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	

Limitations of Generic Scoring Matrix

- Short specific pattern
- Position specific information.

Binary patterns for protein and DNA

Examples:

- **Caspase recognition site:**

[EDQN] X [^RKH] D [ASP]

Observe: Search for potential caspase recognition sites with BaGua

BLAST (&BaGua) are programs implemented in C/C++

```
void BlastTickProc(Int4 sequence_number, BlastThrInfoPtr thr_info)
{
    if(thr_info->tick_callback &&
       (sequence_number > (thr_info->last_db_seq + thr_info->db_incr))) {
        NlmMutexLockEx(&thr_info->callback_mutex);
        thr_info->last_db_seq += thr_info->db_incr;
        thr_info->tick_callback(sequence_number, thr_info->number_of_pos_hits);
    }
    return;
}
/*
          Sends out a message every PERIOD (i.e., 60 secs.) for the index.
          THis function runs as a separate thread and only runs on a threaded
          platform.
```

Should I care ?

If you care:

1.) Data structure and Algorithm

SEQ
- char: name
- char: sequence
- int: seq_length

Identify the best alignment for two sequences (p69-73)

```
Seq1: MA-DSV—WC..
Seq2: MALD-IHWS..
```

Programming language comparison

Translation : C

```
/* TRANSLATION: 3 or 6 frame translate cDNA sequences
                                        */
//---------------------------------------------------------------------------
#include "translation.hpp"

int main(int argc, char **argv)
{   int num_seq=0;
                        char string[MAXLINE];
    DSEQ * dseq;


                        infile.getline (string,MAXLINE);
    if (string[0]=='>') strncpy (dbname,string,MAXLINE);
    while (!infile.eof())
    {   dseq=Get_Lib_Seq ();
        if (dseq->reverse==0)
            Translation (&dseq->name[1], dseq->seq);
        else
            Translation (&dseq->name[1], dseq->r_seq);
                        num_seq++;
        if (num_seq%1000==0)
        {   cout<<num_seq<<endl;
            cout<<dseq->name<<endl;
        }
        delete dseq;
    }

    infile.close();
    outfile.close();
    cout<<num_seq<<" translated"<<endl;
    getch();
                        return 0;
}

DSEQ* Get_Lib_Seq()
{   int i,n;
    char str[MAXLINE];
    DSEQ* dseq;
    n = 0;
    dseq=new DSEQ;
    strcpy (dseq->name, dbname);
```

Translation : Python

```
f#Translation -- read from fasta DNA file and translate into
three frames

#

import string

from Bio import Fasta

from Bio.Tools import Translate

from Bio.Alphabet import IUPAC

from Bio.Seq import Seq

ifile = "S:\\Seq\\test.fasta"

parser = Fasta.RecordParser()

file =open (ifile)

iterator = Fasta.Iterator (file, parser)

cur_rec = iterator.next()

cur_seq = Seq (cur_rec.sequence,IUPACUnambiguousDNA())

translator = Translate.unambiguous_dna_by_id[1]

translator.translate (cur_seq)
```

Programming languages and platforms

Efficiency, Power	Simplicity, Fast Dev.
C/C++	Perl - Bioperl
Java - Biojava	Python - Biopython

Does binary pattern convey all the information ?

Why a BLAST match is refused by the family ?

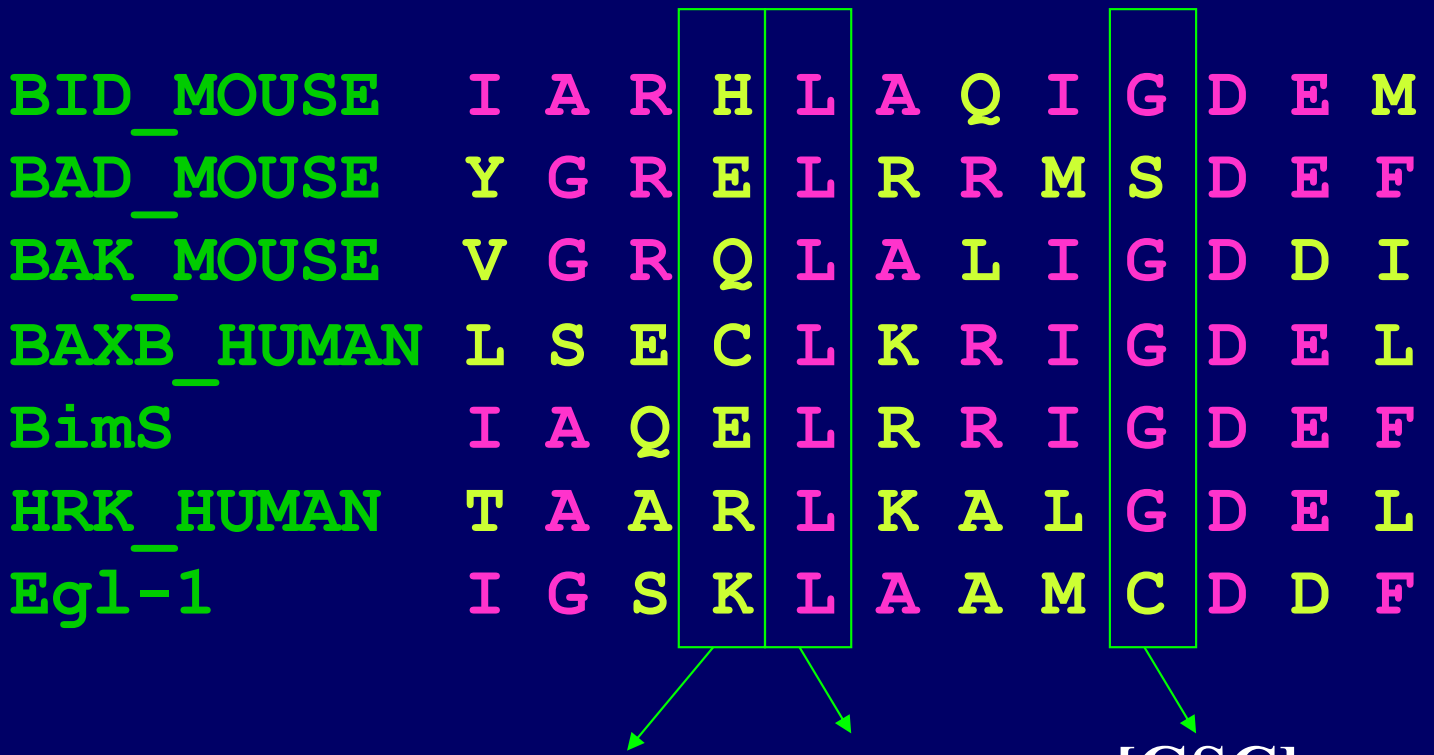
Position –specific information about conserved domains is IGNORED in single sequence –initiated search

```
BID_MOUSE     SESQEEIIHN IARHLAQIGDEM DHNIQPTLVR
BAD_MOUSE     APPNLWAAQR YGRELRRMSDEF EGSFKGLPRP
BAK_MOUSE     PLEPNSILGQ VGRQLALIGDDI NRRYDTEFQN
BAXB_HUMAN    PVPQDASTKK LSECLKRIGDEL DSNMELQRMI
BimS          EPEDLRPEIR IAQELRRIGDEF NETYTRRVFA
HRK_HUMAN     LGLRSSAAQL TAARLKALGDEL HQRTMWRRRA
Egl-1         DSEISSIGYE IGSKLAAMCDDF DAQMMSYSAH

BID_MOUSE     SESQEEIIHN IARHLAQIGDEM DHNIQPTLVR
sequence X    SESSSELLHN SAGHAAQLFDSM RLDIGSTAHR
sequence Y    PGLKSSAANI LSQQLKGIGDDL HQRMMSYSAH
```

Basic concept of motif identification 2.

How do we represent the position specific preference ?



Binary pattern: [HEQCRK] L [GSC]

X

[^ILMFV]

Basic concept of motif identification 2.

How do we represent the position specific preference ?

```
BID_MOUSE   I A R H L A Q I G D E M
BAD_MOUSE   Y G R E L R R M S D E F
BAK_MOUSE   V G R Q L A L I G D D I
BAXB_HUMAN  L S E C L K R I G D E L
BimS        I A Q E L R R I G D E F
HRK_HUMAN   T A A R L K A L G D E L
Egl-1       I G S K L A A M C D D F
```

Statistical representation

G: 5 -> 71%

S: 1 -> 14 %

C: 1 -> 14 %

Protein motif /domain

- Structural unit
- Functional unit
- Signature of protein family

How are they defined?

Practice: Using MEME to identify motifs shared by a set of proteins

1. Load the sequence file to MEME.
2. Make sure you input your email address for results.

Representation of positional information in specific motif

Binary patterns:

M-C-N-S-S-C-[MV]-G-G-M-N-R-R.

Positional matrix:

-2.499	-2.269	-5.001	-4.568	-2.418	-4.589	-3.879	1.971	-4.330	1.477	-1.241	-4.221	-4.590	-4.097	-4.293	-3.808	0.495	2.545	-3.
1.627	-2.453	-1.804	-1.746	-3.528	2.539	1.544	-3.362	-1.440	-3.391	-2.490	-1.435	-3.076	-1.571	0.501	0.201	-1.930	-2.707	-3.
-1.346	-2.872	-1.367	0.699	-2.938	-2.427	-0.936	-2.632	-0.095	1.147	-1.684	-1.111	-2.531	1.174	2.105	1.057	-1.400	-2.255	-2.
1.045	1.754	-1.169	-0.576	-2.756	-2.212	1.686	-2.576	0.951	-2.438	-1.544	0.857	-2.301	1.891	1.556	-1.097	-1.180	-2.155	-2.
-3.385	-2.965	-5.039	-4.313	-1.529	-5.006	-3.577	0.429	-4.094	3.154	-0.121	-4.440	-4.199	-3.292	-3.662	-4.198	-3.281	-1.505	-3.
2.368	-3.197	-2.285	-1.533	-3.721	-2.945	1.815	-3.235	0.067	-3.061	-2.259	-1.680	-3.231	1.195	2.287	-2.009	-2.044	-2.825	-3.
1.046	1.742	0.576	-0.734	-2.072	-2.234	-0.851	0.436	-0.548	-0.129	-0.974	-1.039	-2.318	2.368	0.667	-1.135	-1.076	-1.304	-2.
0.715	-1.778	-3.820	-3.359	-1.535	-3.463	-2.571	3.060	-3.008	0.262	1.566	-2.996	-3.575	0.450	-3.001	-2.571	-1.765	0.193	-2.
-2.053	0.965	-2.767	-3.509	-4.520	3.654	-3.242	-4.548	-3.338	-4.968	-3.789	-2.462	-4.048	-3.738	-3.266	-2.596	-3.573	-3.990	

Scoring sequence based on Model

```
Seq: A S L D E L G D E
```

	1	2	3	4	
A	1	0	-4	.	
C	2	5	-1	.	
D	1	-3	9	.	
…	.	.	.	.	

score @ position_1 = s(A/1) + s(S/2) + s(L/3) + s(D / 4)

An example of position specific matrix

Observe: Search for proteins encodes IL6 motif#2 in Dm genome.

Practice: motif analysis of protein sequence using ScanProsite and Pfam

1. Open two taps for Pfam, input one of the Blast hits and one candidate TNF to each data window.
2. Compare the results

Scan protein for identified motifs

- A service provided by major motif databases such as Prosite,, Pfam, Block, etc.
- The presence of signature motif(s) is often indicative of structural and functional property.
- High frequency motifs may only have suggestive value.

What is the possible function of my protein? Which family my protein belongs to? -- Profile databases

- Pfam (http://pfam.xfam.org/)
- Prosite (https://prosite.expasy.org/)
- InterPro (http://www.ebi.ac.uk/interpro/)

Scan protein for functional motifs

Practice: Scan the top hits from BLAST or Motif-based search in Prosite.

- The presence of motif is a strong indication of potential functional and regulatory mechanisms.
- How to interpret short and high frequency motifs?

Identifying shared motifs using MEME -Multiple EM for Motif Elicitation

- Identifies statistically significant motif(s) in a set of sequences.
- Motifs shared by proteins.
  - Protein family.
  - Mediate interaction between different protein.
- Motifs shared by DNA sequences binding to certain transcription factor (ChIP-Seq).

Two search examples

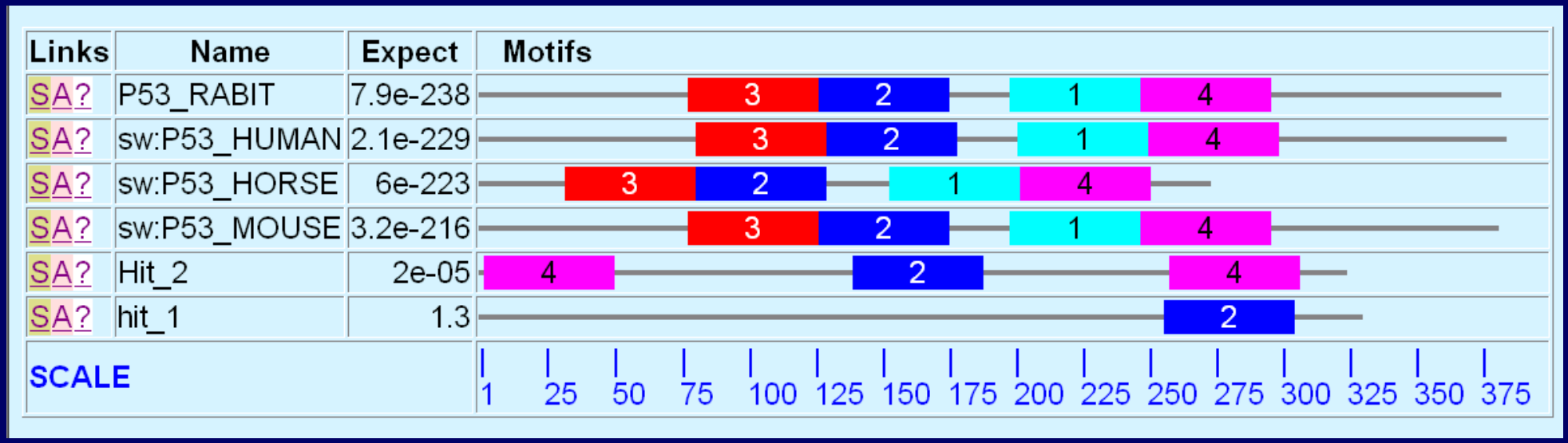
Set1: Mammalian P53 plus mosquito hits

Set2: Diverse set of P53 plus mosquito hits

- The outcome of the search is dependent on the inputting set of sequences.
- Compose the inputting set based on your research needs.

Selection of sequences determines the model

Set1: Mammalian P53 plus mosquito hits



Set2: Diverse set of P53 plus mosquito hits