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)

Overview of homology search strategy

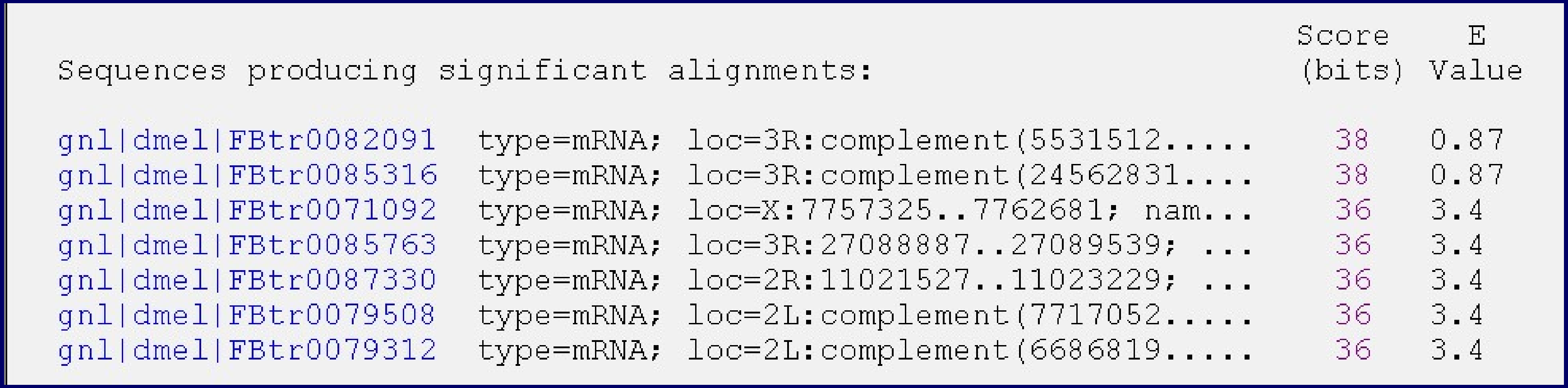
2.) Which sequence should I use as the query?

- Protein
- cDNA
- Genomic

Overview of homology search strategy

2.) Which sequence should I use as the query?

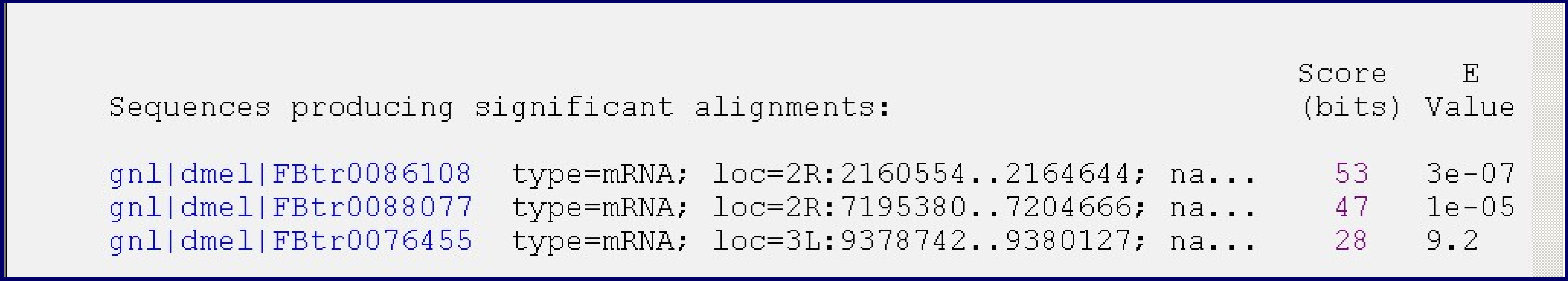
cDNA (BlastN)



```
                                                                  Score    E
Sequences producing significant alignments:                      (bits)  Value

gnl|dmel|FBtr0082091  type=mRNA; loc=3R:complement(5531512.....    38    0.87
gnl|dmel|FBtr0085316  type=mRNA; loc=3R:complement(24562831....    38    0.87
gnl|dmel|FBtr0071092  type=mRNA; loc=X:7757325..7762681; nam...    36    3.4
gnl|dmel|FBtr0085763  type=mRNA; loc=3R:27088887..27089539; ...    36    3.4
gnl|dmel|FBtr0087330  type=mRNA; loc=2R:11021527..11023229; ...    36    3.4
gnl|dmel|FBtr0079508  type=mRNA; loc=2L:complement(7717052.....    36    3.4
gnl|dmel|FBtr0079312  type=mRNA; loc=2L:complement(6686819.....    36    3.4
```

Protein (TblastN)



```
                                                                  Score    E
Sequences producing significant alignments:                      (bits)  Value

gnl|dmel|FBtr0086108  type=mRNA; loc=2R:2160554..2164644; na...    53    3e-07
gnl|dmel|FBtr0088077  type=mRNA; loc=2R:7195380..7204666; na...    47    1e-05
gnl|dmel|FBtr0076455  type=mRNA; loc=3L:9378742..9380127; na...    28    9.2
```

Overview of homology search strategy

2.) Which sequence should I use as the query?

Protein v.s cDNA

```
query: S   A   L     target: S   A   L
query: TCT GCA TTG   target: AGC GCT CTA
```

Protein: 100%

Nucleotide: 33%

<u>Base level identity</u>

Protein: ~ 5%

Nucleotide: ~ 25%

Searching at the protein level is much more sensitive

Overview of homology search strategy

2.) Which sequence should I use as the query?

If you want to identify similar feature at the DNA level. Be Cautious with genomic sequence initiated search

- **Low complexity region**
- **repeats**

Overview of homology search strategy

5.) How to optimize the search ?

- Scoring matrices
- Gap penalty
- Expectation / cut off

Example

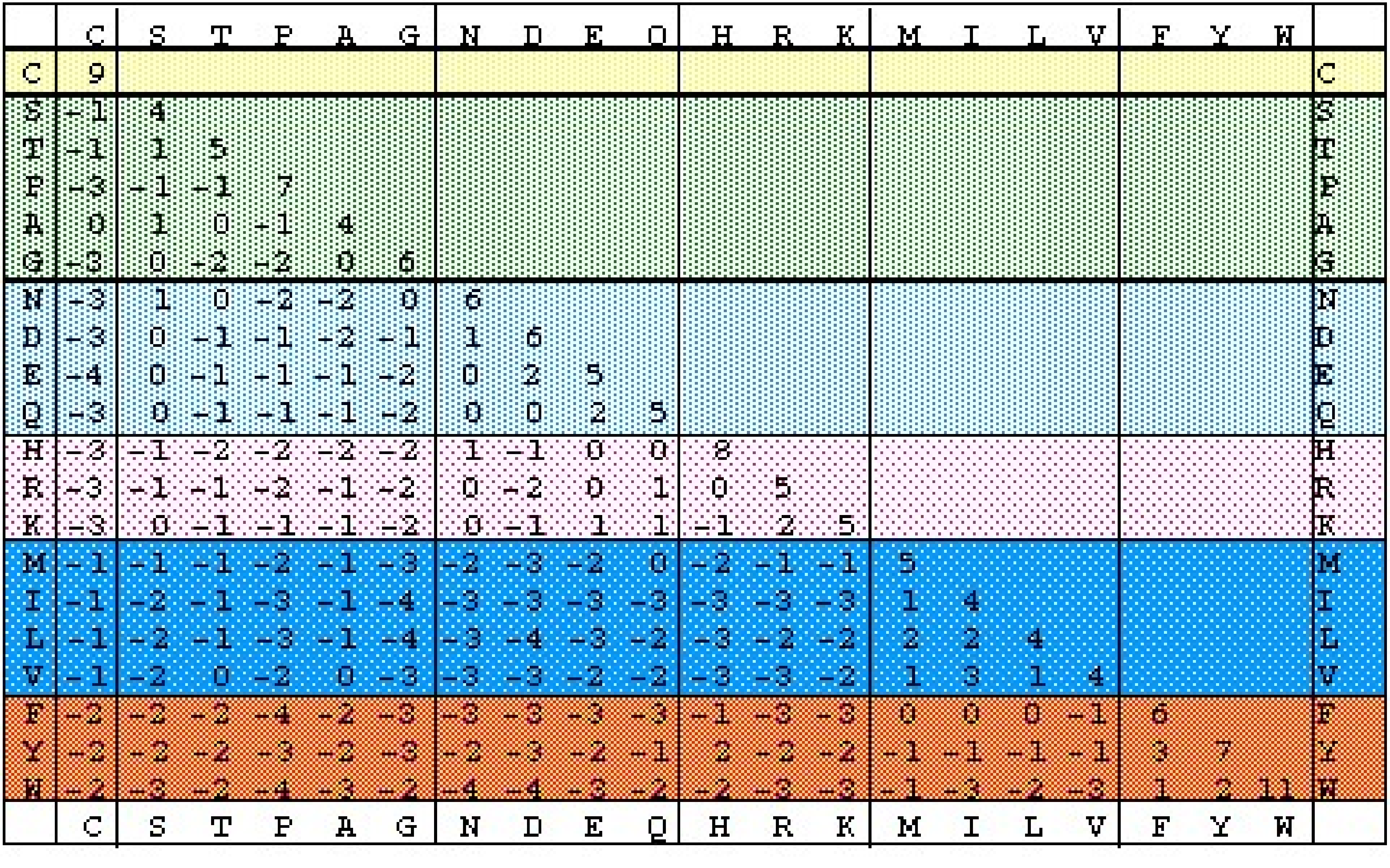
Overview of homology search strategy

8.) How to align (compare) my query and the hits ?

- **Global alignment**
- **Local alignment**

ClustalW/ClustalX

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

Why BLAST may fail to find the real orthologue?

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 ?

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

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).