Finding Function By Sequence Similarity

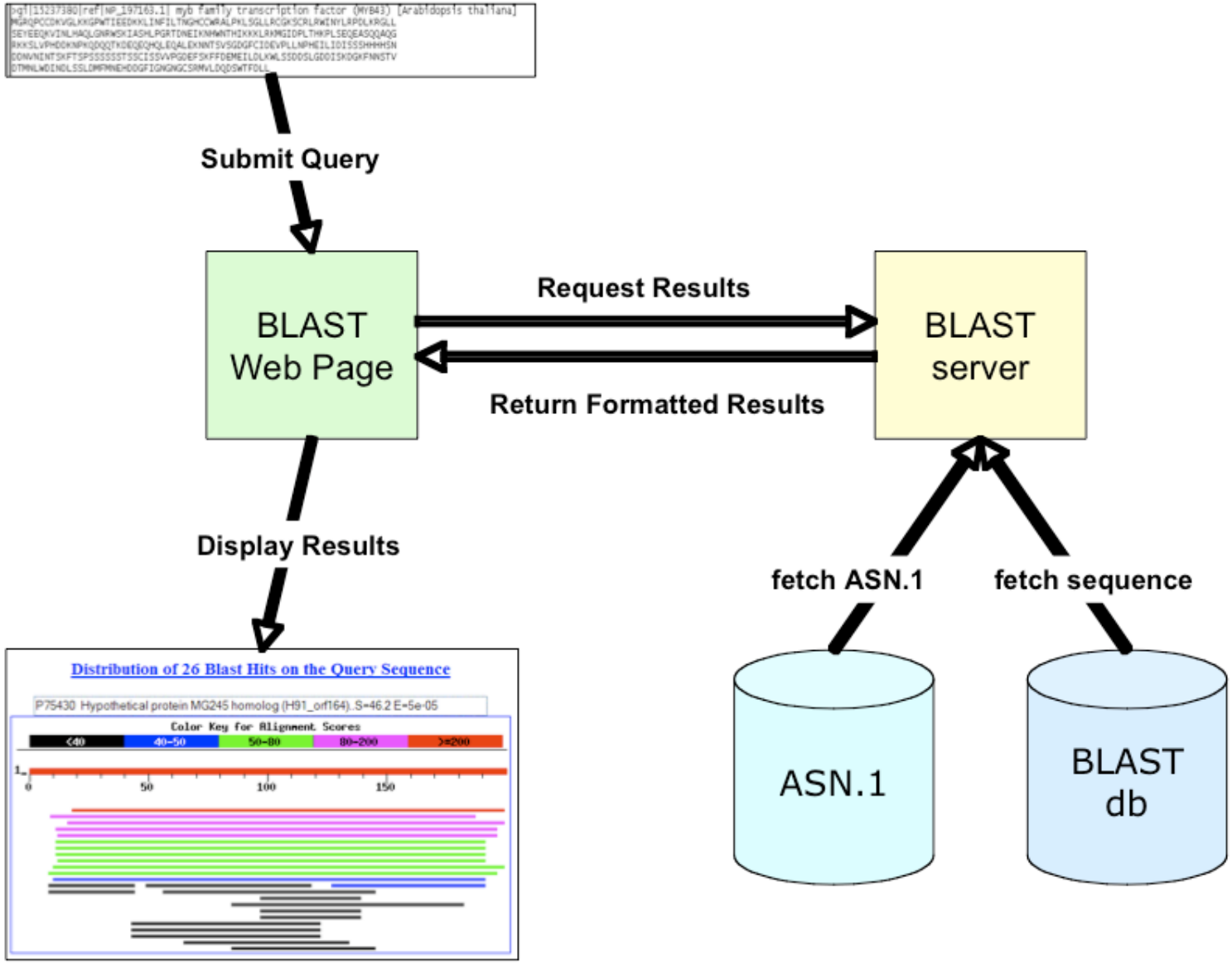
Concepts of Sequence Similarity Searching

- The major goal of sequence analysis is to predict the function and structure of genes and proteins from their sequence similarity.
- One sequence by itself is not informative
- Sequence must be analyzed by comparative methods against existing sequence databases to develop hypothesis concerning relatives and function.

BLAST algorithm

- **B**asic **L**ocal **A**lignment **S**earch **T**ools
- widely used sequence similarity search tool
- set of sequence comparison algorithms used to search sequence databases
- Finds best local alignments to a query
- Heuristic approach based on Smith Waterman algorithm
- Provides statistical significance
- www, standalone and network clients

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (**1990**) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.
Altschul SF, Madden TL, Schaeffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." NAR 25:3389-3402.


Submit Query
BLAST
Web Page
Request Results
Return Formatted Results
BLAST
server
Display Results
fetch ASN.1
fetch sequence
Distribution of 26 Blast Hits on the Query Sequence
P75430 Hypothetical protein MG245 homolog (H91_orf164)..S=46.2 E=5e-05
Color Key for Alignment Scores
<40
40-50
50-80
80-200
>=200
0
50
100
150
ASN.1
BLAST
db

What BLAST tells you ...

- Assumptions
  - Random sequences
  - Constant composition
- reports surprising alignments
  - Different than chance
- Conclusions
  - Surprising similarities imply evolutionary homology

Evolutionary Homology: having a descent from a common ancestor does not always imply similar function

BLAST programs

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
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

BLAST Algorithm

- Scoring of matches done using scoring matrices
- Sequences are split into words (default n=3)
  - Speed, computational efficiency
- BLAST algorithm extends the initial "seed" hit into an HSP
  - HSP = high scoring segment pair = Local optimal alignment

the statistics

- Discriminating between real and artifactual matches is done using an estimate of probability that the match might occur by chance.

- meaning of the scores (S) and e-values (E) that are associated with BLAST hits

score (S) ?

- The quality of each pair-wise alignment is represented as a score and the scores are ranked.
- Scoring matrices are used to calculate the score of the alignment base by base (DNA) or amino acid by amino acid (protein).
- The alignment score will be the sum of the scores for each position.

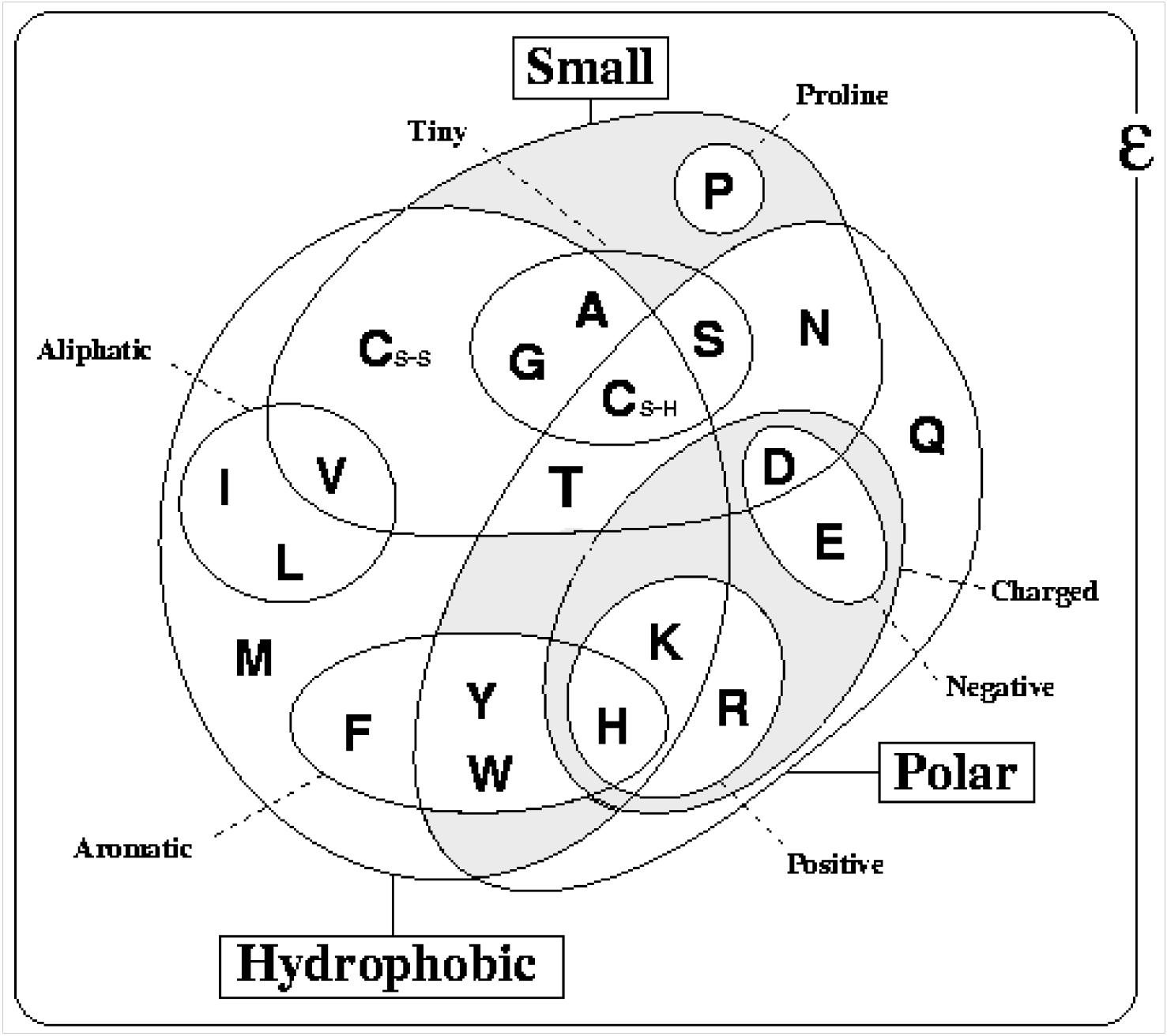
What's a scoring matrix?

- Substitution matrices are used for amino acid alignments.
  - each possible residue substitution is given a score
- A simpler unitary matrix is used for DNA pairs (+1 for match, -2 mismatch)

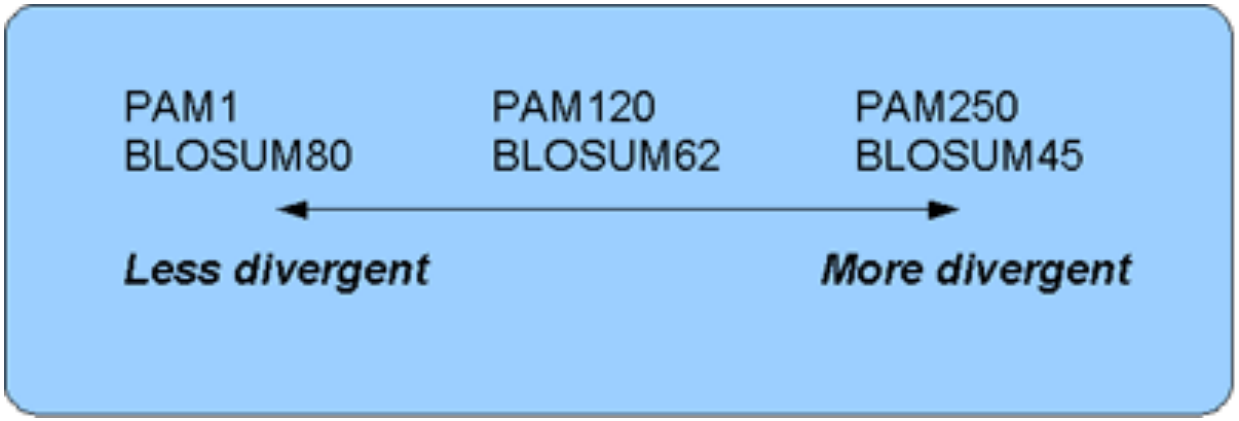
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

TABLE 27.3. DNA substitution matrix and some word matches

Query Sequence	Database Sequence A	C	G	T
A	1	0	0	0
C	0	1	0	0
G	0	0	1	0
T	0	0	0	1


Small
Proline
Tiny
ε
P
A
S
N
Aliphatic
Cs-s
G
Cs-H
Q
D
I
V
T
E
L
Charged
K
M
Negative
Y
R
F
H
W
Polar
Aromatic
Positive
Hydrophobic

BLOSUM vs PAM



- BLOSUM 62 is the default matrix in BLAST.
- This works well to identify moderately distant proteins, and performs well in detecting closer relationships.
- A search for distant relatives may be more sensitive with a different matrix.

Score and the e-value?

- The quality of the alignment is represented by the Score (S).
- The score of an alignment is calculated as the sum of substitution and gap scores. Substitution scores are given by a look-up table (PAM, BLOSUM) whereas gap scores are assigned empirically .
- The significance of each alignment is computed as an E value (E).
- Expectation value. The number of different alignments with scores equivalent to or better than S that are expected to occur in a database search by chance. The lower the E value, the more significant the score.

Notes on E-values

- Low E-values suggest that sequences are homologous
  - Can't show non-homology
- Statistical significance depends on both the size of the alignments and the size of the sequence database
  - Important consideration for comparing results across different searches
  - E-value increases as database gets bigger
  - E-value decreases as alignments get longer

Homology

- Similarity can be indicative of homology
  - if two sequences are significantly similar over entire length they are likely homologous
- Low complexity regions can be highly similar without being homologous
- Homologous sequences not always highly similar

BLAST Cutoffs

- nucleotide based searches
  - look for hits with E-values of 10^{-6} or less and sequence identity of 70% or more
- protein based searches
  - look for hits with E-values of 10^{-3} or less and sequence identity of 25% or more

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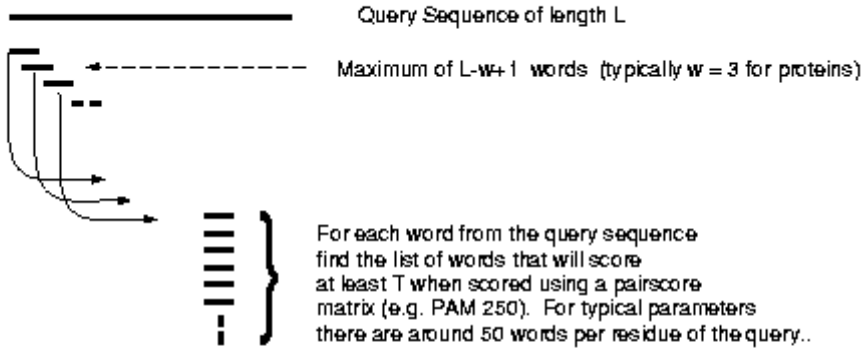
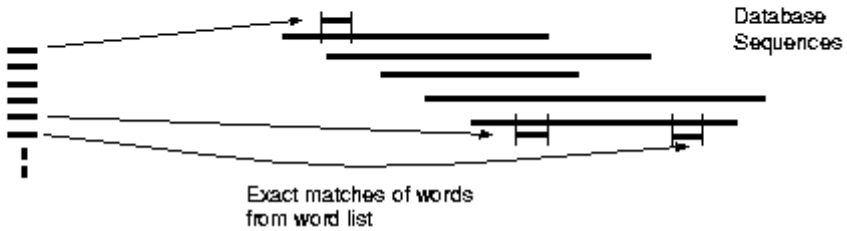
How does BLAST work?

BLAST Algorithm

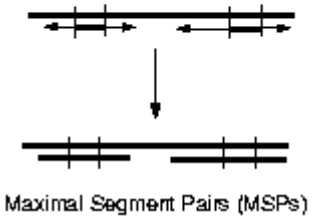
(1) For the query find the list of high scoring words of length w.



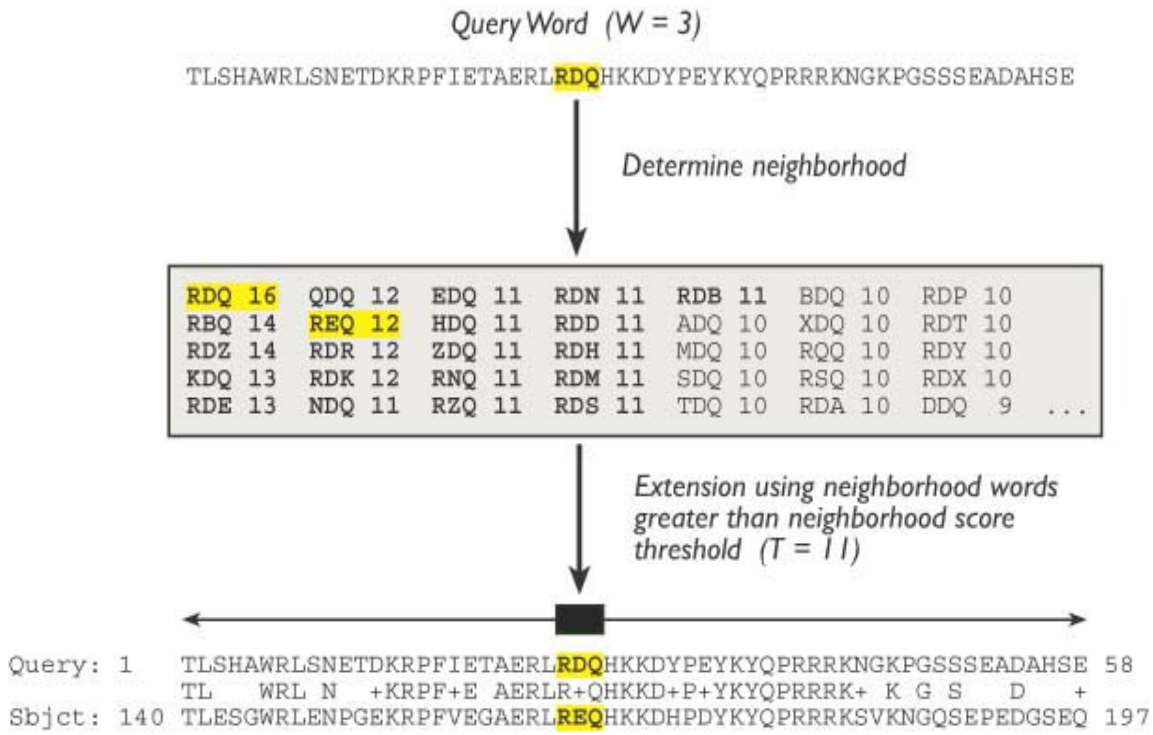
(2) Compare the word list to the database and identify exact matches.



(3) For each word match, extend alignment in both directions to find alignments that score greater than score threshold S.



BLAST Algorithm



```
>gb|AAL08419.1|  PTEN [Takifugu rubripes]
Length=412

 Score =  197 bits (501),  Expect = 2e-49, Method: Composition-based stats.
 Identities = 95/100 (95%), Positives = 98/100 (98%), Gaps = 0/100 (0%)

Query  2    IVSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHKNHYKI  61
            +VSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHKNHYKI
Sbjct  8    MVSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHKNHYKI  67

Query  62   YNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFKQN  101
            YNLCAERHYD AKFNCRVAQYPFEDHNPPQLELIKPF ++
Sbjct  68   YNLCAERHYDAAKFNCRVAQYPFEDHNPPQLELIKPFCED  107


 Score = 83.6 bits (205),  Expect = 4e-15, Method: Composition-based stats.
 Identities = 60/103 (58%), Positives = 68/103 (66%), Gaps = 32/103 (31%)

Query  99   KQNKMLKKDKMFHFWVNTFFIPGPEEV--------------------------------D  126
            KQNKM+KKDKMFHFWVNTFFIPGPEE                                  +
Sbjct  260  KQNKMMKKDKMFHFWVNTFFIPGPEESRDKLENGAVNNADSQQGVPAPGQGQPQSAECRE  319

Query  127  NDKEYLVLTLTkndldkankdkanRYFSPNFKVKLYFTKTVEE  169
            +D++YL+LTL+KND DKANKDKANRYFSPNFKVKL F+KTVEE
Sbjct  320  SDRDYLILTLSKNDRDKANKDKANRYFSPNFKVKLCFSKTVEE  362


>gb|AAH93110.1|  Ptenb protein [Danio rerio]
Length=289

 Score =  197 bits (500),  Expect = 2e-49, Method: Composition-based stats.
 Identities = 95/99 (95%), Positives = 98/99 (98%), Gaps = 0/99 (0%)

Query  3    VSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHKNHYKIY  62
            VSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHK+HYKIY
Sbjct  9    VSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKHKDHYKIY  68

Query  63   NLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFKQN  101
            NLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPF ++
Sbjct  69   NLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFCED  107
```

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- Additional slides

Extending the High Scoring Segment Pair (HSP)