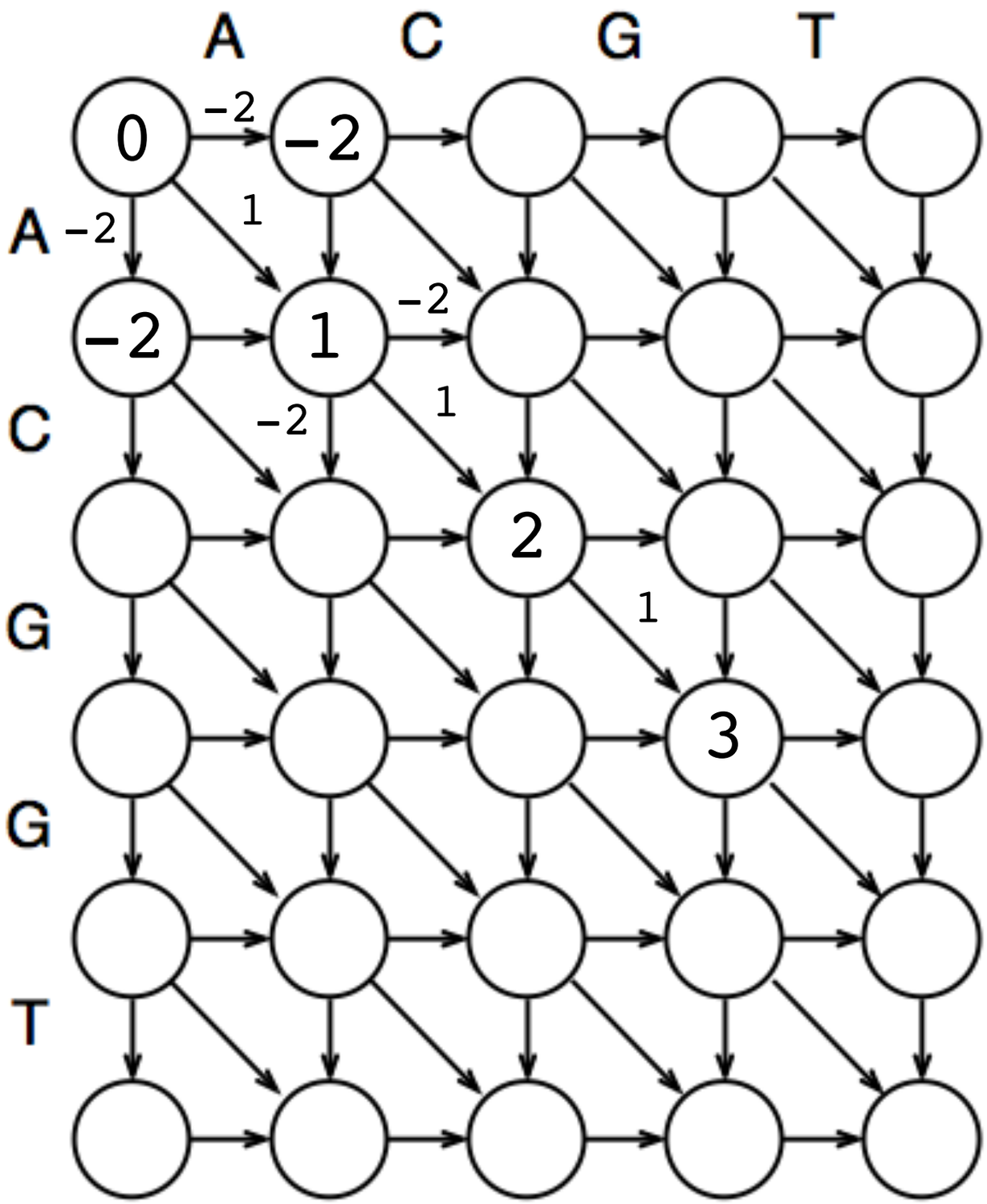
AC-GT

ACGGT



Local Alignments

Match: 1
Mismatch: -1
Gap: -2



Local Alignments

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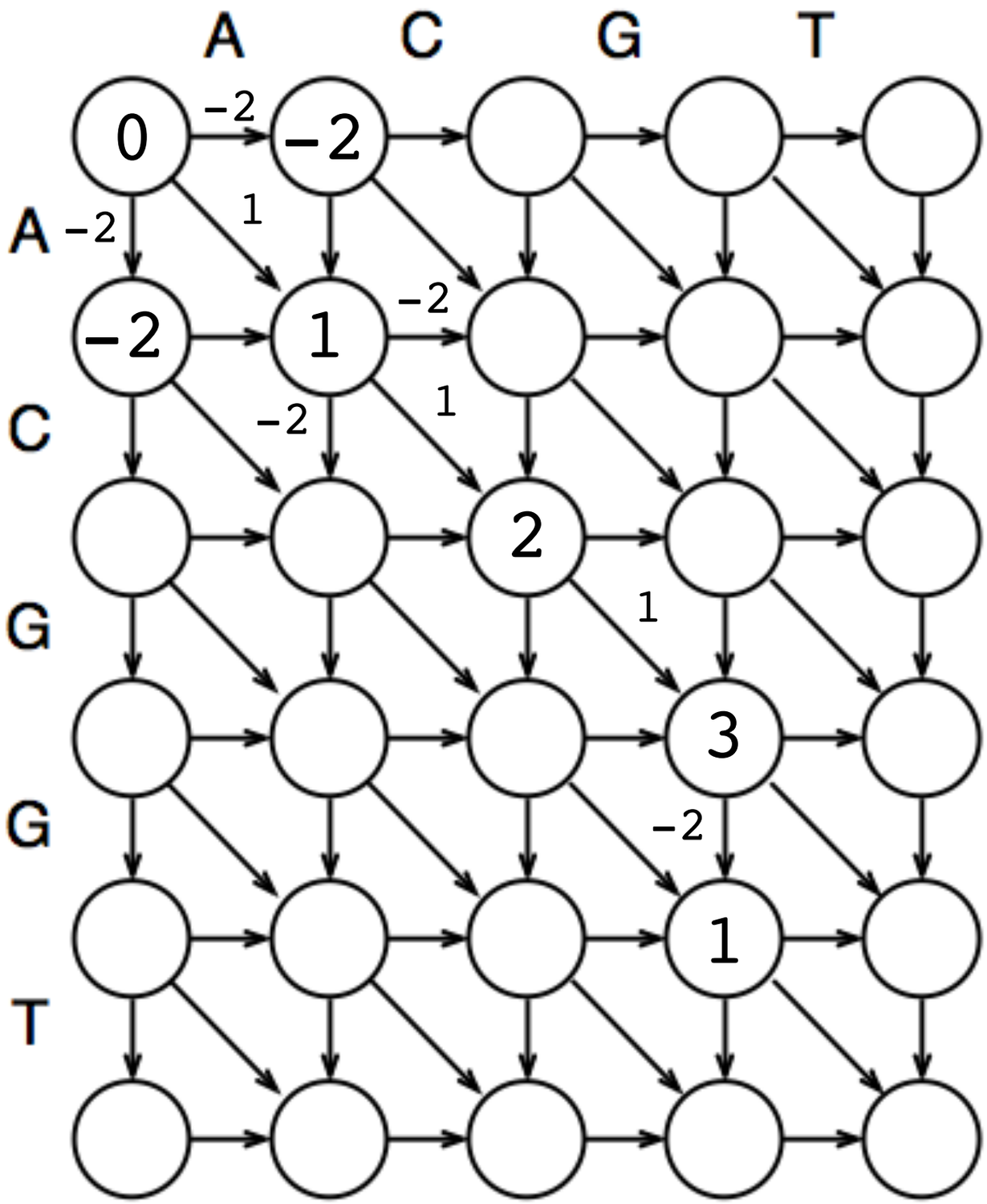
Mismatch: -1
Gap: -2

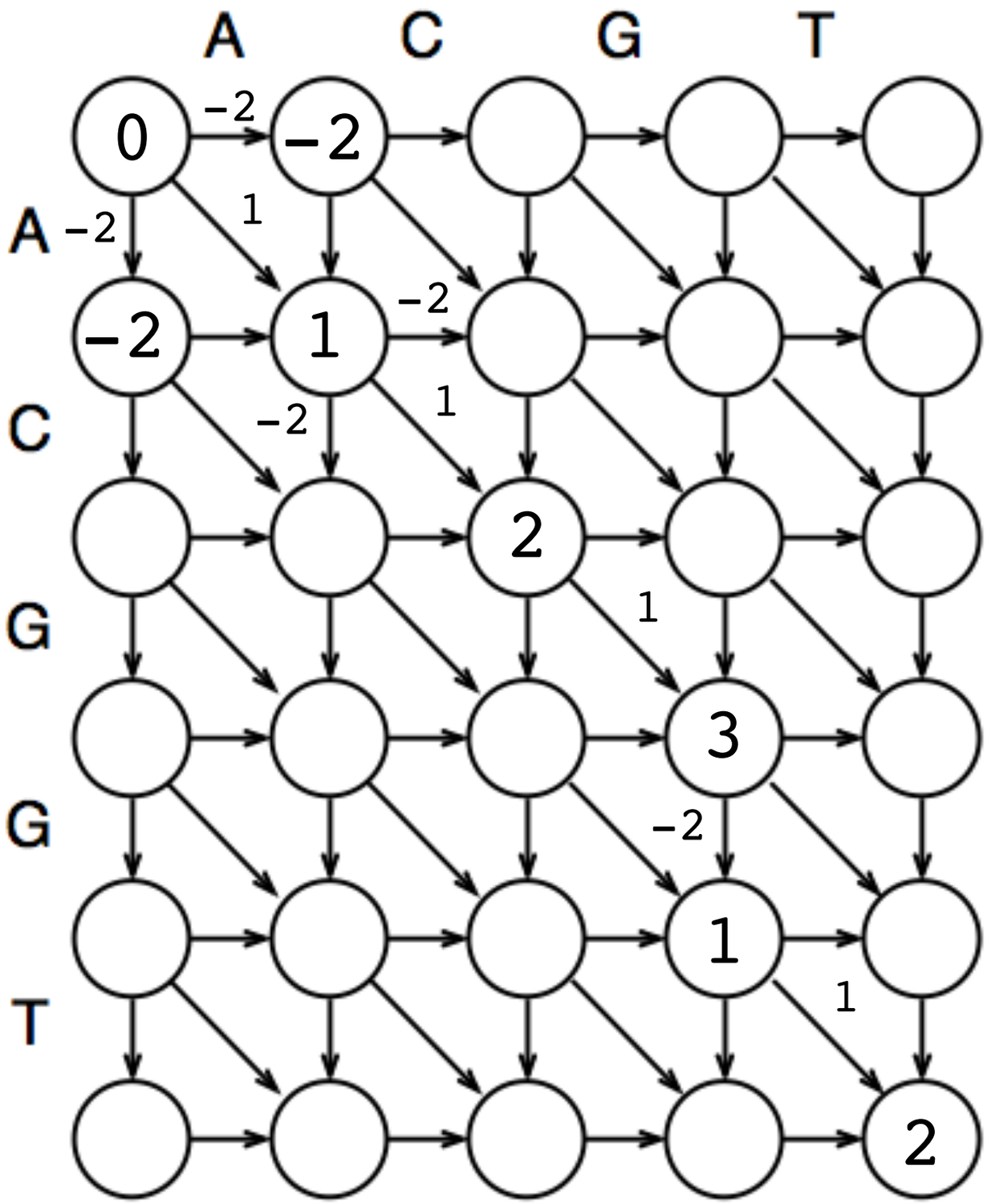
ACG-T

ACGGT



Local Alignments

Match: 1
Mismatch: -1
Gap: -2



ACG-T

ACGGT

Scoring Matrices For Proteins

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

PAM Matrices

- The PAM matrices were introduced by Margret Dayhoff in 1978
- They were based on 1572 observed mutations in 71 families of closely related proteins.
- Each matrix has the twenty standard amino acids in its twenty rows and columns
- The value in a given cell represents the probability of a substitution of one amino acid for another.

PAM Matrices

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

- S is the replacement score of i to j
- λ term is used to scale the matrix so that individual scores can be accurately represented with integers
- qij is Replacement frequency of i to j
- pi is the expected frequency of i

Table 1: Relative mutabilities and the distribution of amino acids in M. Dayhoff's database of observed amino acid changes.

		mut_i	f_i				mut_i	f_i
Ala	A	100	0.087		Leu	L	40	0.085
Arg	R	65	0.041		Lys	K	56	0.081
Asn	N	134	0.040		Met	M	94	0.015
Asp	D	106	0.047		Phe	F	41	0.040
Cys	C	20	0.033		Pro	P	56	0.051
Gln	Q	93	0.038		Ser	S	120	0.070
Glu	E	102	0.050		Thr	T	97	0.058
Gly	G	49	0.089		Trp	W	18	0.010
His	H	66	0.034		Tyr	Y	41	0.030
Ile	I	96	0.037		Val	V	20	0.065

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

PAM1: Predicts one mutation per 100 aa

PAM40: Predicts 40 mutations per 100 aa

PAM250: Predicts 250 mutations per 100 aa

PAM Matrices

	G	A	V	L	I	P	S	T	D	E	N	Q	K	R	H	F	Y	W	M	C	B	Z	X	*	
G	5																								G
A	1	2																							A
V	-1	0	4																						V
L	-4	-2	2	6																					L
I	-3	-1	4	2	5																				I
P	0	1	-1	-3	-2	6																			P
S	1	1	-1	-3	-1	1	2																		S
T	0	1	0	-2	0	0	1	3																	T
D	1	0	-2	-4	-2	-1	0	0	4																D
E	0	0	-2	-3	-2	-1	0	0	3	4															E
N	0	0	-2	-3	-2	0	1	0	2	1	2														N
Q	-1	0	-2	-2	-2	0	-1	-1	2	2	1	4													Q
K	-2	-1	-2	-3	-2	-1	0	0	0	0	1	1	5												K
R	-3	-2	-2	-3	-2	0	0	-1	-1	-1	0	1	3	6											R
H	-2	-1	-2	-2	-2	0	-1	-1	1	1	2	3	0	2	6										H
F	-5	-3	-1	2	1	-5	-3	-3	-6	-5	-3	-5	-5	-4	-2	9									F
Y	-5	-3	-2	-1	-1	-5	-3	-3	-4	-4	-2	-4	-4	-4	0	7	10								Y
W	-7	-6	-6	-2	-5	-6	-2	-5	-7	-7	-4	-5	-3	-2	-3	0	0	17							W
M	-3	-1	2	4	2	-2	-2	-1	-3	-2	-2	-1	0	0	-2	0	-2	-4	6						M
C	-3	-2	-2	-6	-2	-3	0	-2	-5	-5	-4	-5	-5	-4	-3	-4	0	-8	-5	12					C
B	0	0	-2	-3	-2	-1	0	0	3	3	2	1	1	-1	1	-4	-3	-5	-2	-4	3				B
Z	0	0	-2	-3	-2	0	0	-1	3	3	1	3	0	0	2	-5	-4	-6	-2	-5	2	3			Z
X	-1	0	-1	-1	-1	-1	0	0	-1	-1	0	-1	-1	-1	-1	-2	-2	-4	-1	-3	-1	-1	-1		X
*	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	-8	1	*
	G	A	V	L	I	P	S	T	D	E	N	Q	K	R	H	F	Y	W	M	C	B	Z	X	*	

PAM 250

Details on Scoring Matrices

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

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

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

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

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

$\lg_2 = 1/3$; $S_{R:N(\ 40)} = -2.333/\lg_2 = -7$

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

Details on Scoring Matrices

PAM

- 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

•Empirically determined, no extrapolation (no model)

•BLOSUM45-50 - distant (1/3 bits)

•BLOSUM80 -very highly conserved (not small change), high info/position

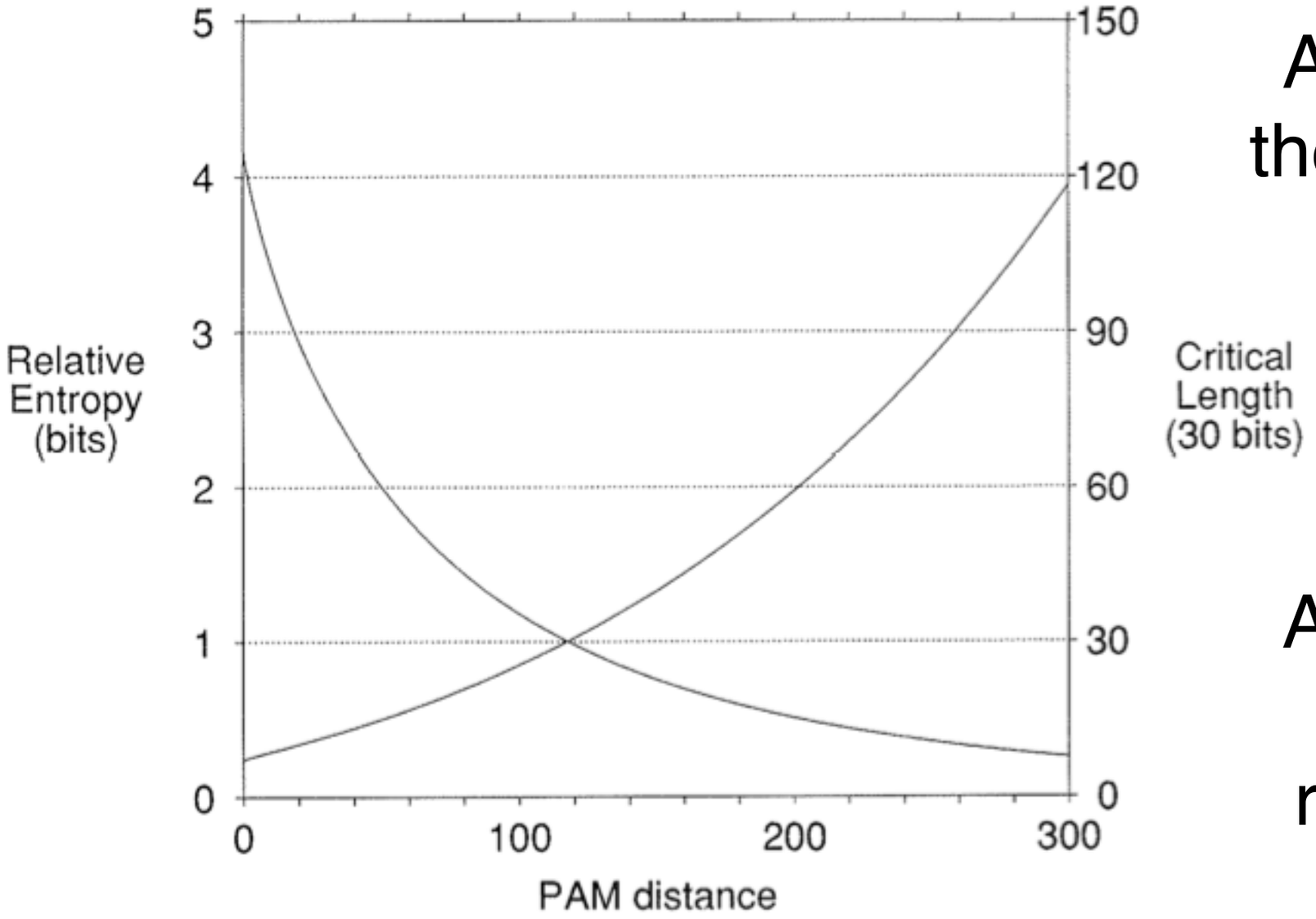
•BLOSUM62 - 1/2 bits

Scoring Matrices

- 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 (closer)
- Shallow matrices set maximum look-back time

PAM	BLOSUM
PAM100:	BLOSUM90
PAM120:	BLOSUM80
PAM160:	BLOSUM60
PAM200:	BLOSUM52
PAM250:	BLOSUM45

Details on Scoring Matrices

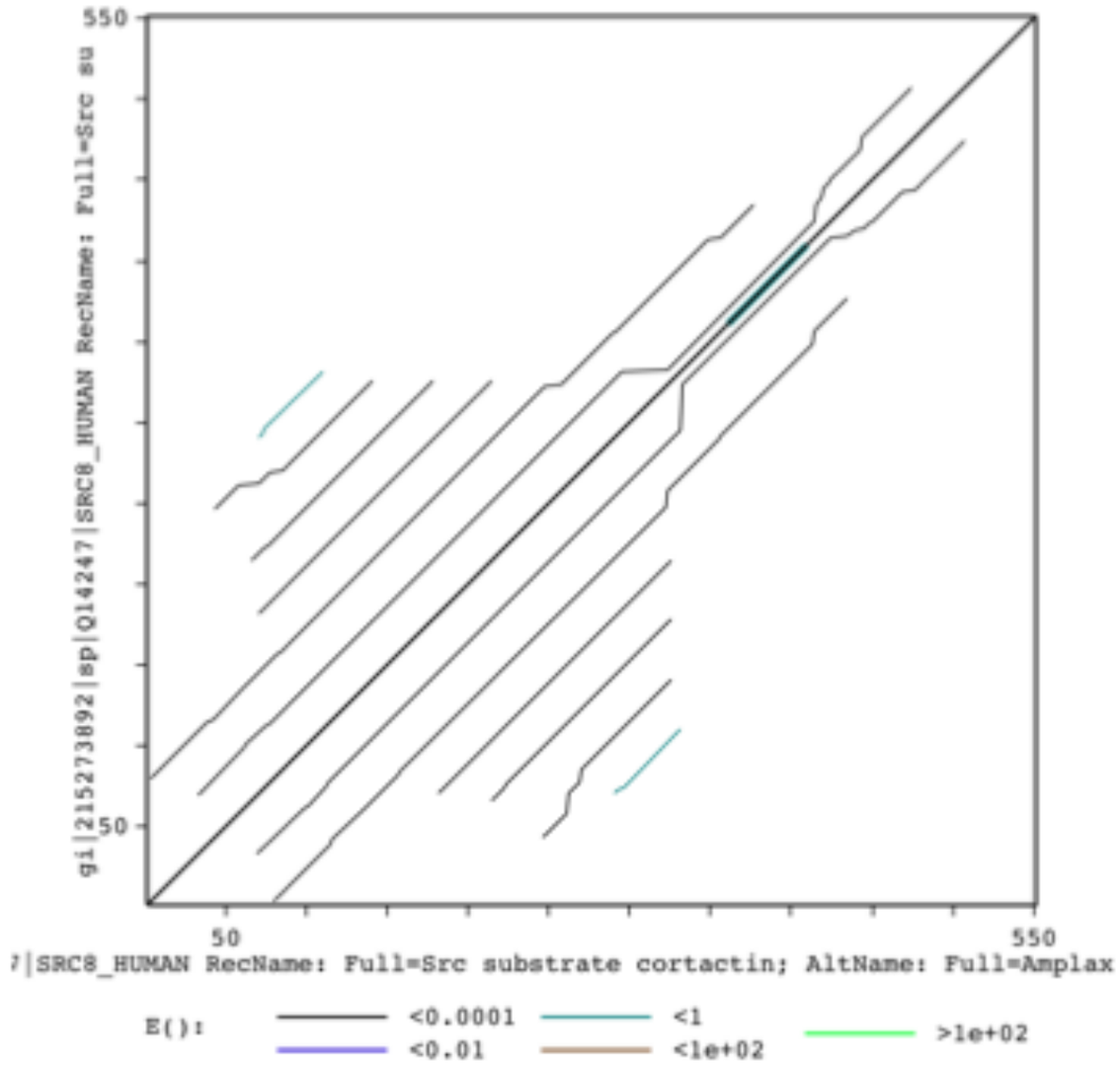


As sequences diverge, there is less information per position

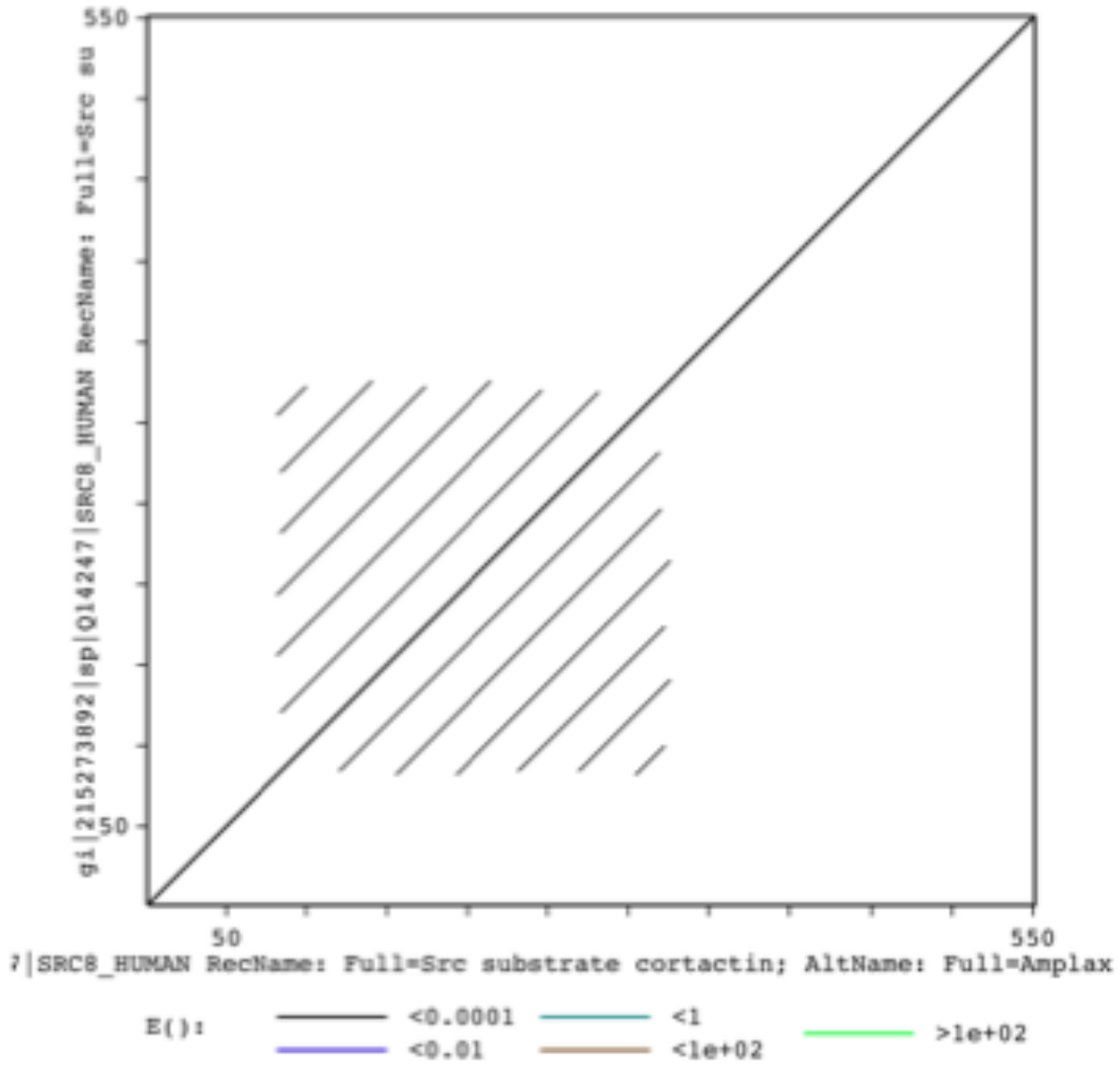
As sequences diverge, longer alignments are required to contain the score threshold

Stringent Score Leads to Short Alignments

BLOSUM62 -11/-1



MD20 -26/-4



Scoring Alignments

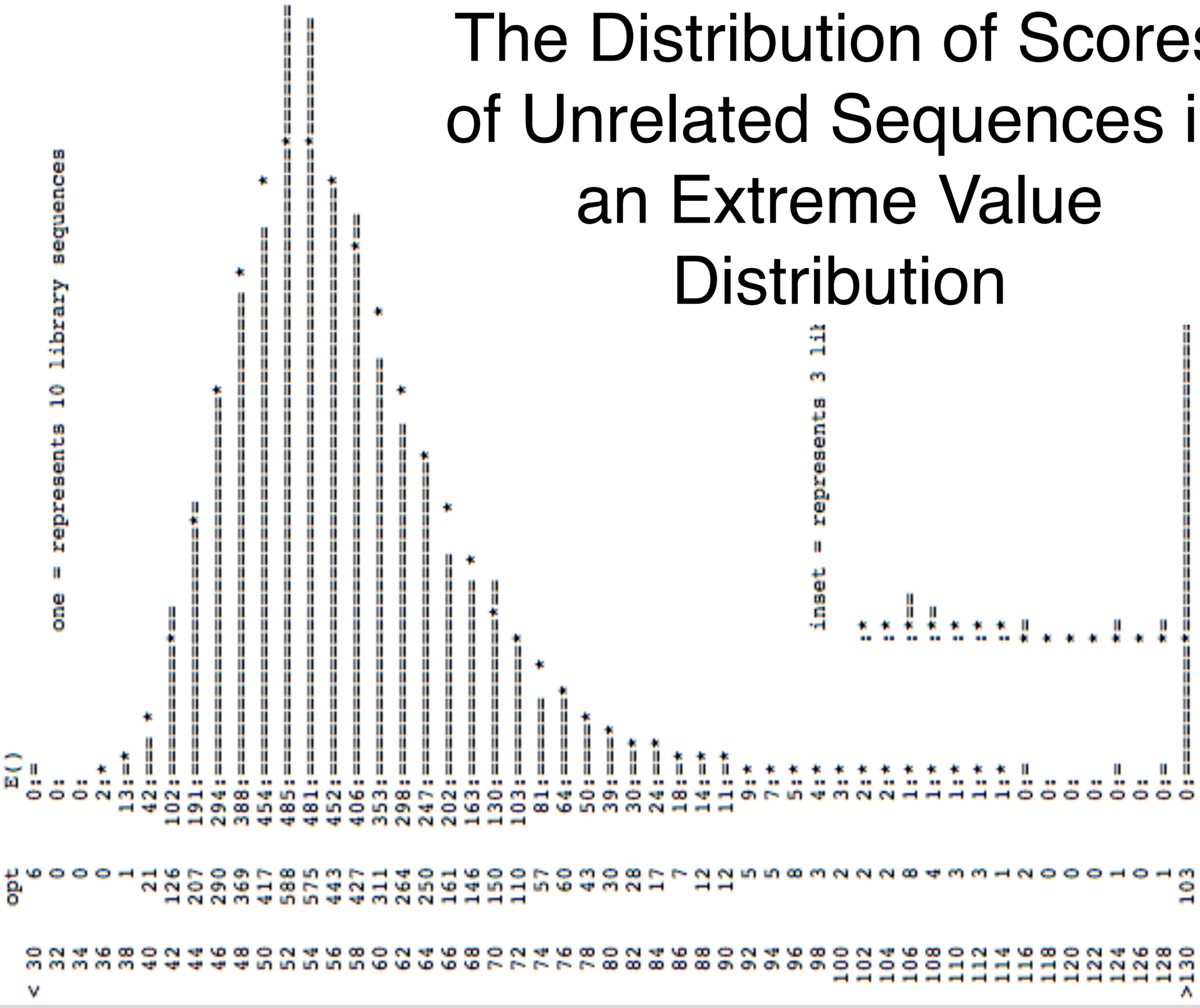
```
>>sp|P07925|ATP6_MAIZE ATP synthase a chain (ATPase protein 6)                (291 aa)
 initn:  96 init1:  56 opt: 116  Z-score: 161.2  bits: 37.6 E(13351): 0.0048
Smith-Waterman score: 175; 24.7% identity (57.9% similar) in 247 aa overlap (16-251:31-259)
Entrez Lookup  Re-search database  General re-search
                              10        20        30        40        50        60
gi|231                  MKIVLLYYFVNMFISGIFQIANVEVGQHFYWSILGFQIHGQVLINSWIVILIIGFLSIYTTKNL-
                                     ::  : ....:. .: :. ....  ..:..  .:.:.. :  . : :.
sp|P07 MERNGEivnngsiiipggggpvTESPLDQFGIHPILDLNIGK-YYVSFtnlsl--smlltlglvlllv-f--vvtkkggg
               10        20        30        40          50          60          70

          70        80        90          100       110       120       130       140
gi|231 TLVPANKQIFIELVTEFITDISKTQIGEKEYS---KWVPYIGTMFLFIFVSNWSGALIPWKIIELPNGELGAPTNDINTT
        ::   : ..::. .:. .. . :::    .   :. : :.. : : .  : .: .::...          . :. .  :
sp|P07 ksvPNAFQSLVELIYDFVPNLVNEQIGGLSGNVKHKFFPCISVTFTFSLFRNPQG-MIPFSF---------TVTSHFLIT
           80        90        100       110       120       130                   140

             150       160       170             180           190       200       210
gi|231 AGLAILTSLAYFYAGLNKKGLTYFKKYVQPTPILLPIN----ILEDFT---KPLSLSFRLFGNILADELVVAVLVSLVPL
        .:..   ..   .:....:: .:. .. :. . ::.     .:: ..   . :: ..:::.:..: .  : .: ...
sp|P07 LALSFSIFIGITIVGFQRHGLHFFS-fllpagvplplapflvllelISHCFRALSSGIRLFANMMAGHSSVKILSGFAWT
          150       160       170       180       190       200       210       220

          220       230       240       250
gi|231 IVPVPLIFLGLFTSGIQALIFATLSGSYIGEAMEGHH
       .. .  ::  :   :   ...: :.:  .: :.   :
sp|P07 MLFLNNIFYFLGDLGPLFIVLA-LTGLELGVAISQAHVSTISICIYLNDATNLHQNESFHNCIKTRSQS
          230       240       250       260       270       280       290
```

Alignments are scored using the scoring matrix

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

The Distribution of Scores of Unrelated Sequences is an Extreme Value Distribution



What is an Expectation Value

The best scores are:		opt	bits	E(13351)	%_id	%_sim	alen	
sp|P0AB98|ATP6_ECOLI	ATP synthase a chain (ATPase prote (271)	1650	428.4	1.1e-120	1.000	1.000	271	align
sp|P06451|ATPI_SPIOL	Chloroplast ATP synthase a chain p (247)	161	49.1	1.5e-06	0.270	0.616	211	align
sp|P06289|ATPI_MARPO	Chloroplast ATP synthase a chain p (248)	161	49.1	1.5e-06	0.261	0.621	211	align
sp|P06452|ATPI_PEA	Chloroplast ATP synthase a chain pre (247)	158	48.3	2.6e-06	0.274	0.614	223	align
sp|P69371|ATPI_ATRBE	Chloroplast ATP synthase a chain p (247)	156	47.8	3.7e-06	0.270	0.607	211	align
sp|P00848|ATP6_MOUSE	ATP synthase a chain (ATPase prote (226)	149	46.0	1.2e-05	0.259	0.617	193	align
sp|P00846|ATP6_HUMAN	ATP synthase a chain (ATPase prote (226)	148	45.7	1.4e-05	0.237	0.589	236	align
sp|P30391|ATPI_EUGGR	Chloroplast ATP synthase a chain p (251)	139	43.4	7.6e-05	0.298	0.596	225	align
sp|P00847|ATP6_BOVIN	ATP synthase a chain (ATPase prote (226)	138	43.2	8.1e-05	0.233	0.581	236	align
sp|P0C2Y5|ATPI_ORYSA	Chloroplast ATP synthase a chain p (247)	132	41.7	0.00026	0.259	0.603	239	align
sp|P68526|ATP6_TRITI	ATP synthase a chain (ATPase prote (386)	121	38.9	0.0028	0.259	0.603	239	align
sp|P27178|ATP6_SYNY3	ATP synthase a chain (ATPase prote (276)	116	37.6	0.0048	0.264	0.578	258	align
sp|P00854|ATP6_YEAST	ATP synthase a chain precursor (AT (259)	113	36.8	0.0077	0.235	0.578	277	align
sp|P08444|ATP6_SYNP6	ATP synthase a chain (ATPase prote (261)	113	36.8	0.0077	0.267	0.600	240	align
sp|P00852|ATP6_EMENI	ATP synthase a chain precursor (AT (256)	111	36.3	0.011	0.209	0.590	244	align
sp|P07925|ATP6_MAIZE	ATP synthase a chain (ATPase prote (291)	109	35.8	0.017	0.259	0.578	232	align
sp|P00851|ATP6_DROYA	ATP synthase a chain (ATPase prote (224)	98	33.0	0.094	0.225	0.549	253	align
sp|P14862|ATP6_COCHE	ATP synthase a chain (ATPase prote (257)	91	31.2	0.37	0.204	0.608	265	align
ref|NP_008281.1|ATP6_10704	ATP synthase F0 subunit 6 [D (224)	90	31.0	0.39	0.230	0.576	165	align
sp|P09716|US17_HCMVA	Hypothetical protein HVLF1 (293)	91	31.2	0.42	0.260	0.565	131	align
sp|P12446|MAT_INCJJ	Polyprotein p42 [Contains: Protein (374)	85	29.7	1.5	0.247	0.559	93	align
sp|P00849|ATP6_XENLA	ATP synthase a chain (ATPase prote (226)	79	28.2	2.7	0.261	0.630	165	align
sp|P06974|FLIM_ECOLI	Flagellar motor switch protein fli (334)	81	28.7	2.8	0.308	0.673	52	align
sp|P05499|ATP6_TOBAC	ATP synthase a chain (ATPase prote (395)	81	28.7	3.3	0.220	0.582	268	align
sp|P07864|LDHC_HUMAN	L-lactate dehydrogenase C chain (L (332)	80	28.4	3.3	0.478	0.783	23	align
sp|Q02655|CYB_PODAN	Cytochrome b (387)	80	28.4	3.9	0.241	0.517	116	align
sp|P00162|CYB_NEUCR	Cytochrome b (385)	79	28.2	4.6	0.244	0.543	234	align
sp|P06320|TVB7_MOUSE	T-cell receptor beta chain V regio (134)	73	26.6	4.7	0.360	0.540	50	align
sp|P09261|CELF_VZVD	Cell fusion protein precursor (340)	78	27.9	4.8	0.204	0.512	162	align
sp|P05841|VNS3_BMDNV	Putative nonstructural protein (OR (167)	74	26.9	4.9	0.464	0.750	28	align
ref|YP_220570.1|	NADH dehydrogenase subunit 3 [Mus musc (115)	71	26.1	5.8	0.290	0.508	124	align
sp|P02980|TCR2_ECOLI	Tetracycline resistance protein, c (401)	77	27.7	6.8	0.325	0.650	40	align
sp|P03921|NU5M_MOUSE	NADH-ubiquinone oxidoreductase cha (607)	79	28.2	7.1	0.321	0.605	81	align
sp|P00160|CYB_XENLA	Cytochrome b (380)	76	27.4	7.7	0.248	0.545	165	align
sp|P45014|NRFD_HAEIN	Protein nrfD homolog (321)	75	27.2	7.8	0.221	0.579	145	align
sp|Q00098|KR16_ICHV1	Gene 16 protein kinase (387)	76	27.4	7.8	0.250	0.484	128	align

Highest Scoring Unrelated Sequenced E() ~ 1

The best scores are:			s-w bits	E(13351)	%_id	%_sim	alen	
sp|P26205|BGLT_TRIRP	Cyanogenic beta-glucosidase precur	(425)	1187	281.9	4.3e-76	0.452	0.763	392	align
sp|P26204|BGLS_TRIRP	Non-cyanogenic beta-glucosidase pr	(493)	1179	279.9	1.9e-75	0.406	0.704	497	align
sp|P11546|LACG_LACLA	6-phospho-beta-galactosidase (Beta	(468)	712	171.6	7.5e-43	0.326	0.603	494	align
sp|P12614|BGLS_AGRSA	Beta-glucosidase (Gentiobiase) (Ce	(459)	699	168.6	5.9e-42	0.302	0.590	483	align
sp|P31835|CDGT2_PAEMA	Cyclomaltodextrin glucanotransfer	(713)	110	31.7	1.5	0.251	0.561	187	align
sp|P26537|VL1_HPV5B	Major capsid protein L1	(525)	106	30.9	1.9	0.245	0.504	139	align
sp|P02667|CS2LA_RAT	Alpha-S2-casein-like A precursor (C	(179)	97	29.2	2.1	0.288	0.652	66	align
sp|Q03763|DSG1_BOVIN	Desmoglein-1 precursor (Desmosomal	(1043)	109	31.3	2.8	0.206	0.497	286	align
sp|P09282|UL32_VZVD	Probable major envelope glycoprotei	(585)	101	29.7	4.8	0.237	0.568	118	align
sp|Q92040|ANX12_COLLI	Annexin A1 isoform p37 (Annexin I	(343)	96	28.7	5.5	0.251	0.508	179	align
sp|P16330|CN37_MOUSE	2',3'-cyclic-nucleotide 3'-phospho	(420)	97	28.9	6.1	0.227	0.529	172	align
ref|NP_276832.1|	transcriptional regulator Icc related	(262)	91	27.7	8.8	0.285	0.455	123	align

Highest Scoring Unrelated Protein

The best scores are:		opt	bits	E(13351)	%_id	%_sim	alen	
sp|P00846|ATP6_HUMAN	ATP synthase a chain (ATPase prote (226)	1124	289.8	4.1e-79	1.000	1.000	226	align
sp|P00847|ATP6_BOVIN	ATP synthase a chain (ATPase prote (226)	1075	277.5	2e-75	0.779	0.951	226	align
sp|P00848|ATP6_MOUSE	ATP synthase a chain (ATPase prote (226)	1057	273.0	4.5e-74	0.757	0.916	226	align
sp|P00849|ATP6_XENLA	ATP synthase a chain (ATPase prote (226)	499	133.4	4.7e-32	0.533	0.847	229	align
sp|P00854|ATP6_YEAST	ATP synthase a chain precursor (AT (259)	357	97.9	2.7e-21	0.353	0.694	232	align
sp|P00851|ATP6_DROYA	ATP synthase a chain (ATPase prote (224)	323	89.4	8.3e-19	0.378	0.721	222	align
ref|NP_008281.1|ATP6_10704	ATP synthase F0 subunit 6 [D (224)	321	88.9	1.2e-18	0.375	0.710	224	align
sp|P00852|ATP6_EMENI	ATP synthase a chain precursor (AT (256)	266	75.1	1.9e-14	0.304	0.691	230	align
sp|P14862|ATP6_COCHE	ATP synthase a chain (ATPase prote (257)	221	63.8	4.7e-11	0.313	0.650	214	align
sp|P68526|ATP6_TRITI	ATP synthase a chain (ATPase prote (386)	204	59.5	1.5e-09	0.289	0.651	235	align
sp|P05499|ATP6_TOBAC	ATP synthase a chain (ATPase prote (395)	185	54.7	4e-08	0.283	0.635	233	align
sp|P07925|ATP6_MAIZE	ATP synthase a chain (ATPase prote (291)	182	54.0	4.7e-08	0.311	0.667	180	align
sp|P0AB98|ATP6_ECOLI	ATP synthase a chain (ATPase prote (271)	166	50.1	7e-07	0.233	0.585	236	align
sp|P15993|AROP_ECOLI	Aromatic amino acid transport prot (457)	103	34.2	0.072	0.234	0.622	111	align
sp|P27178|ATP6_SYNY3	ATP synthase a chain (ATPase prote (276)	92	31.5	0.27	0.265	0.571	170	align
sp|P00329|ADH1_MOUSE	Alcohol dehydrogenase 1 (Alcohol d (375)	89	30.7	0.64	0.344	0.607	61	align
sp|P06757|ADH1_RAT	Alcohol dehydrogenase 1 (Alcohol deh (376)	85	29.7	1.3	0.339	0.629	62	align
sp|P00161|CYB_EMENI	Cytochrome b (387)	83	29.2	1.9	0.308	0.593	91	align
sp|P29631|CYB_POMTE	Cytochrome b (308)	81	28.8	2	0.274	0.584	113	align
sp|P00328|ADH1S_HORSE	Alcohol dehydrogenase S chain (374)	82	29.0	2.2	0.328	0.590	61	align
sp|P00327|ADH1E_HORSE	Alcohol dehydrogenase E chain (375)	82	29.0	2.2	0.328	0.590	61	align
sp|P11599|HLYB_PROVU	Alpha-hemolysin translocation ATP- (707)	86	29.8	2.3	0.277	0.625	112	align
sp|P03880|ANI1_EMENI	Intron-encoded DNA endonuclease I- (488)	83	29.1	2.5	0.389	0.630	54	align
sp|P07327|ADH1A_HUMAN	Alcohol dehydrogenase 1A (Alcohol (375)	79	28.2	3.6	0.265	0.556	117	align
sp|P41680|ADH1_PERMA	Alcohol dehydrogenase 1 (Alcohol d (375)	79	28.2	3.6	0.241	0.583	108	align
sp|P24956|CYB_EQUGR	Cytochrome b (379)	79	28.2	3.7	0.315	0.576	92	align
sp|P10724|ALR_BACST	Alanine racemase (388)	79	28.2	3.8	0.233	0.535	86	align
sp|P03046|CIM_BPMU	Cim protein (Kil protein) (74)	66	25.4	5.1	0.208	0.623	53	align
sp|P72588|DNLJ_SYNY3	DNA ligase (Polydeoxyribonucleotid (669)	81	28.6	5.1	0.250	0.570	128	align

Significance Thresholds

```
>>sp|P68526|ATP6_TRITI ATP synthase a chain (ATPase protein 6) g              (386 aa)
 initn: 108 initl:  70 opt: 121  Z-score: 165.5  bits: 38.9 E(13351): 0.0028
Smith-Waterman score: 209; 25.9% identity (60.3% similar) in 239 aa overlap (39-271:150-362)
Entrez Lookup  Re-search database  General re-search
                  10        20        30        40        50        60        70
gi|811   MASENMTPQDYIGHHLNNLQLDLRTFSLVDPQNPPATFWTINIDSMFFSVVLGLLFLVLFRSVAKKATSGVPGKFQTA
                                                .: ..  :...::: ..:: .:  . : . ..::. .:.
sp|P68 AGGGMDNFIQNLPGAYPETPLDQFAIIPIIDLHVGNFYLSFTNEVLYMLltvvl-vvfl-ffvvtkkgggkSVPNAWQSL
     110       120       130       140       150       160       170       180

       80        90              100       110       120       130       140       150
gi|811 IELVIGFVNGSVKDMYHG-----KSKLIAPLALTIFVWVFLMNLMDLLPIDLLPYIAEHVLGLPALRVVPSADVNVTLSM
       .::.  :: . :...  :     :.:..  ...: :.. .. : . ..:...   .. : :               .::..
sp|P68 VELIYDFVLNLVNEQIGGLSGNVKQKFFPRISVT-FTFSLFRNPQGMIPFSFT--VTSHFL--------------ITLAL
       190       200       210       220       230       240                       250

           160       170       180       190       200       210       220       230
gi|811 ALGVFILILFYSIKMKGIGGFTKELTLQPFNHWAFIPVNLILEGVSLLSKPVSLGLRLFGNMYAGELIFILIAGLLPWWS
       ....:: : . ... .:.  :.  . :       . :  ..:: .:   . .:::.:::.::.::. .  ...:.   :.
sp|P68 SFSIFIGITIVGFQRHGLHFFS--fllpagvplplapflvllelISYCFRALSLGIRLFANMMAGHSLVKILSGFA--WT
              260       270       280       290       300       310       320

           240       250       260       270
gi|811 QWILNVPWAIFHILIITLQAFIFMVLTIVYLSMA-SEEH
       . .::    ::...      :: ..:: . :..: :. :
sp|P68 MLFLN---NIFYFIGDLGPLFIVLALTGLELGVAISQAHVSTISICIYLNDATNLHQNESFHN
        330           340       350       360       370       380
```

Unrelated or Too Distance

The best scores are:		opt	bits	E(13351)	%_id	%_sim	alen	
sp|P0AB98|ATP6_ECOLI ATP synthase a chain (ATPase prote	(271)	1650	428.4	1.1e-120	1.000	1.000	271	align
sp|P06451|ATPI_SPIOL Chloroplast ATP synthase a chain p	(247)	161	49.1	1.5e-06	0.270	0.616	211	align
sp|P06289|ATPI_MARPO Chloroplast ATP synthase a chain p	(248)	161	49.1	1.5e-06	0.261	0.621	211	align
sp|P06452|ATPI_PEA Chloroplast ATP synthase a chain pre	(247)	158	48.3	2.6e-06	0.274	0.614	223	align
sp|P69371|ATPI_ATRBE Chloroplast ATP synthase a chain p	(247)	156	47.8	3.7e-06	0.270	0.607	211	align
sp|P00848|ATP6_MOUSE ATP synthase a chain (ATPase prote	(226)	149	46.0	1.2e-05	0.259	0.617	193	align
sp|P00846|ATP6_HUMAN ATP synthase a chain (ATPase prote	(226)	148	45.7	1.4e-05	0.237	0.589	236	align
sp|P30391|ATPI_EUGGR Chloroplast ATP synthase a chain p	(251)	139	43.4	7.6e-05	0.298	0.596	225	align
sp|P00847|ATP6_BOVIN ATP synthase a chain (ATPase prote	(226)	138	43.2	8.1e-05	0.233	0.581	236	align
sp|P0C2Y5|ATPI_ORYSA Chloroplast ATP synthase a chain p	(247)	132	41.7	0.00026	0.259	0.603	239	align
sp|P68526|ATP6_TRITI ATP synthase a chain (ATPase prote	(386)	121	38.9	0.0028	0.259	0.603	239	align
sp|P27178|ATP6_SYNY3 ATP synthase a chain (ATPase prote	(276)	116	37.6	0.0048	0.264	0.578	258	align
sp|P00854|ATP6_YEAST ATP synthase a chain precursor (AT	(259)	113	36.8	0.0077	0.235	0.578	277	align
sp|P08444|ATP6_SYNP6 ATP synthase a chain (ATPase prote	(261)	113	36.8	0.0077	0.267	0.600	240	align
sp|P00852|ATP6_EMENI ATP synthase a chain precursor (AT	(256)	111	36.3	0.011	0.209	0.590	244	align
sp|P07925|ATP6_MAIZE ATP synthase a chain (ATPase prote	(291)	109	35.8	0.017	0.259	0.578	232	align
sp|P00851|ATP6_DROYA ATP synthase a chain (ATPase prote	(224)	98	33.0	0.094	0.225	0.549	253	align
sp|P14862|ATP6_COCHE ATP synthase a chain (ATPase prote	(257)	91	31.2	0.37	0.204	0.608	265	align
ref|NP_008281.1|ATP6_10704 ATP synthase F0 subunit 6 [D	(224)	90	31.0	0.39	0.230	0.576	165	align
sp|P09716|US17_HCMVA Hypothetical protein HVLF1	(293)	91	31.2	0.42	0.260	0.565	131	align
sp|P12446|MAT_INCJJ Polyprotein p42 [Contains: Protein	(374)	85	29.7	1.5	0.247	0.559	93	align
sp|P00849|ATP6_XENLA ATP synthase a chain (ATPase prote	(226)	79	28.2	2.7	0.261	0.630	165	align
sp|P06974|FLIM_ECOLI Flagellar motor switch protein fli	(334)	81	28.7	2.8	0.308	0.673	52	align
sp|P05499|ATP6_TOBAC ATP synthase a chain (ATPase prote	(395)	81	28.7	3.3	0.220	0.582	268	align

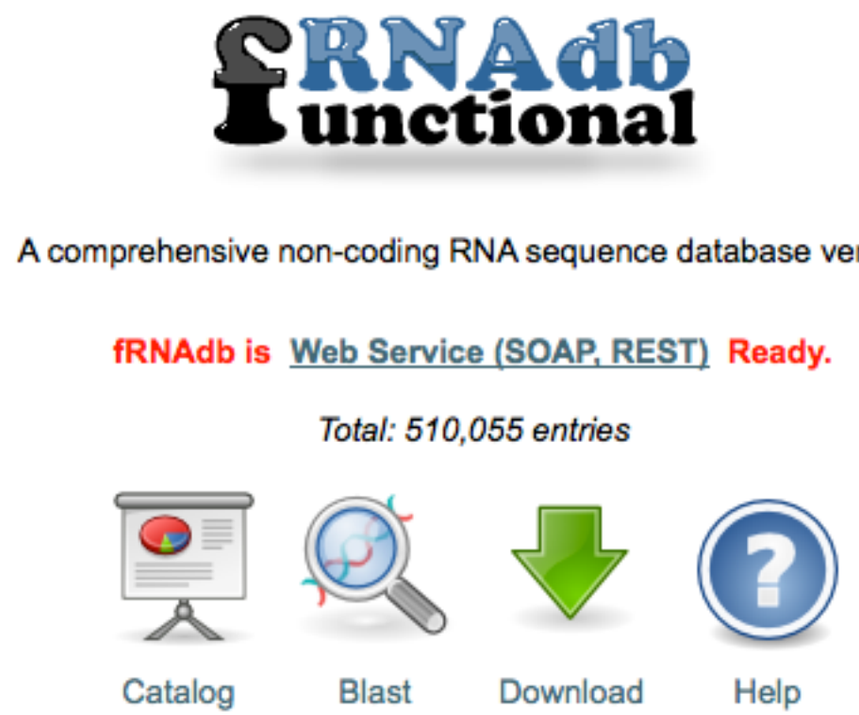
Search Algorithms

Algorithm	Value Calculated	Scoring Matrix	Gap penalty	Time Requirement	Reference
Needleman-Wunsch	Global similarity	Any	Penalty/Gap	O(n2)	Needleman and Wunsch, 1970
Sellers	Global distance	Unity	Penalty/Gap	O(n2)	Sellers, 1974
Smith-Waterman	Local Similarity	Sij < 0.0	Affine (q+rk)	O(n2)	Smith and Waterman, 1981 Gotoh, 1982
SRCHN	Approx. local similarity	diagonal	Penalty/Gap	O(n) – O(n2)	Wilbur and Lipman, 1983
FASTP/FASTA	Approx. local similarity	Sij < 0.0	Limit Size (q+rk)	O(n2)/K	Lipman and Pearson, 1985, Pearson and Lipman, 1988
BLAST	Maximum Segment Score	Sij < 0.0	Multiple Segment	O(n2)/K	Altschul et al 1990
BLAST2.0	Approx. local similarity	Sij < 0.0	(q+rk)	O(n2)/K	Altschul et al 1997

Proteins Sequences Are Better for Comparing Divergent or Not Well Conserved Genes.

UT Southwestern
Medical Center
Lyda Hill Department of Bioinformatics

DNA vs Protein Comparison



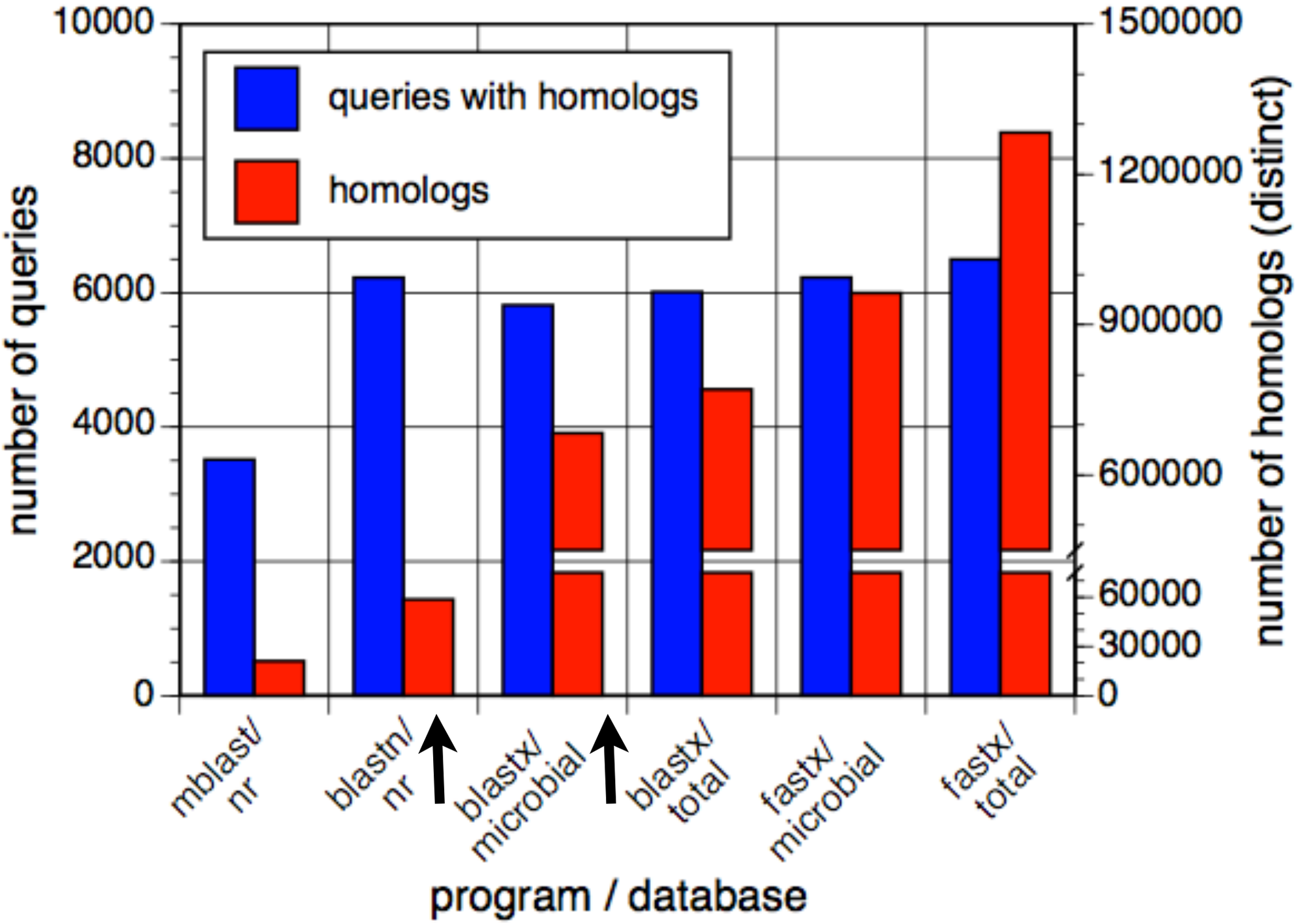
ncRNAs

Population Genetics

DNA vs Protein 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

BlastX vs BlastN



Tips for Improving Results

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

What question to ask?

Is there an homologous protein?

- Does that homologous protein have a similar domain?
- 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 the same function/ modification/antigenic site?

What program do I use?

- What is your query sequence?
  - protein: BLAST (NCBI), SSEARCH (EBI)
  - DNA vs Protein: 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
- Is Sequence X homologous to Y?
  - BL2SEQ (NCBI), LALIGN, PRSS
- Does my protein contain repeated domains?
  - LALIGN

Sequence Alignment Via the Web

BLAST® Home Rec

BLAST finds regions of similarity between biological sequences. more...

BLAST Assembled Genomes

Find Genomic BLAST pages:

Enter organism name or id--completions will be suggested GO

- Human
- Mouse
- Rat
- Cow
- Pig
- Dog
- Rabbit
- Chimp
- Guinea pig
- Fruit fly
- Honey bee
- Chicken
- Zebrafish
- Clawed frog
- *Arabidopsis*
- Rice
- Yeast
- Microbes

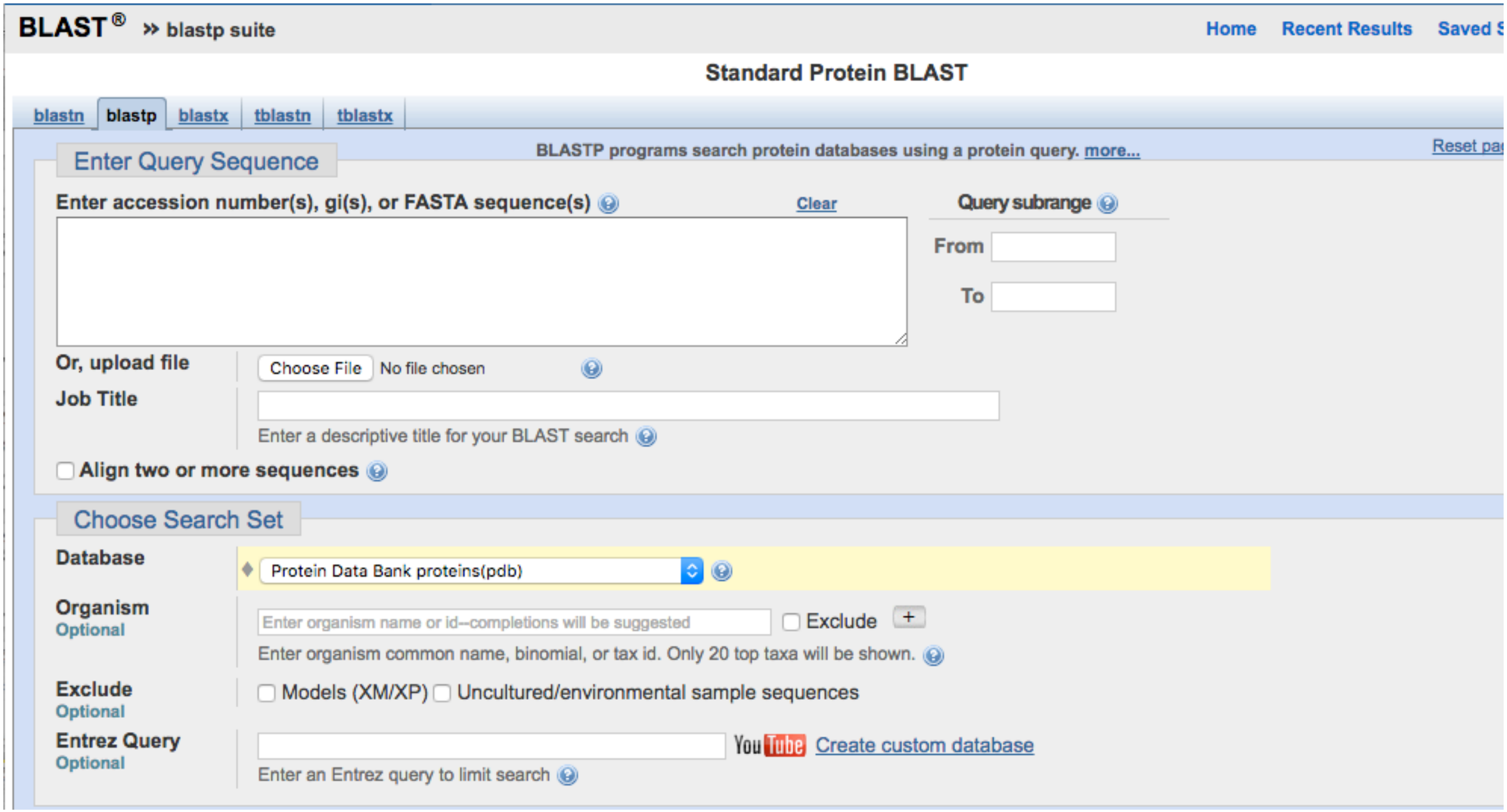
Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast, delta-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

http://blast.ncbi.nlm.nih.gov/

Sequence Alignment Via the Web



http://blast.ncbi.nlm.nih.gov

Sequence Alignment Via the Web

FASTA

FASTA

FASTA is another commonly used sequence similarity search tool which uses heuristics for fast **local** alignment searching.

Protein Nucleotide Genomes Whole Genome Shotgun

SSEARCH

SSEARCH is an optimal (as opposed to heuristics-based) **local** alignment search tool using the Smith-Waterman algorithm. Optimal searches guarantee you find the best alignment score for your given parameters.

Protein Nucleotide Genomes Whole Genome Shotgun

PSI-Search

PSI-Search combines the sensitivity of the Smith-Waterman search algorithm (SSEARCH) with the PSI-BLAST profile construction strategy to find distantly related protein sequences.

Protein

GGSEARCH

GGSEARCH performs optimal **global-global** alignment searches using the Needleman-Wunsch algorithm.

Protein Nucleotide

BLAST

NCBI BLAST

NCBI BLAST is the most commonly used sequence similarity search tool. It uses heuristics to perform fast **local** alignment searches.

Protein Nucleotide Vectors

PSI-BLAST

PSI-BLAST allows users to construct and perform a BLAST search with a custom, position-specific, scoring matrix which can help find distant evolutionary relationships. PHI-BLAST functionality is also available to restrict results using patterns.

Protein

http://www.ebi.ac.uk/Tools/sss/

Sequence Alignment Via the Web

STEP 1 - Select your databases

PROTEIN DATABASES

1 Databank Selected — X Clear Selection

- UniProt Knowledgebase
- UniProtKB/Swiss-Prot
- UniProtKB/Swiss-Prot isoforms
- UniProtKB/TrEMBL
- **UniProtKB Taxonomic Subsets**
- **UniProt Clusters**
- **Patents**
- **Structure**
- **Other Protein Databases**
  - UniProt Archive
  - IntAct
  - IMGT/HLA
  - IPD-KIR
  - IPD-MHC
  - MACiE Annot Pub

STEP 2 - Enter your input sequence

Enter or paste a PROTEIN sequence in any supported format:

http://www.ebi.ac.uk/Tools/sss/

or Upload a file: Choose File No file chosen

STEP 3 - Set your parameters

PROGRAM

FASTA

Sequence Alignment Via the Web

UVa FASTA Server

New: Annotation features available for SwissProt/PIR1 library searches.

About

- Getting started
- fasta_guide.pdf

Other FASTA Servers

- EMBL-EBI
- KEGG (Japan)

References

- FASTA
- FASTX/FASTY
- Statistics
- FASTS/FASTF

Software

- FASTA v36 ChangeLog
- Downloads
- Sequence Libraries
- Developer Mailing list

Other resources

- CHAPS - Convert HMMs and Profiles
- Near optimal alignments
- FASTA Exercises
- NCBI BLAST server
- EMBL-EBI Server

The **FASTA** programs find regions of local or global similarity between Protein or DNA sequences, either by searching Protein or DNA databases, or by identifying local duplications within a sequence. Other programs provide information on the statistical significance of an alignment. Like **BLAST**, **FASTA** can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Protein

- Protein-protein **FASTA**
- Protein-protein Smith-Waterman (**ssearch**)
- Global Protein-protein (Needleman-Wunsch) (**ggsearch**)
- Global/Local protein-protein (**glsearch**)
- Protein-protein with unordered peptides (**fasts**)
- Protein-protein with mixed peptide sequences (**fastf**)

Nucleotide

- Nucleotide-Nucleotide (DNA/RNA **fasta**)
- Ordered Nucleotides vs Nucleotide (**fastm**)
- Un-ordered Nucleotides vs Nucleotide (**fasts**)

fasta.bioch.virginia.edu

Translated

- Translated DNA (with frameshifts, e.g. ESTs) vs Proteins (**fastx/fasty**)
- Protein vs Translated DNA (with frameshifts) (**tfastx/tfasty**)
- Peptides vs Translated DNA (**tfasts**)

Statistical Significance

- Protein vs Protein shuffle (**prss**)
- DNA vs DNA shuffle (**prss**)
- Translated DNA vs Protein shuffle (**prfx**)

Local Duplications

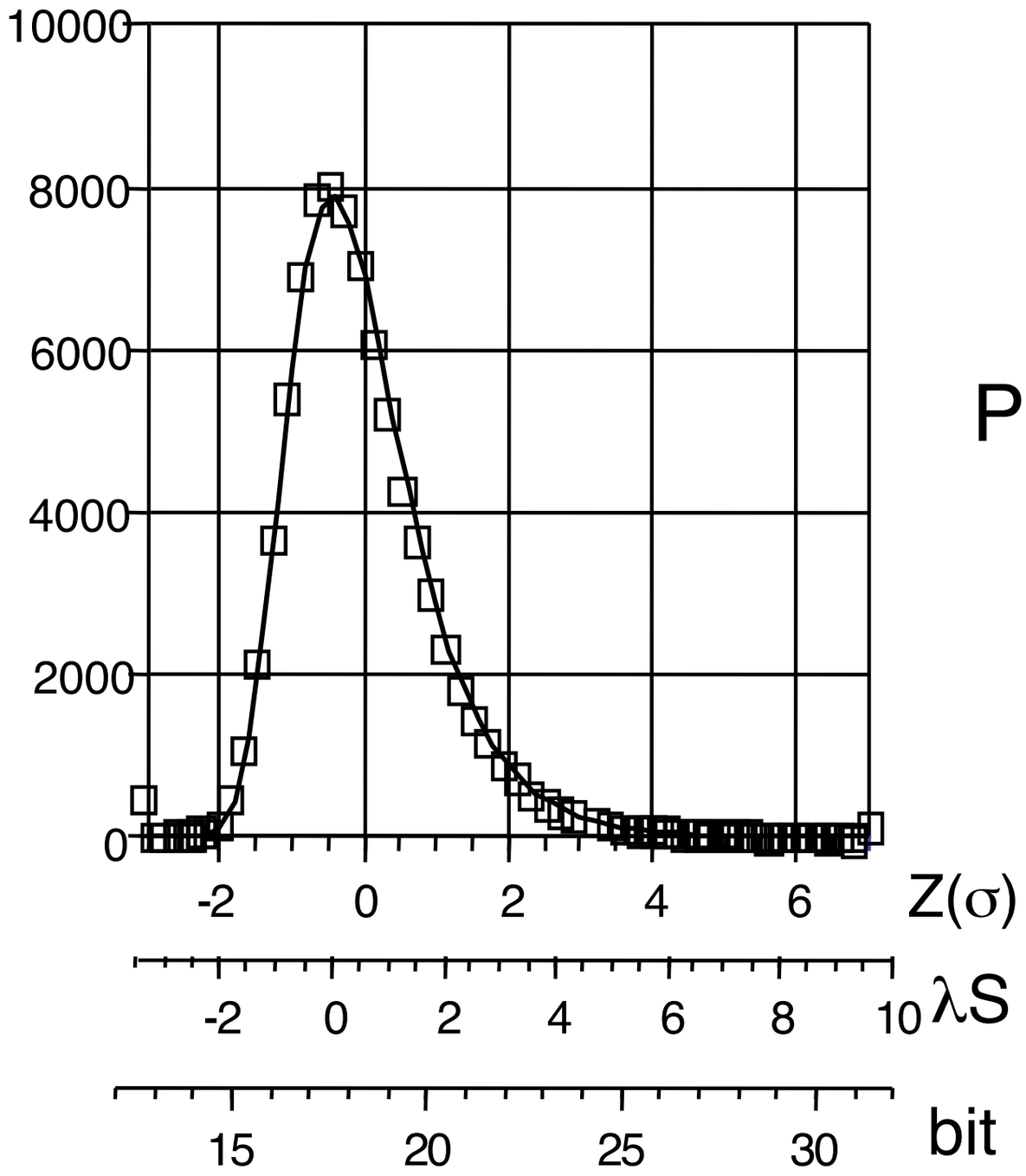
- Local Protein alignments (**lalign**)
- Plot Protein alignment "dot-plot" (**plalign**)
- Local DNA alignments (**lalign**)
- Plot DNA alignment "dot-plot" (**plalign**)

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)

DB Size Matters
Smaller is Better



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

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

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

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

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

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

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

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

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

DB Size Matters
Smaller is Better

```
gi|114443|sp|P00846.1|ATP6_HUMAN ATP synthase subunit a; F-ATPase - 226 aa
                                                  vs
gi|16131606|ref|NP_418194.1| F0 sector of membrane-bound ATP synthase, subunit a [Escherichia
coli str. K-12 subst - 271 aa

 initn: 159 init1: 104 opt: 148  Z-score: 212.5  bits: 46.8
Smith-Waterman score: 178; 23.7% identity (58.9% similar) in 236 aa overlap (45-264:8-222)
```

Database	Entries	Length	E()	Time (s)
E.Coli	4237	1350094	3.8E-07	<0.5
Human Ref	38000	17401176	1.9E-05	1
SwissProt	445410	165796297	0.0015	10
RefSeq	711441	261324908	NS	16

How Can I Choose my DB?

BLAST® » blastp suite

Home Recent Results Saved S

Standard Protein BLAST

blastn | blastp | blastx | tblastn | tblastx

Enter Query Sequence

BLASTP programs search protein databases using a protein query. more... Reset pag

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

Query subrange

From

To

Or, upload file Choose File No file chosen

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database: Protein Data Bank proteins(pdb)

Organism Optional: Enter organism name or id--completions will be suggested Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude Optional: Models (XM/XP) Uncultured/environmental sample sequences

Entrez Query Optional: YouTube Create custom database

Enter an Entrez query to limit search

How can you tell what is the Highest Scoring Unrelated Hit?

```
Query: TMP.q
  1>>>gi|28200469|gb|AAO31759.1| endo-b1,4-mannanase 5A [Cellvibrio  - 430 aa
Library: Swissprot (NCBI)
  165796297 residues in 445410 sequences

Statistics:  Expectation_n fit: rho(ln(x))= 7.6630+/-0.000201; mu= 3.3292+/- 0.012
 mean_var=63.4892+/-13.027, 0's: 51 Z-trim(131.3): 79  B-trim: 0 in 0/68
 Lambda= 0.160962
 statistics sampled from 60000 (180148) to 445316 sequences
Algorithm: Smith-Waterman (SSE2, Michael Farrar 2006) (7.2 Nov 2010)
Parameters: BL50 matrix (15:-5)xS, open/ext: -10/-2
 Scan time: 29.700
```

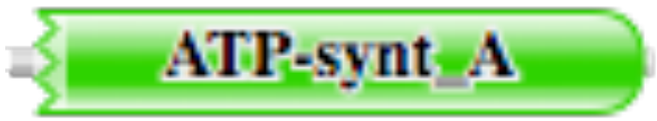
The best scores are:				s-w bits	E(445410)	%_id	%_sim	alen	
sp|P51529.2|MANA_STRLI	Mannan endo-1,4-beta-mannosidase	(383)	1225	291.3	1.5e-77	0.520	0.789	375	align
sp|P22533.2|MANB_CALSA	Beta-mannanase/endoglucanase A;	(1331)	896	214.5	7.1e-54	0.403	0.686	382	align
sp|P14768.2|XYNA_CELJU	Endo-1,4-beta-xylanase A; Xylan	(611)	226	59.1	1.9e-07	0.330	0.614	176	align
sp|P10476.2|GUNA_CELJU	Endoglucanase A; EGA; Cellulase	(962)	227	59.2	2.8e-07	0.350	0.657	137	align
sp|P27033.2|GUNC_CELJU	Endoglucanase C; Cellodextrinase	(747)	223	58.4	3.9e-07	0.286	0.636	206	align
sp|P18126.1|GUNB_CELJU	Endoglucanase B; EGB; Cellulase	(511)	201	53.4	8.3e-06	0.327	0.619	202	align
sp|O74706.1|EGLB_ASPNG	Endo-beta-1,4-glucanase B; Endo	(331)	190	51.0	2.9e-05	0.275	0.558	233	align
sp|Q12647.1|GUNB_NEOPA	Endoglucanase B; Cellulase B; En	(473)	183	49.2	0.00014	0.229	0.469	414	align
sp|Q96WQ8.1|EGLB_ASPKA	Probable endo-beta-1,4-glucanase	(332)	179	48.4	0.00017	0.278	0.543	234	align
sp|P23661.1|GUNB_RUMAL	Endoglucanase B; Cellulase B; En	(409)	166	45.3	0.0018	0.227	0.508	299	align
sp|P54937.1|GUNA_CLOLO	Endoglucanase A; Cellulase A; En	(517)	166	45.3	0.0024	0.209	0.520	406	align

Perform a search with your "suspect"

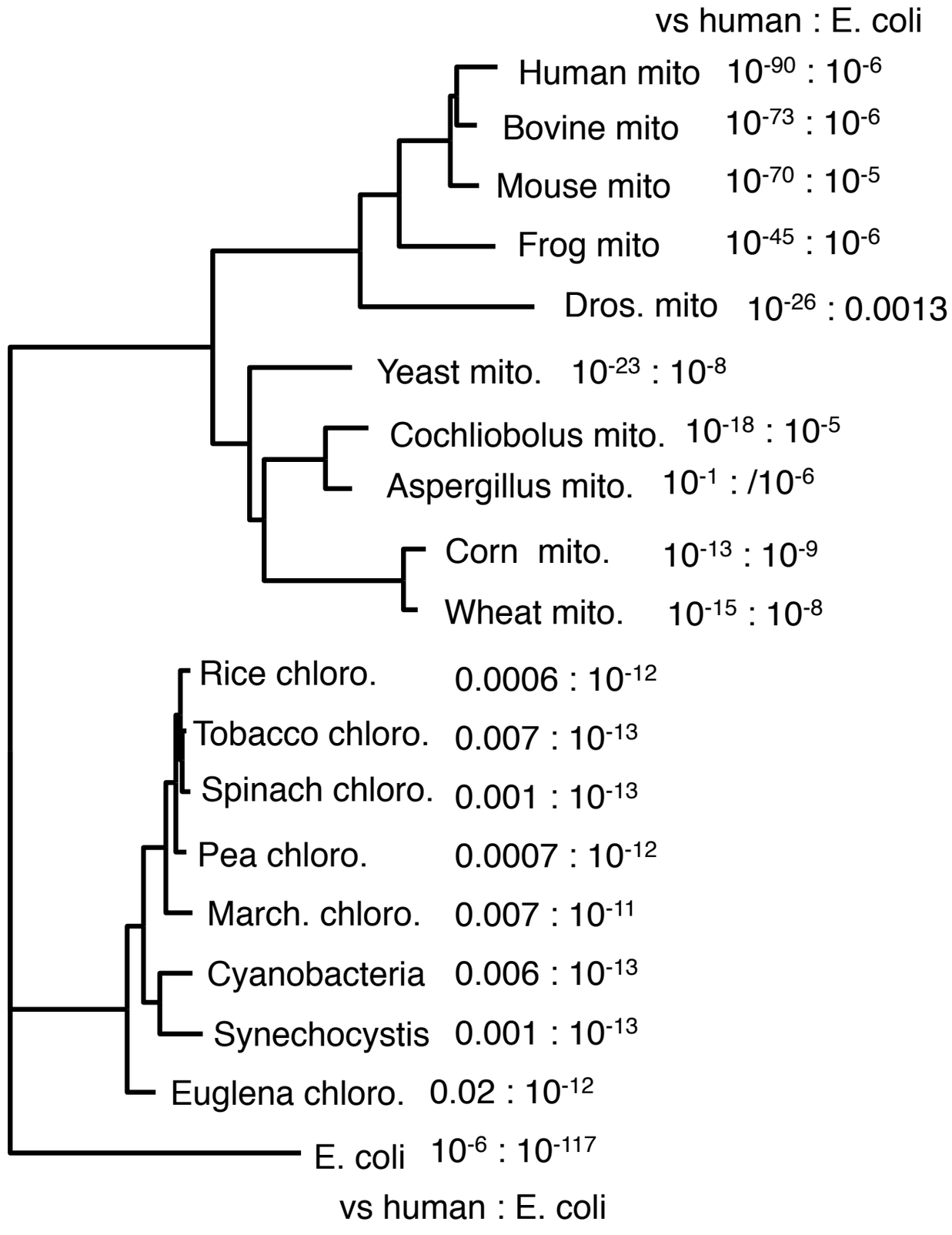
The best scores are:										s-w bits	E(445410)	%_id	%_sim	alen	
sp|P23661.1|GUNB_RUMAL	Endoglucanase B; Cellulase B; En	(	409)	2549	597.9	7.6e-170	1.000	1.000	409	align
sp|P16216.1|GUN1_RUMAL	Endoglucanase 1; Cellulase; Endo	(	406)	2186	513.7	1.7e-144	0.806	0.934	407	align
sp|P23660.1|GUNA_RUMAL	Endoglucanase A; Cellulase A; En	(	364)	992	236.7	3.7e-61	0.461	0.723	343	align
sp|P54937.1|GUNA_CLOLO	Endoglucanase A; Cellulase A; En	(	517)	984	234.7	2.1e-60	0.431	0.727	355	align
sp|Q12647.1|GUNB_NEOPA	Endoglucanase B; Cellulase B; En	(	473)	895	214.1	3.1e-54	0.433	0.693	342	align
sp|P10477.1|GUNE_CLOTM	Endoglucanase E; Cellulase E; En	(	814)	898	214.5	3.9e-54	0.368	0.679	408	align
sp|P28623.2|GUND_CLOC7	Endoglucanase D; Cellulase D; En	(	515)	894	213.8	4e-54	0.413	0.707	334	align
sp|P17901.1|GUNA_CLOCE	Endoglucanase A; Cellulase A; EG	(	475)	875	209.4	7.7e-53	0.403	0.679	380	align
sp|P20847.1|GUN1_BUTFI	Endoglucanase 1; Cellulase 1; En	(	547)	855	204.7	2.3e-51	0.389	0.664	378	align
sp|P28621.1|GUNB_CLOC7	Endoglucanase B; Cellulase B; En	(	440)	853	204.4	2.4e-51	0.388	0.703	340	align
sp|P23550.1|GUNB_PAELA	Endoglucanase B; Cellulase B; En	(	566)	601	145.8	1.3e-33	0.314	0.638	354	align
sp|P25472.1|GUND_CLOCE	Endoglucanase D; Cellulase D; EG	(	584)	570	138.6	2e-31	0.334	0.638	329	align
sp|O08342.1|GUNA_PAEBA	Endoglucanase A; Cellulase A; En	(	400)	538	131.3	2.1e-29	0.303	0.612	356	align
sp|P16218.1|GUNH_CLOTH	Endoglucanase H; Cellulase H; En	(	900)	507	123.8	9e-27	0.317	0.609	363	align
sp|P19570.1|GUN3_BACS4	Endoglucanase C; Cellulase C; En	(	825)	208	54.4	6.2e-06	0.217	0.506	397	align
sp|Q04469.1|GUN1_CRYFL	Endoglucanase 1; Carboxymethyl-c	(	341)	185	49.5	7.8e-05	0.232	0.547	254	align
sp|P07982.1|GUN2_TRIRE	Endoglucanase EG-II; EGLII; Cel	(	418)	185	49.4	0.0001	0.224	0.568	340	align
sp|Q2UPQ4.1|EGLB_ASPOR	Probable endo-beta-1,4-glucanase	(	333)	181	48.6	0.00014	0.209	0.538	273	align
sp|P06564.1|GUN_BACS1	Endoglucanase; Alkaline cellulase	(	800)	188	49.8	0.00015	0.256	0.555	211	align
sp|P19424.1|GUN_BACS6	Endoglucanase; Alkaline cellulase	(	941)	186	49.3	0.00025	0.263	0.577	194	align
sp|P54583.1|GUN1_ACIC1	Endoglucanase E1; Cellulase E1;	(	562)	176	47.2	0.00064	0.251	0.498	307	align

Is a hit from your original search in the re-search?

Homology through Transitivity



How do you pick the right sequence homologous to both?



Unrelated ≠ Random

low complexity sequence

The best scores are:			s-w bits	E(13351)	% id	% sim	alen	
sp|P17343|GBB1_CAEEL	Guanine nucleotide-binding protein	(340)	251	45.2	8.4e-05	0.227	0.531	277	align
sp|P16520|GBB3_HUMAN	Guanine nucleotide-binding protein	(340)	250	45.0	9.2e-05	0.236	0.528	288	align
sp|P26308|GBB1_DROME	Guanine nucleotide-binding protein	(340)	249	44.9	0.0001	0.219	0.559	288	align
sp|P62871|GBB1_BOVIN	Guanine nucleotide-binding protein	(340)	248	44.8	0.00011	0.243	0.558	267	align
sp|P29387|GBB4_MOUSE	Guanine nucleotide-binding protein	(340)	241	43.8	0.00022	0.234	0.543	265	align
sp|P11017|GBB2_BOVIN	Guanine nucleotide-binding protein	(326)	240	43.7	0.00023	0.230	0.543	265	align
sp|P04280|PRP1_HUMAN	Basic salivary proline-rich protei	(392)	242	43.9	0.00023	0.268	0.423	291	align
sp|P62879|GBB2_HUMAN	Guanine nucleotide-binding protein	(340)	240	43.7	0.00024	0.230	0.543	265	align
sp|P04258|CO3A1_BOVIN	Collagen alpha-1(III) chain	(1049)	246	44.4	0.00044	0.288	0.454	302	
+-			197	37.7	0.046	0.267	0.470	285	
+-			182	35.6	0.19	0.246	0.460	313	align
sp|P29829|GBB2_DROME	Guanine nucleotide-binding protein	(346)	232	42.6	0.00052	0.233	0.574	258	align
sp|P04474|PRP3_RAT	Acidic proline-rich protein PRP33 pr	(206)	224	41.5	0.00064	0.300	0.511	190	align
sp|P23232|GBB_LOLFO	Guanine nucleotide-binding protein	(341)	220	40.9	0.0016	0.215	0.548	279	align
ref|NP_203699.1|	alpha 5 type IV collagen isoform 2, pr	(1691)	225	41.5	0.0054	0.256	0.445	308	
+-			208	39.1	0.027	0.256	0.465	301	
+-			202	38.3	0.048	0.280	0.467	321	
+-			183	35.7	0.29	0.251	0.438	347	align

Filter Low Complexity (SEG)

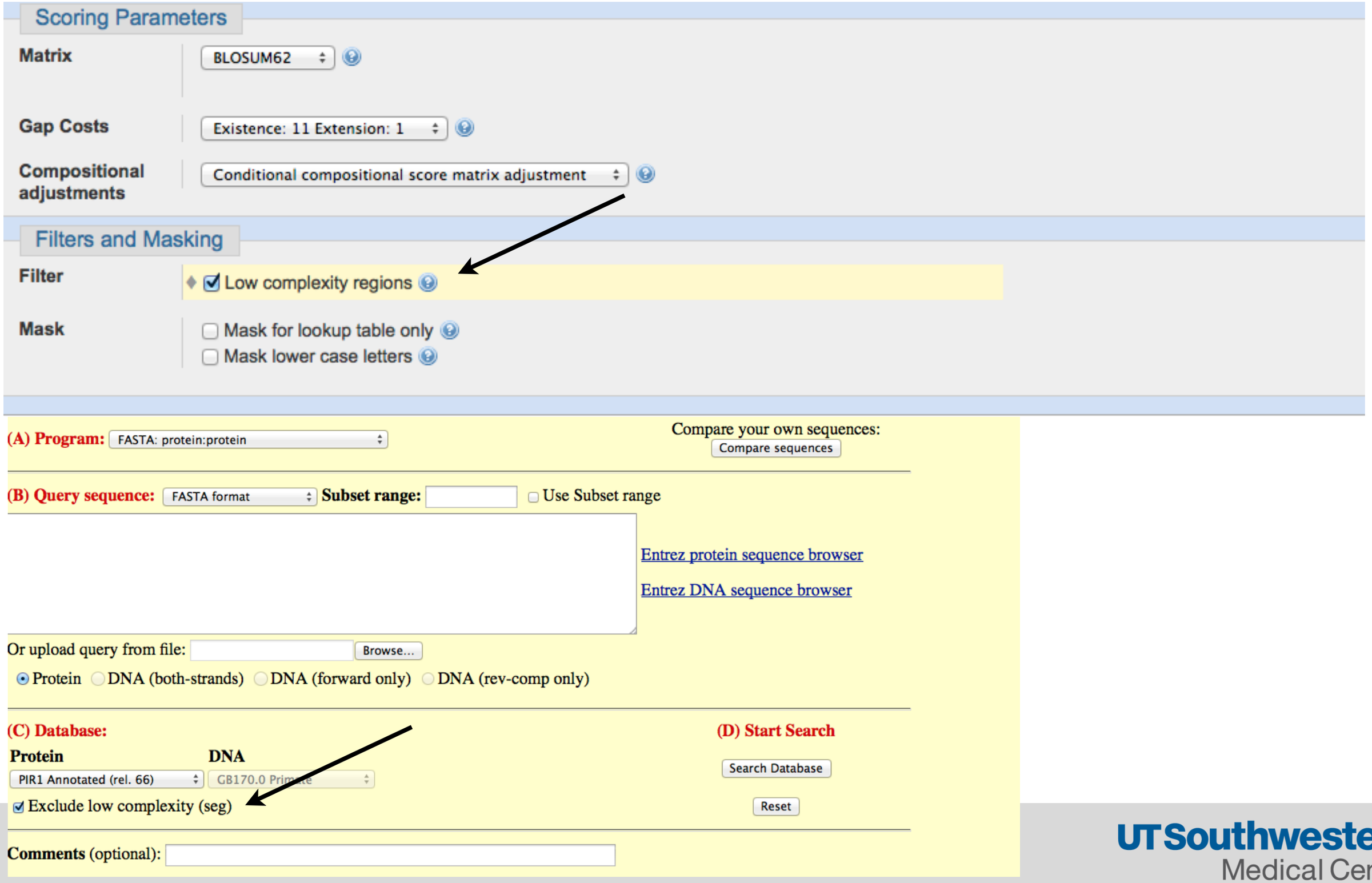
sp|P62871|GBB1_BOVIN	Guanine nucleotide-binding protein	(340)	225	52.9	4e-07	0.243	0.558	267	align
sp|P23232|GBB_LOLFO	Guanine nucleotide-binding protein	(341)	220	51.9	8.1e-07	0.215	0.548	279	align
sp|P13712|MSI1_YEAST	Chromatin assembly factor 1 subuni	(422)	147	37.2	0.026	0.207	0.515	309	align
sp|P53622|COPA_YEAST	Coatomer subunit alpha (Alpha-coat	(1201)	142	35.8	0.2	0.201	0.479	234	align
sp|P11269|GAG_MLVRD	Gag polyprotein (Core polyprotein)	(537)	134	34.5	0.22	0.252	0.482	226	align
sp|P29674|LHX1_XENLA	LIM/homeobox protein Lhx1 (LIM hom	(403)	129	33.6	0.3	0.299	0.538	117	align
sp|P09256|VGLC_VZVD	Glycoprotein GPV	(560)	132	34.1	0.3	0.248	0.482	141	align
sp|O13528|YA11A_YEAST	Transposon Ty1-A/Ty1-PR1 Gag poly	(440)	127	33.2	0.44	0.246	0.508	183	align
sp|P53621|COPA_HUMAN	Coatomer subunit alpha (Alpha-coat	(1224)	134	34.1	0.63	0.199	0.534	146	align

SEG Remove Low Complexity

```
>gi|122065196|sp|P16371.3|GROU_DROME Protein groucho; Enhancer of split m9/10 protein;  E(spl)m9/10

                                1-8     MYPSPVRH
                      paaggpppqgp       9-19
                               20-122   IKFTIADTLERIKEEFNFLQAQYHSIKLEC
                                        EKLSNEKTEMQRHYVMYYEMSYGLNVEMHK
                                        QTEIAKRLNTLINQLLPFLQADHQQQVLQA
                                        VERAKQVTMQELN
liighqqqhgiqqllqqihaqqvpggppqp  123-154
                             mg
                               155-292  ALNPFGALGATMGLPHGPQGLLNKPPEHHR
                                        PDIKPTGLEGPAAAEERLRNSVSPADREKY
                                        RTRSPLDIENDSKRRKDEKLQEDEGEKSDQ
                                        DLVVDVANEMESHSPRPNGEHVSMEVRDRE
                                        SLNGERLEKPSSSGIKQE
          rppsrsgssssrstps  293-308
                               309-321  LKTKDMEKPGTPG
        akartptpnaaapapgvnpk  322-341
      qmmpqgpppagypgapyqrpa  342-362
                               363-730  DPYQRPPSDPAYGRPPPMPYDPHAHVRTNG
                                        IPHPSALTGGKPAYSFHMNGEGSLQPVPFP
                                        PDALVGVGIPRHARQINTLSHGEVVCAVTI
                                        SNPTKYVYTGGKGCVKVWDISQPGNKNPVS
                                        QLDCLQRDNYIRSVKLLPDGRTLIVGGEAS
                                        NLSIWDLASPTPRIKAELTSAAPACYALAI
                                        SPDSKVCFSCCSDGNIAVWDLHNEILVRQF
                                        QGHTDGASCIDISPDGSRLWTGGLDNTVRS
                                        WDLREGRQLQQHDFSSQIFSLGYCPTGDWL
                                        AVGMENSHVEVLHASKPDKYQLHLHESCVL
                                        SLRFAACGKWFVSTGKDNLLNAWRTPYGAS
                                        IFQSKETSSVLSCDISTDDKYIVTGSGDKK
                                        ATVYEVIY
```

http://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=misc1&pgm=seg

SEG Remove Low Complexity



Validating Stats

- 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

Validating Stats

Choose: (A) program and (B, C) sequences to compare:

(A) Program: PRSS: protein:protein

(B) Number of shuffles: 200
Uniform Window

(B.1) Enter first (query) sequence: FASTA format **Subset range:**

Annotate Query Sequence (SwissProt accessions)
No annotation
Upload annotation file: Choose File No file chosen

Entrez protein / Entrez DNA sequence browser
Uniprot sequence browser

(B.2) Or upload sequence from file: Choose File No file chosen

Protein DNA (both-strands) DNA (forward only) DNA (rev-comp only) Use PSSM:

(C.1) Enter the second sequence: FASTA format **Subset range:**

Annotate Target Sequence (SwissProt accessions)
No annotation
Upload annotation file: Choose File No file chosen

Shuffle Sequence Reset Form

(C.2) Or choose file of sequences/accessions: Choose File No file chosen

Other comparison options:

Scoring matrix: open: ext: Ktup:
Blosum50 (25%) -10 -2 ktup = 2

Alignment Summary

- Compare Protein Sequences for long distances, DNA for close relationships.
- Sequence statistical significance estimates are accurate (verify this yourself)10-6 < E() < 10-3 is statistically significant
- Local sequence alignments find the best region (so that extending the region reduces the score). Global alignments go from end-to-end.
- The Smith-Waterman algorithm produces local alignments with affine gaps in time O(nm) and space O (n).
- BLAST and FASTA try to approximate Smith- Waterman scores for homologous sequences
- Smaller databases increase search sensitivity
- Statistical accuracy can be evaluated by examining the “highest scoring unrelated sequence” or by random shuffles

Workshop Time

https://bcantarel.github.io/cshl_homology_workshop1