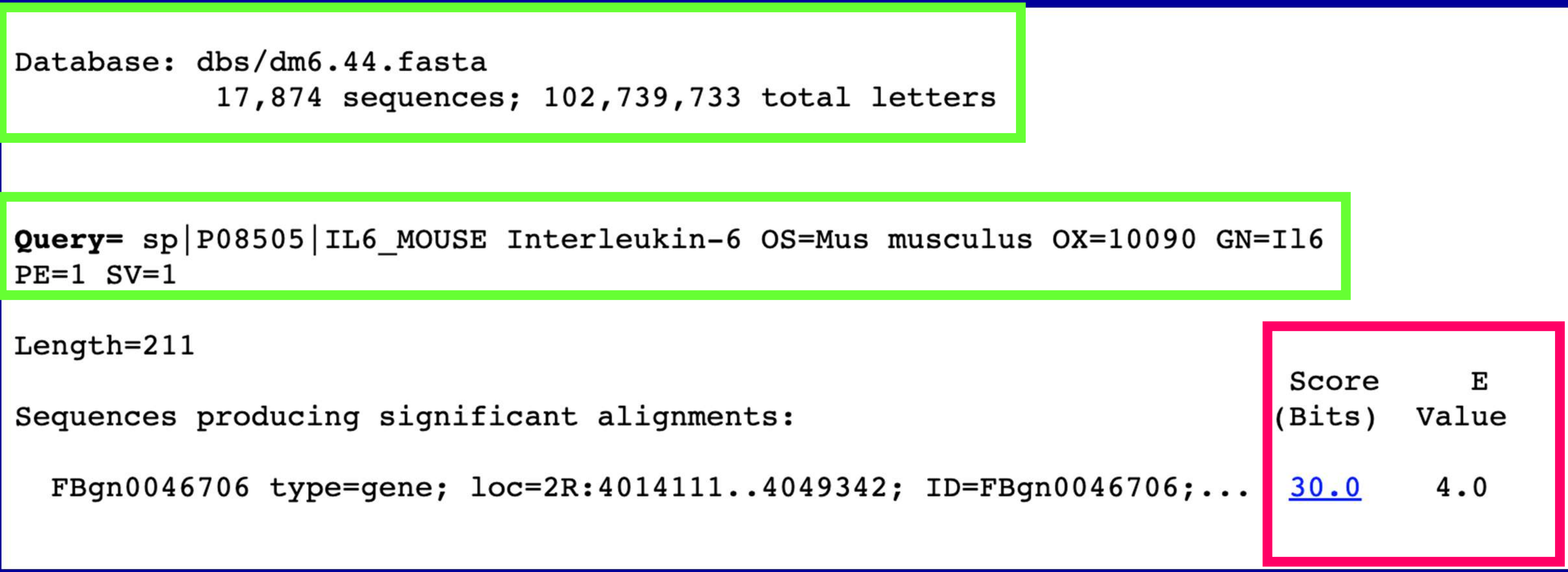
Blast output



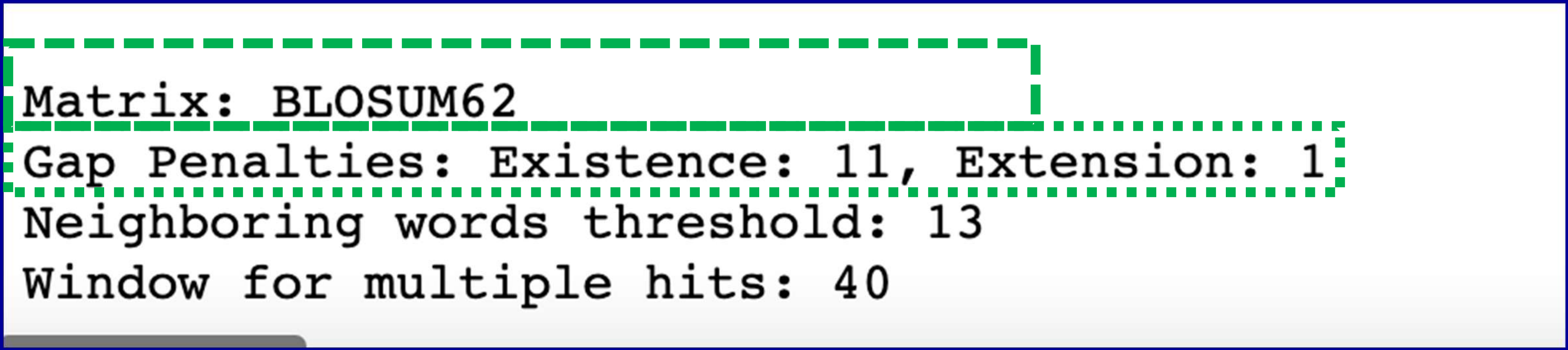
```
Database: dbs/dm6.44.fasta
           17,874 sequences; 102,739,733 total letters

Query= sp|P08505|IL6_MOUSE Interleukin-6 OS=Mus musculus OX=10090 GN=Il6
PE=1 SV=1

Length=211
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value

  FBgn0046706 type=gene; loc=2R:4014111..4049342; ID=FBgn0046706;...  30.0     4.0
```

Questions after the Blast search

Questions:

- How are the hits identified?
- What is the meaning of the score?

Blast output



```
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Neighboring words threshold: 13
Window for multiple hits: 40
```

Questions after the Blast search

Questions:

- How are the hits identified?
- What is the meaning of the score?

Observe & Practice: Scoring the similarity between two sequences.

How to measure the similarity between two sequences

Q: which one is a better match to the query ?

Query: **M A T W L**

Seq_A: **M A T P P**

Seq_B: **M P P W I**

Judging the match using “Scoring Matrix”

Q: which one is a better match to the query ?

Query: M A T W L

Seq_A: M A T P P

Score: 5 4 5 -4 -3

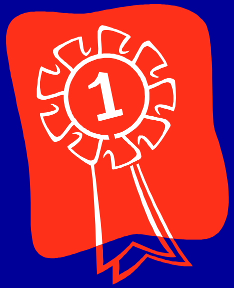
Total: 7

Query: M A T W L

Seq_B: M P P W I

Score: 5-1-1 112

Total: 16



“Scoring Matrix” assigns a score to each pair of amino acids

	A	S	T	L	I	V	K	D	...
L	-1	-2	-2	4	3	1	-2	-4	

BLOSUM-62

BLOSUM - Blocks Substitution Matrices

Block: very well conserved region of a protein family. – perform the same (similar) function.

```
ASLDEFL
SALEDFL
ASLDDYL
ASIDEFY
ASIDEFY
…
```

$$\text{Score}(a1/a2) = 2 * \log 2 \frac{\textbf{observed frequency of } \mathbf{a1/a2}}{\textbf{predicated frequency of } \mathbf{a1/a2}}$$

```
AA:  6

AS:  4

AT:  0
```

BLOSUM - Blocks Substitution Matrices

Block: very well conserved region of a protein family. –
perform the same (similar) function.

ASLDEFL
ASLEDFL
ASLDDYL
SALEEFL
ASLDDYL
SALEEFL
…

$$\text{Score (a1/a2)} \begin{cases} > 0 & \textbf{observed frequency of a1/a2} > \textbf{predicated frequency of a1/a2} \\ = 0 & \\ < 0 & \textbf{observed frequency of a1/a2} < \textbf{predicated frequency of a1/a2} \end{cases}$$

BLOSUM - Blocks Substitution Matrices

Block: very well conserved region of a protein family. –
perform the same (similar) function.

```
ASLDEFL
ASLEDFL
ASLDDYL
SALEEFL
ASLDDYL
SALEEFL
…
```

observed frequency of L / I
i.e: 0.03

>

predicated frequency of L / I
i.e: 0.1*0.1 = 0.01

Score (L/I) > 0

Substitution of L / I is common in conserved sequences

BLOSUM - Blocks Substitution Matrices

Block: very well conserved region of a protein family. – perform the same (similar) function.

```
ASLDEFL
ASLEDFL
ASLDDYL
SALEEFL
ASLDDYL
SALEEFL
…
```

observed frequency of L / K
i.e: 0.0002

<

predicated frequency of L / K
i.e: $0.1*0.1 = 0.01$

Score (L/K) < 0

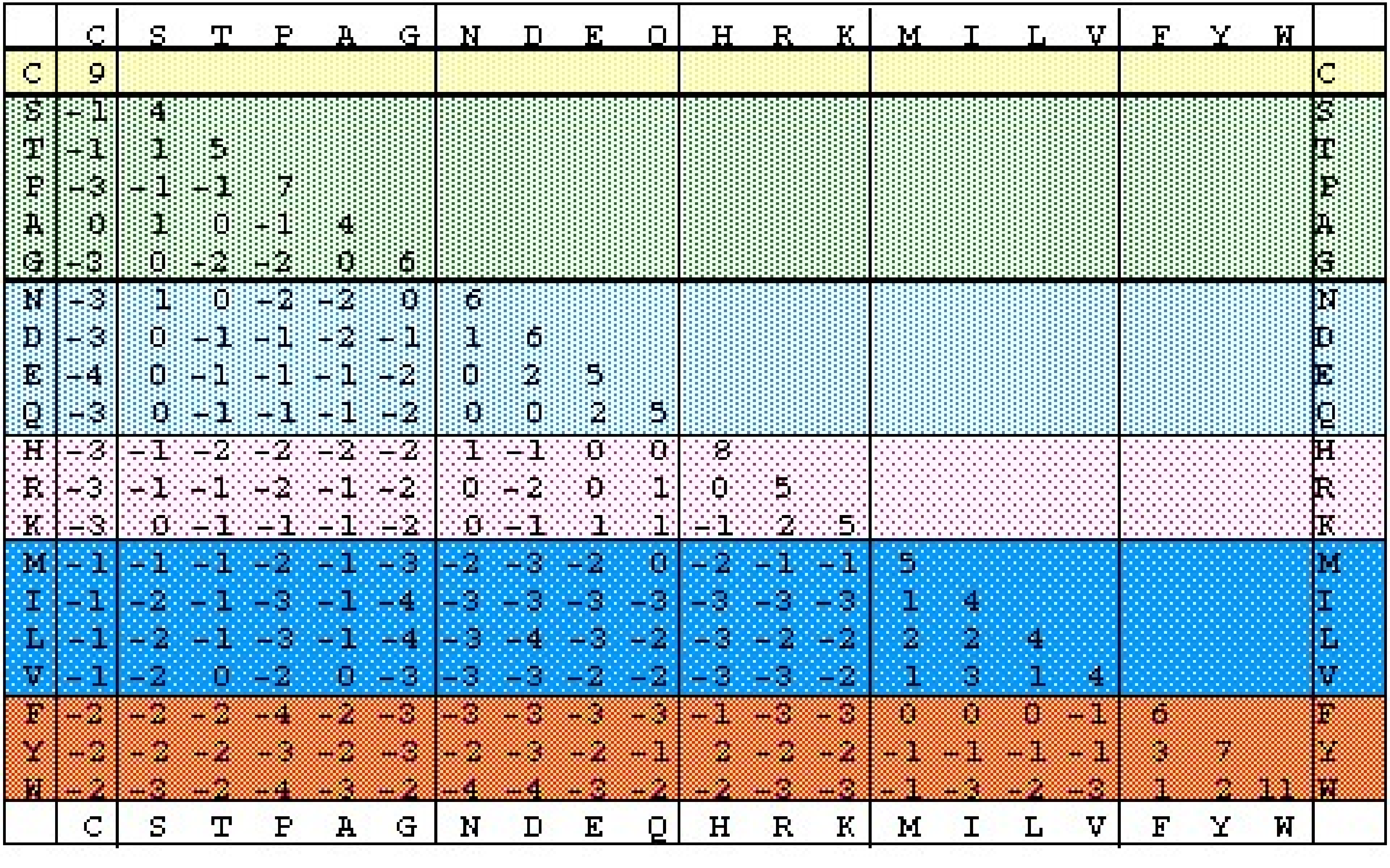
Substitution of L / K is rare in conserved sequences

“Scoring Matrix” assigns a score to each pair of amino acids

	A	S	T	L	I	V	K	D	...
L	-1	-2	-2	4	3	1	-2	-4	

BLOSUM-62

Scoring matrix –BLOSUM 62



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

BLOSUM - Blocks Substitution Matrices

-- Clustering threshold

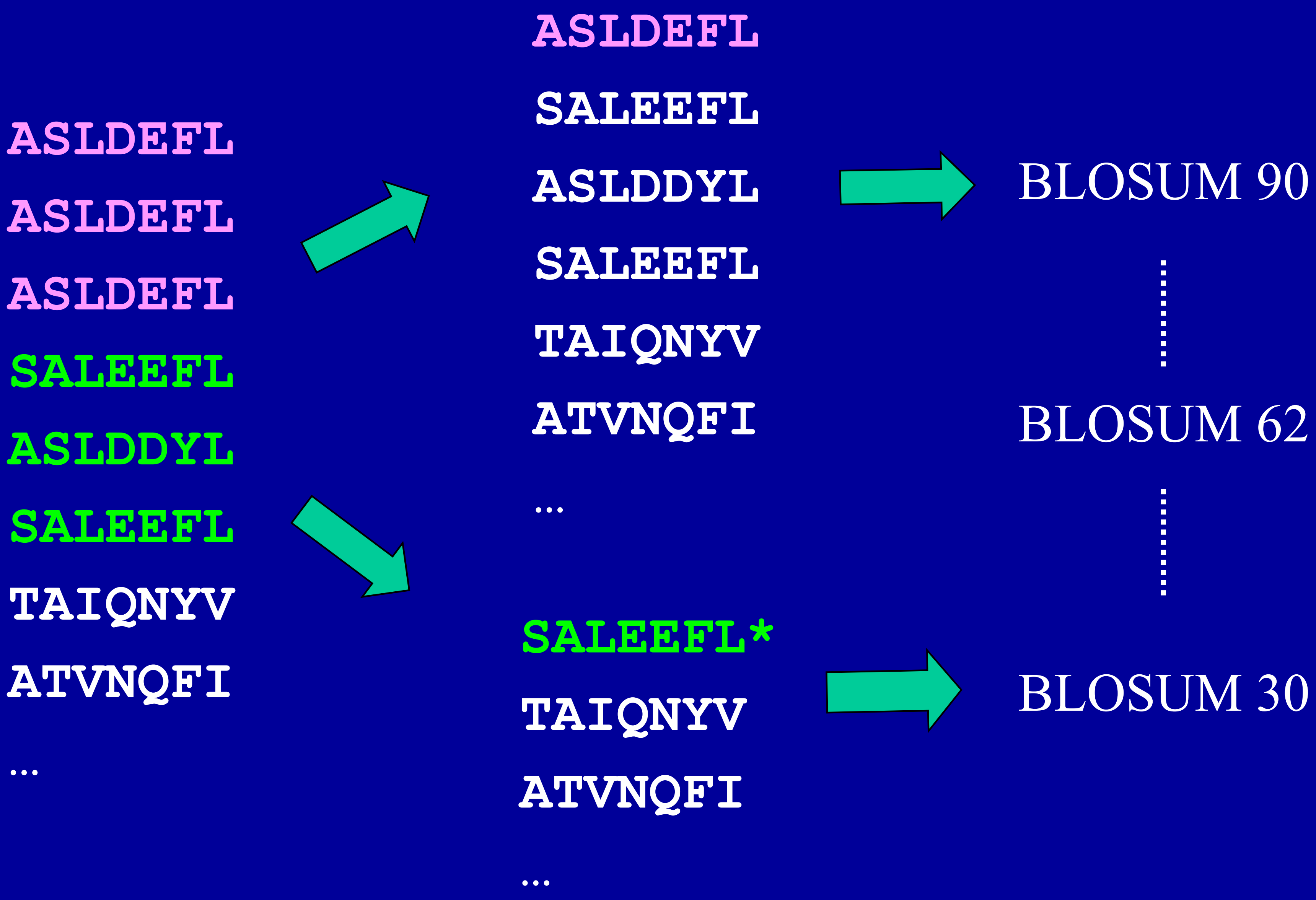
BLOSUM 90 – Blocks with >= **90%** identity are counted as one to compute the substitution score

BLOSUM 62

BLOSUM 30 – Blocks with >= **30 %** identity are counted as one to compute the substitution score

BLOSUM - Blocks Substitution Matrices

-- Clustering threshold



ASLDEFL
ASLDEFL
ASLDEFL
SALEEFL
ASLDDYL
SALEEFL
TAIQNYV
ATVNQFI
…

ASLDEFL
SALEEFL
ASLDDYL
SALEEFL
TAIQNYV
ATVNQFI
…

SALEEFL*
TAIQNYV
ATVNQFI
…

BLOSUM 90

BLOSUM 62

BLOSUM 30

Comparison of Blosum matrixes

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F
L	-2	-3	-4	-5	-2	-3	-4	-5	-4	1	5	-3	2	0
Blosum 90														
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0
Blosum 62														
L	-1	-2	-2	-1	0	-2	-1	-2	-1	2	4	-2	2	2
Blosum 30														

Q:

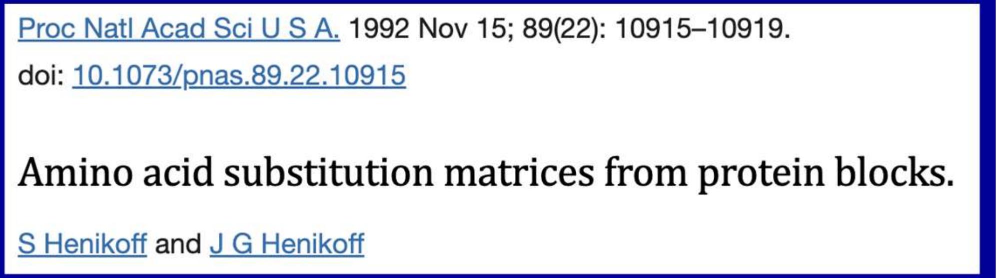
Which substitution matrix will you use to identify a distant ortholog ?

a.) Blosum 40

b.) Blosum 60

c.) Blosum 90

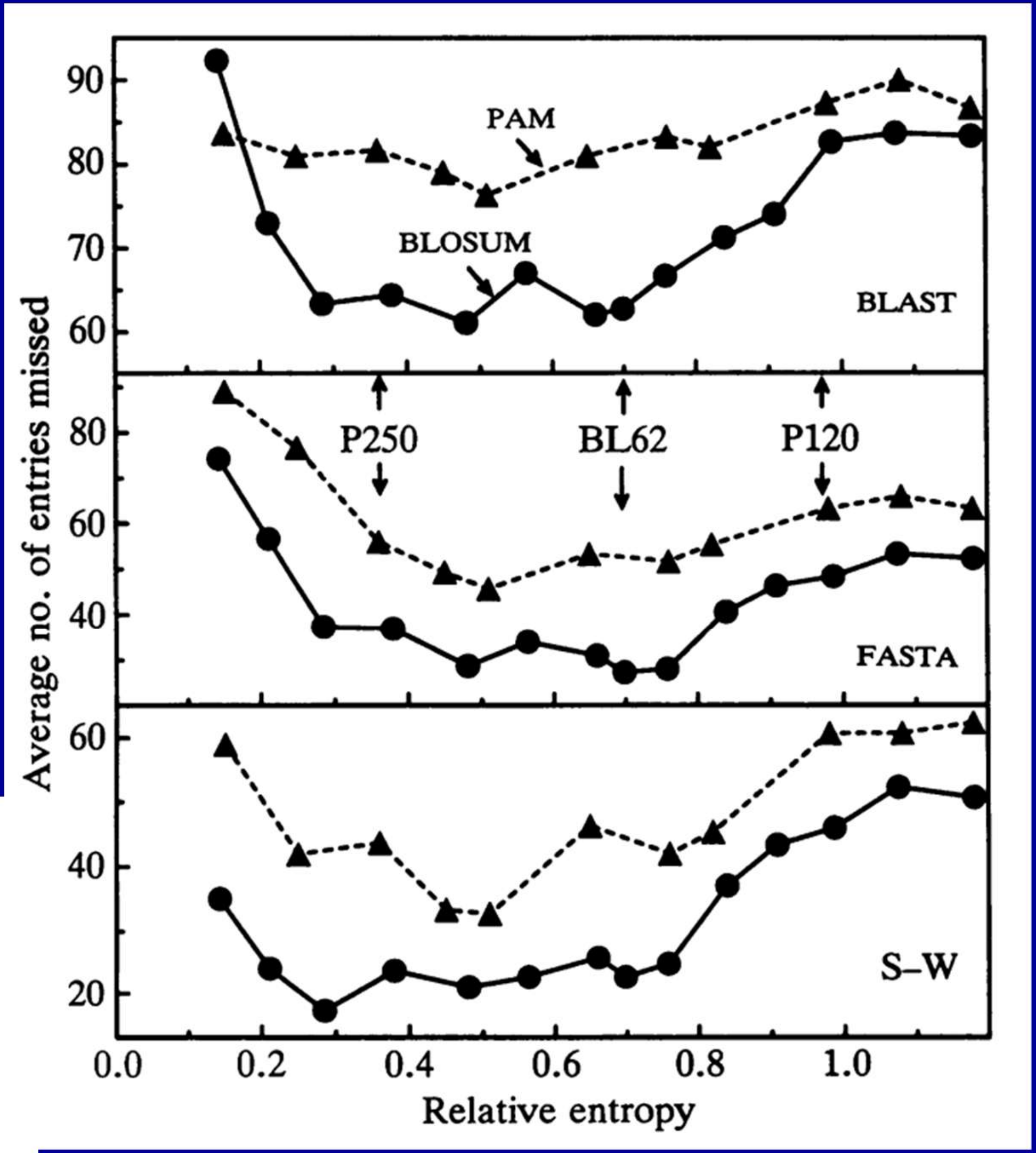
Why BLAST uses BLOSUM62 as the Default.



Proc Natl Acad Sci U S A. 1992 Nov 15; 89(22): 10915–10919.
doi: 10.1073/pnas.89.22.10915

Amino acid substitution matrices from protein blocks.

S Henikoff and J G Henikoff



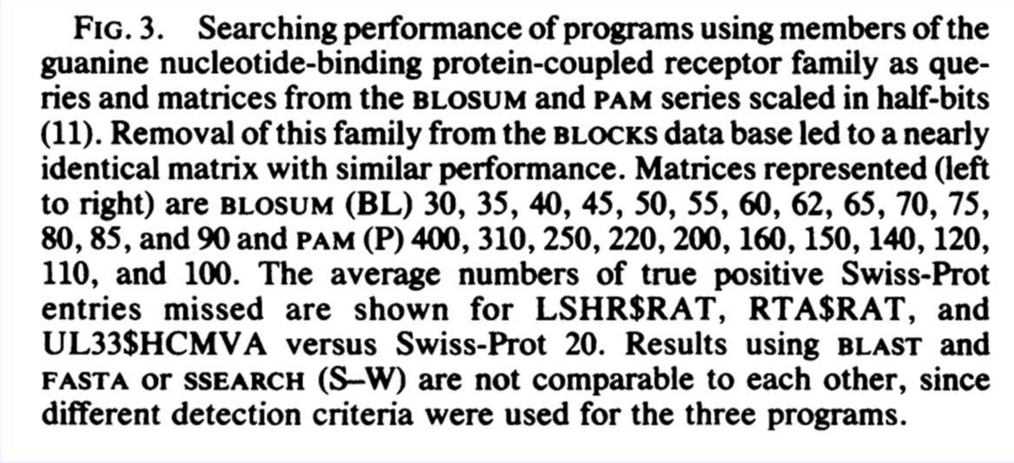


FIG. 3. Searching performance of programs using members of the guanine nucleotide-binding protein-coupled receptor family as queries and matrices from the BLOSUM and PAM series scaled in half-bits (11). Removal of this family from the BLOCKS data base led to a nearly identical matrix with similar performance. Matrices represented (left to right) are BLOSUM (BL) 30, 35, 40, 45, 50, 55, 60, 62, 65, 70, 75, 80, 85, and 90 and PAM (P) 400, 310, 250, 220, 200, 160, 150, 140, 120, 110, and 100. The average numbers of true positive Swiss-Prot entries missed are shown for LSHRRAT, RTARAT, and UL33$HCMVA versus Swiss-Prot 20. Results using BLAST and FASTA or SSEARCH (S–W) are not comparable to each other, since different detection criteria were used for the three programs.

Finding the best alignment = Get the highest score

The consideration on whether to open/extend a gap is weighed by its effect on the **total score** of the alignment.

Optimization - Dynamic programming

Effect of matrices on Local Alignment

Observe: effect of matrices on the outcome of local alignment

First name initial > L -- Align seq1 and seq 2 with “blosum62”

Others -- Align seq1 and seq 2 with “blosum35”

Effect of matrices on Local Alignment

```
Score: 156 at (seq1)[10..36] :
(seq2)[64..90]
10 EPTEVFMDLWPEDHSNWQELSPLEPSD
   |||||||||||||||||||||||||||
64 EPTEVFMDLWPEDHSNWQELSPLEPSD
```

Blosum 62:

P / H: -2

L/M: 2

```
Score: 206 at (seq1)[10..38] :
(seq2)[64..92]
10 EPTEVFMDLWPEDHSNWQELSPLEPSDPL
   |||||||||||||||||||||||||||  .
64 EPTEVFMDLWPEDHSNWQELSPLEPSDHM
```

Blosum 35:

P / H: -1

L/M: 3

Gap open -15; Gap ext -3

Introducing a gap

Q: M A T W L I .

A: M A W T V A .

Scr: 5 4 -2 -2 1 -1

Total: 5

Q: M A T W L I .

A: M A - W T V .

Scr: 5 4 -? 11 -1 3

Total = 22 - ?

Blosum 62:

Gap openning: -6 ~ -15

Gap Extension: -2 ~ -6

Effect of gap penalty on Local Alignment

Practice : effect of gap penalty on local alignment

Set matrix to “blosum62”

Column 1,3,5, align seq1 and seq2 with “gap=15, ext=3,”

Column 2 and 4, align seq1 and seq2 with “gap=5, ext=1”

Effect of gap penalty on Local Alignment

Blosum 62

```
Score: 156 at (seq1)[10..36] :
(seq2)[64..90]
10 EPTEVFMDLWPEDHSNWQELSPLEPSD
   |||||||||||||||||||||||||||
64 EPTEVFMDLWPEDHSNWQELSPLEPSD
```

Gap: -15

Ex: -3

Gap: -5 Ex: - 1

```
Score: 161 at (seq1)[2..36] : (seq2)[53..90]
2  ASTV----TSCLEPTEVFMDLWPEDHSNWQELSPLEPSD
   || |    |   |||||||||||||||||||||||||||
53 ASSVSVGATEA-EPTEVFMDLWPEDHSNWQELSPLEPSD
```

BLAST – Basic Local Alignment Search Tool

It is based on local alignment, -- highest score is the only priority in terms of finding alignment match.

-- determined by scoring matrix, gap penalty

It is **optimized** for searching large data set instead of finding the best alignment for two sequences

BLAST – Basic Local Alignment Search Tool

1. A high similarity core (2-4aa)
2. Often without gap

```
Query: M A T W L I .
Word : M A T
         A T W
           T W L
        W L I
```

1. **For each word, find matches with Score > T.**
2. **Extend the match as long as profitable.**

   - High Scoring segment Pair (best local alignment)

3. **Find the P and E value for HSP(s) with Score > cut off*.**

* Cut off value can be automatically calculated based on E

BLAST – Basic Local Alignment Search Tool

The P and E value for HSP(s) : based on the **total score (S)** of the identified "best" local alignment.

P (S) : the probability that two random sequences, one the length of the query and the other the entire length of the database, could achieve the score S.

E (S) : The expectation of observing a score >= S in the target database.

For a given database, there is a one to one correspondence between S and E(s) -- choosing E determines cut off score

BLAST – Basic Local Alignment Search Tool

BLASTN

BLASTP

TBLASTN

compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames.

BLASTX

compares a nucleotide query sequence translated in all reading frames against a protein sequence database

TBLASTX

compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. Please note that tblastx program cannot be used with the nr database on the BLAST Web page.

BLAST – Advanced options : all adjustable in stand alone BLAST

-F Filter query sequence [String] default = T

-M Matrix [String] default = BLOSUM62

-G Cost to open gap [Integer] default = 5 for nucleotides 11 proteins

-E Cost to extend gap [Integer] default = 2 nucleotides 1 proteins

-q Penalty for nucleotide mismatch [Integer] default = -3

-r reward for nucleotide match [Integer] default = 1

-e expect value [Real] default = 10

-W wordsize [Integer] default = 11 nucleotides 3 proteins

-T Produce HTML output [T/F] default = F

Overview of homology search strategy

1.) Where should I search?

- **NCBI**

  Has pretty much every thing that has been available for some time

- **Genome projects**

  Has the updated information (DNA sequence as well as analysis result)