Sequence alignments and scoring matrices

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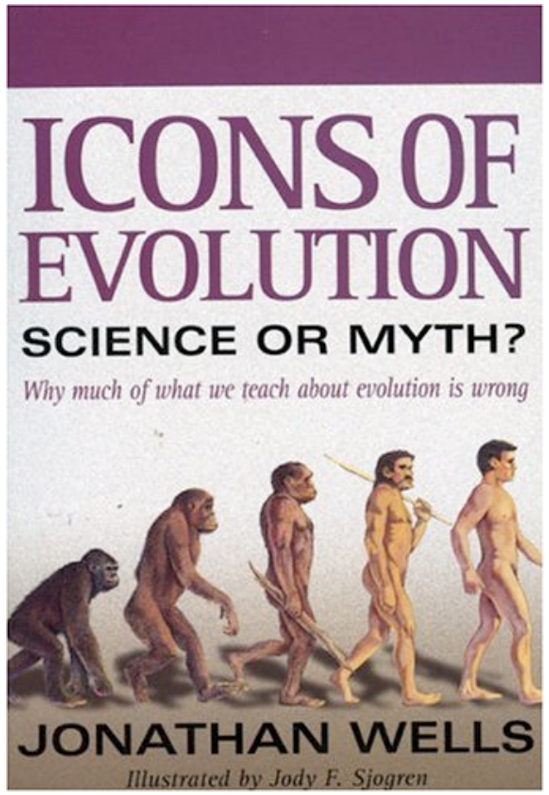
To read: http://perso.fundp.ac.be/~lambertc/DEA-bioinfo/CLambert_curr_gen_2003.pdf

To read: Wikipidea about Sequence Alignment

Why alignments ?

```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                    ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .***************    :  *.: :
```

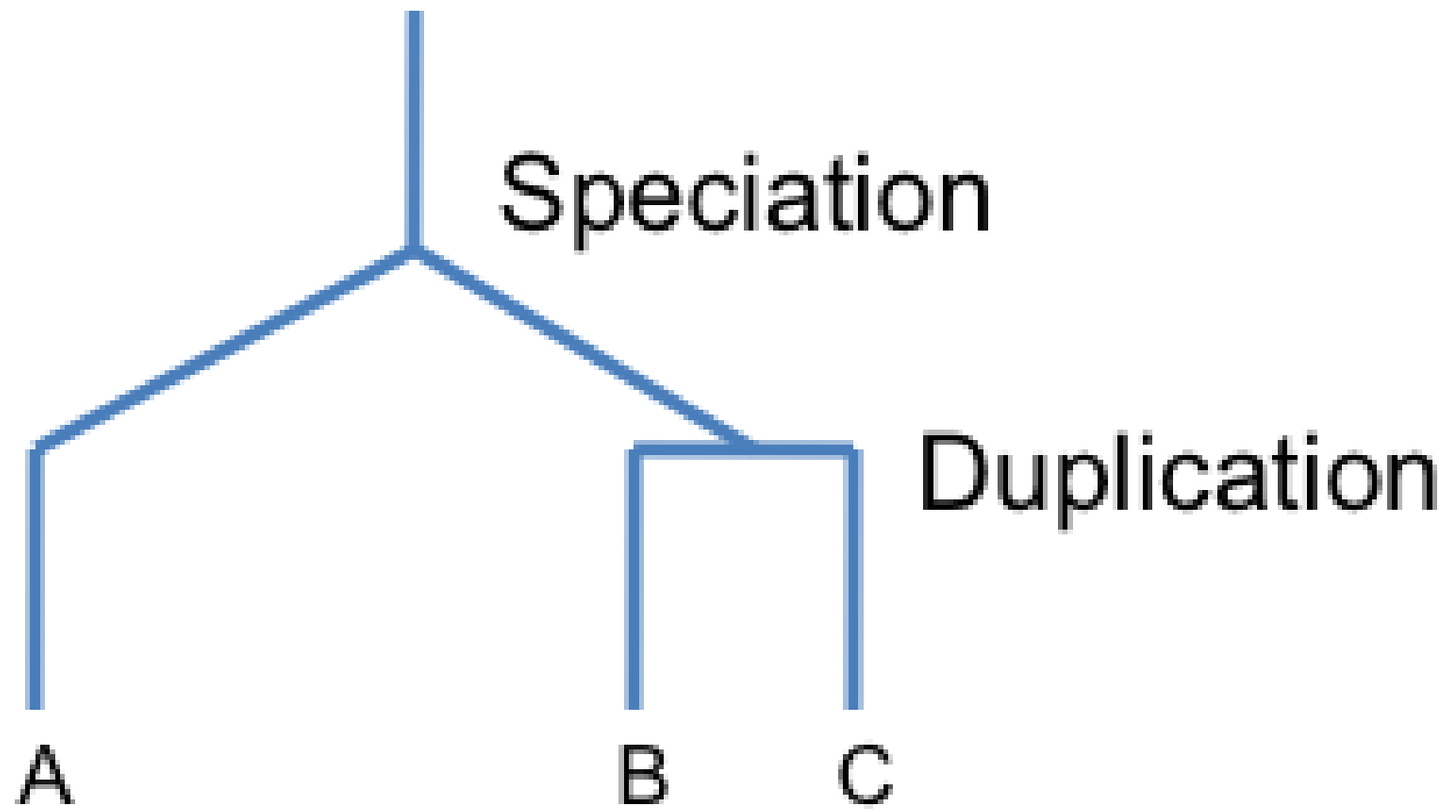
- Detect homology
- Study evolution
- Predict functions
- Model 3D-structure



Sequence similarity

- Homologs have a common ancestor
- Gene duplication or speciation
- High sequence similarity indicates homology
- Homologs have similar 3D-structure

Homology

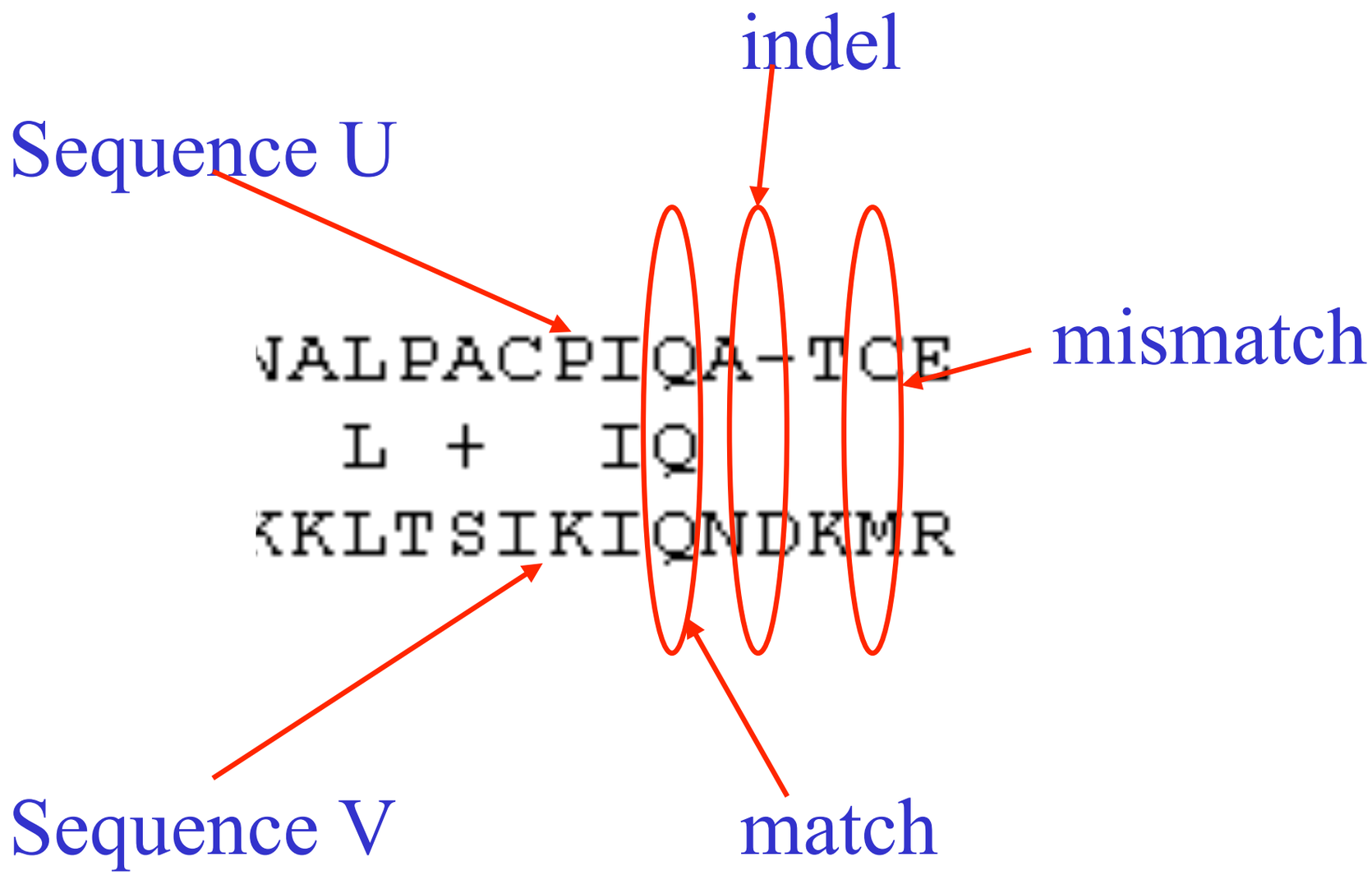


Convergent evolution

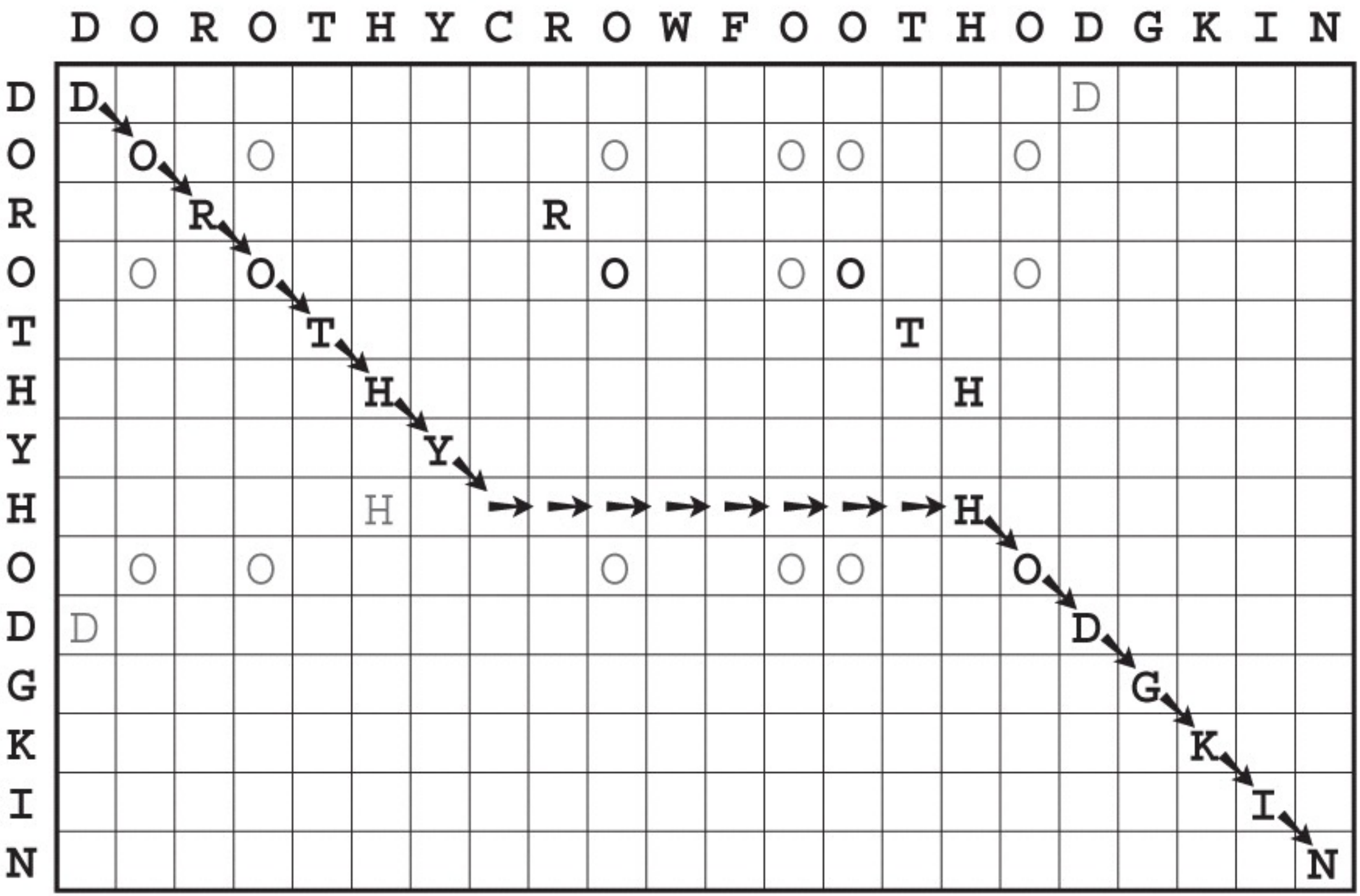
What is an alignment

```
THISSEQUENCE
||  ||||||||      10/12 Identical
THATSEQUENCE

THATSEQUENCE
||      |  |      4/12 Identical
THISISASEQUENCE

THISISA-SEQUENCE
||    | ||||||||  11/12 Identical
TH----ATSEQUENCE
```

What can an alignment say ?

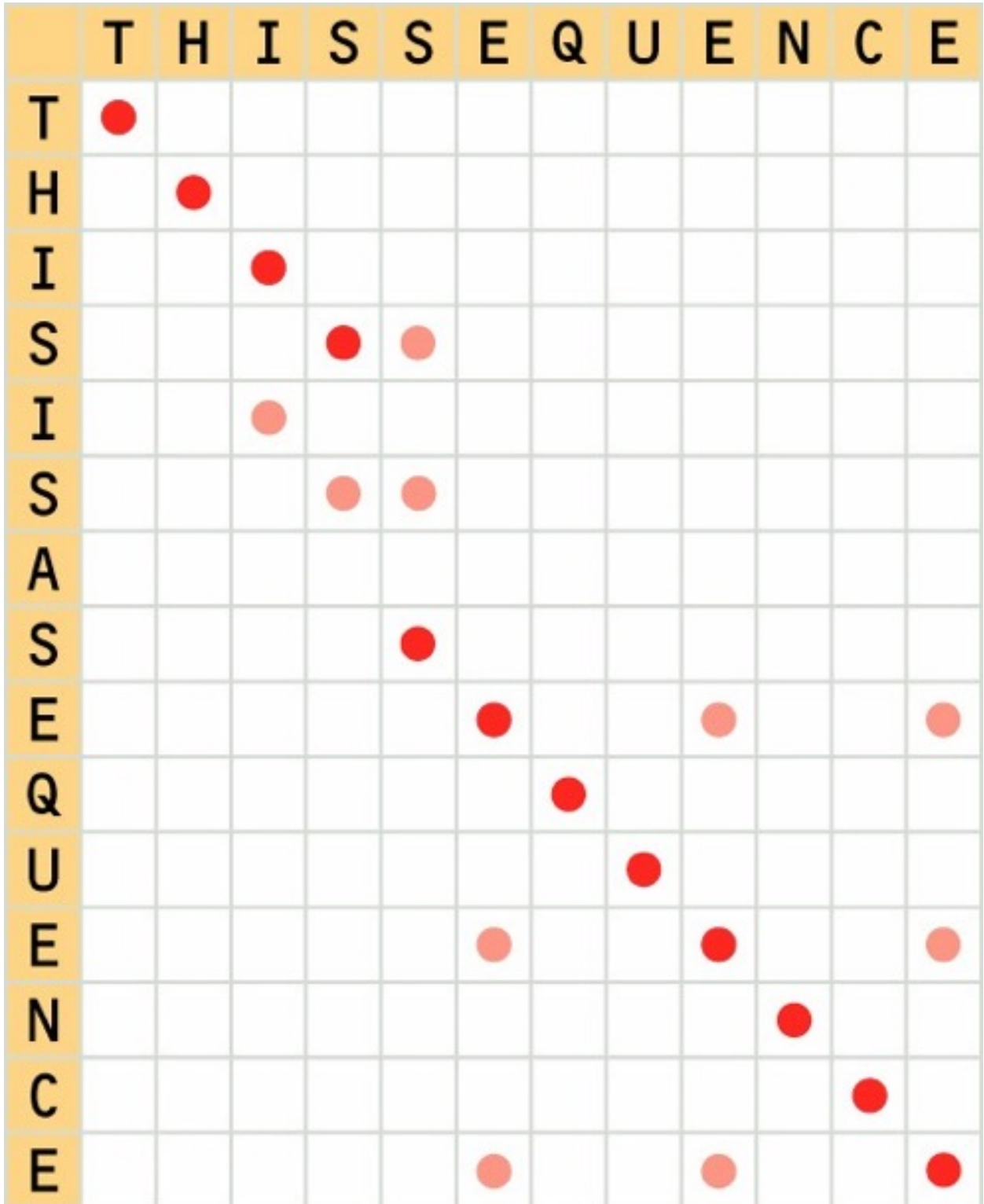


An alignment matrix



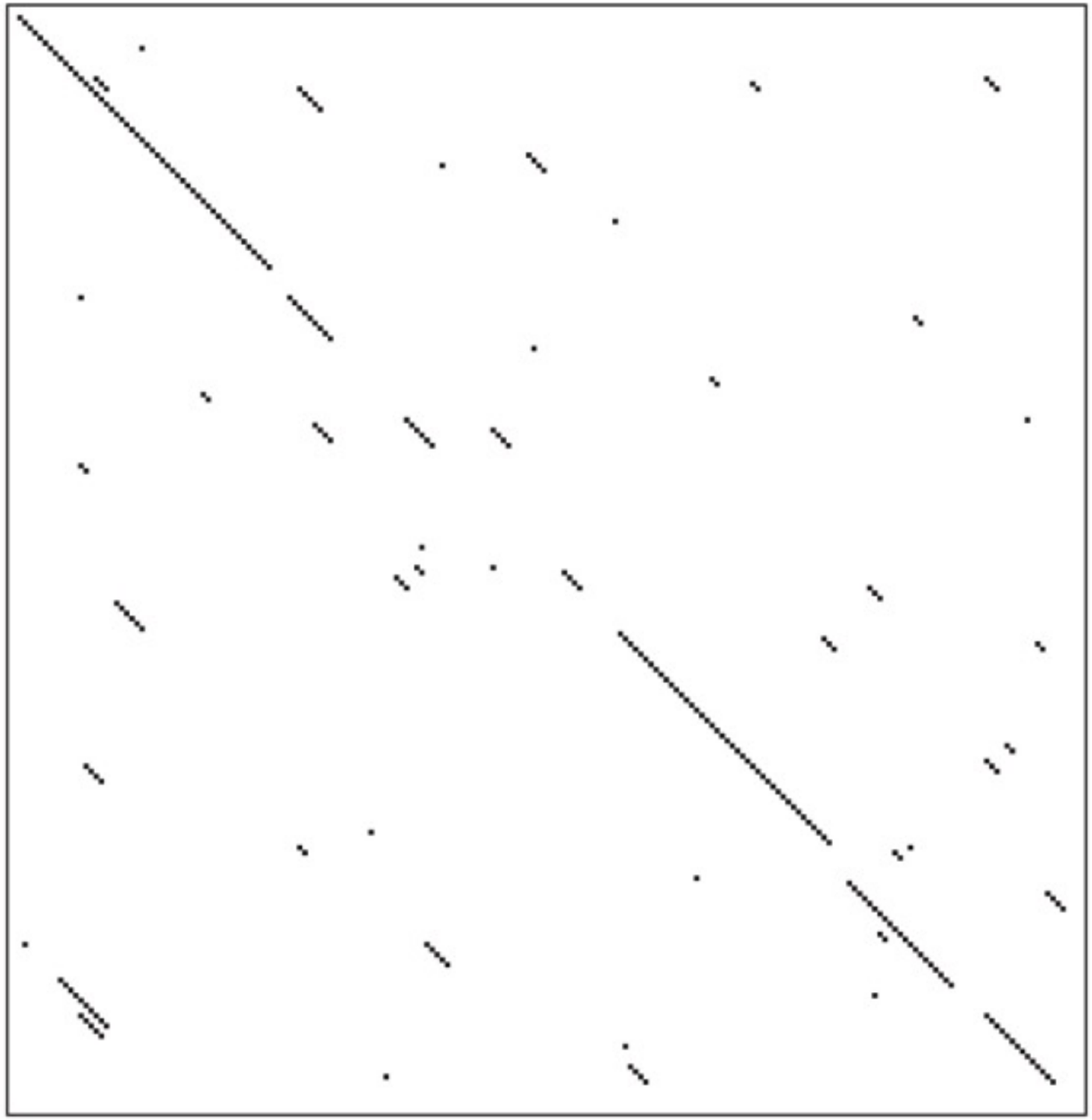
Dotplots

Dotplots



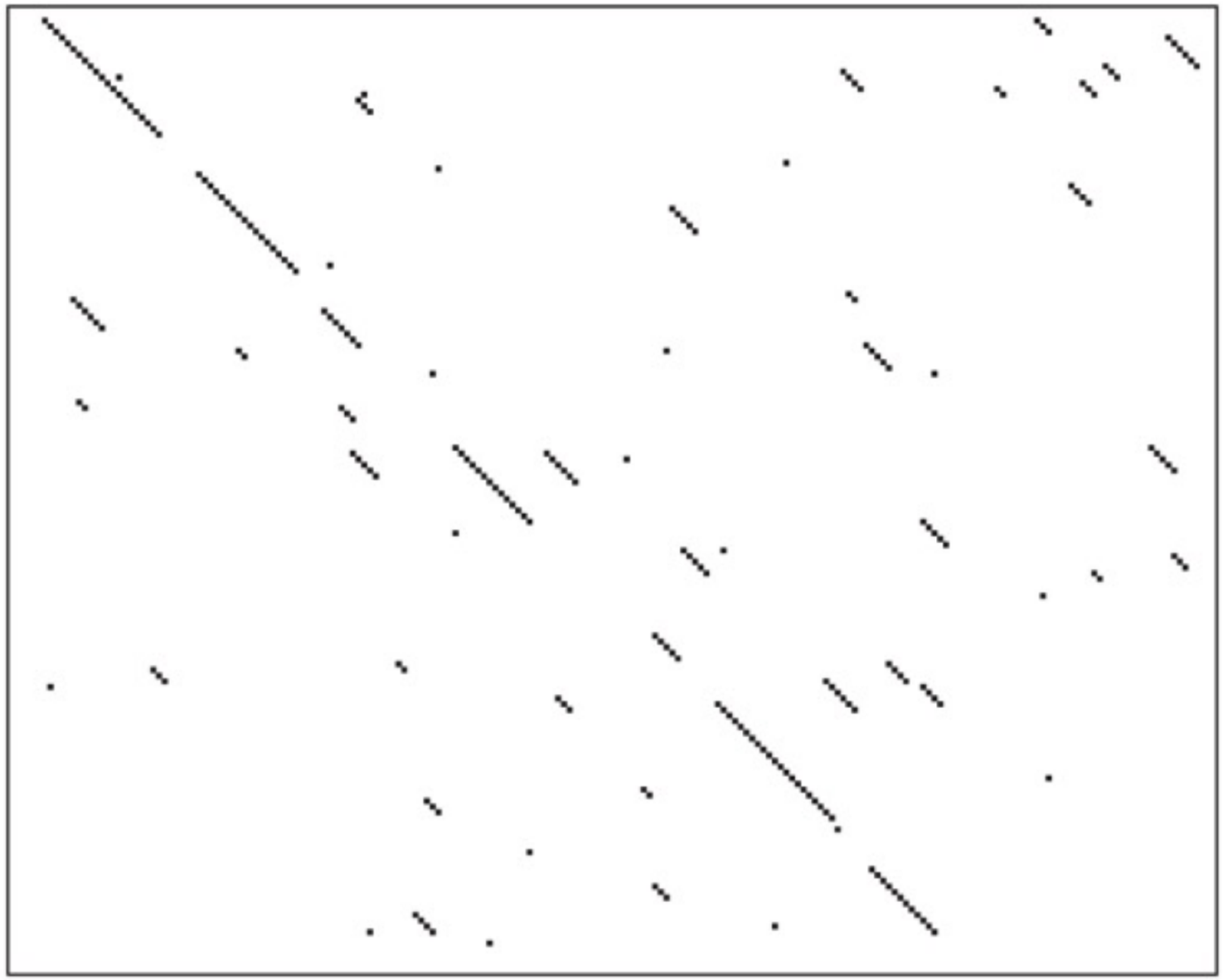
Dotplots

PAPA_CARPA / ACTN_ACTCH



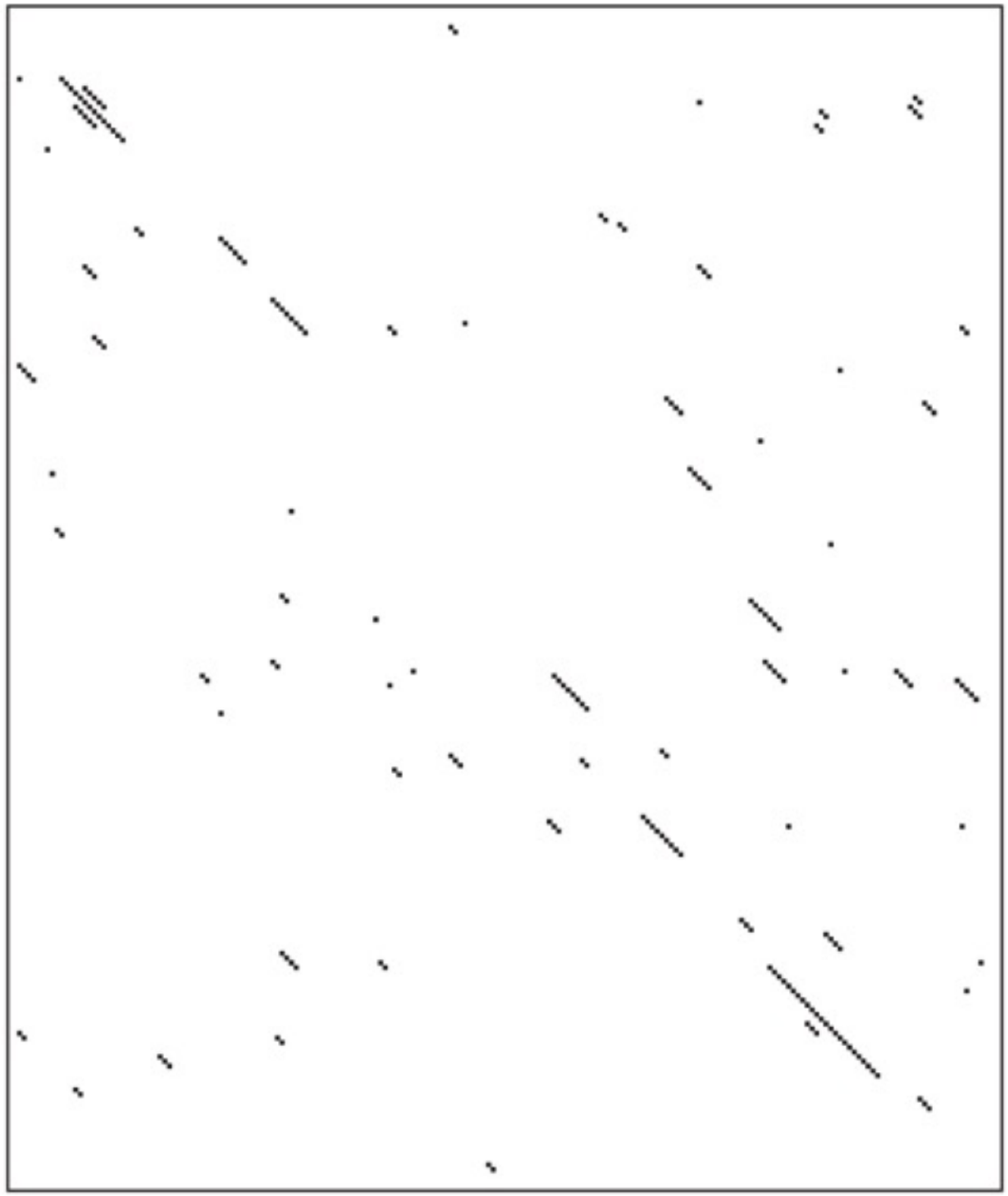
Dotplots

PAPA_CARPA / CATL_HUMAN



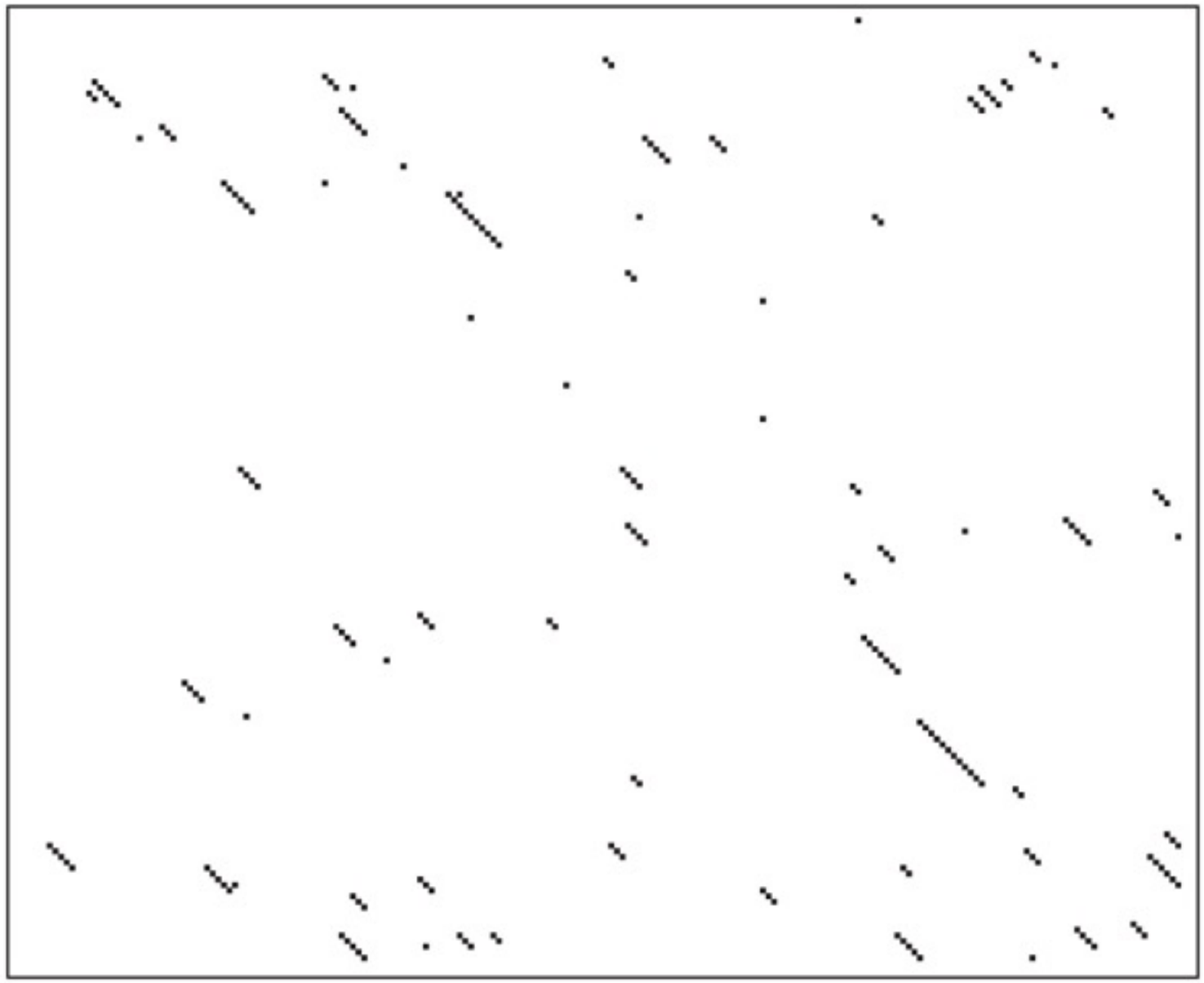
Dotplots

PAPA_CARPA / CATB_HUMAN

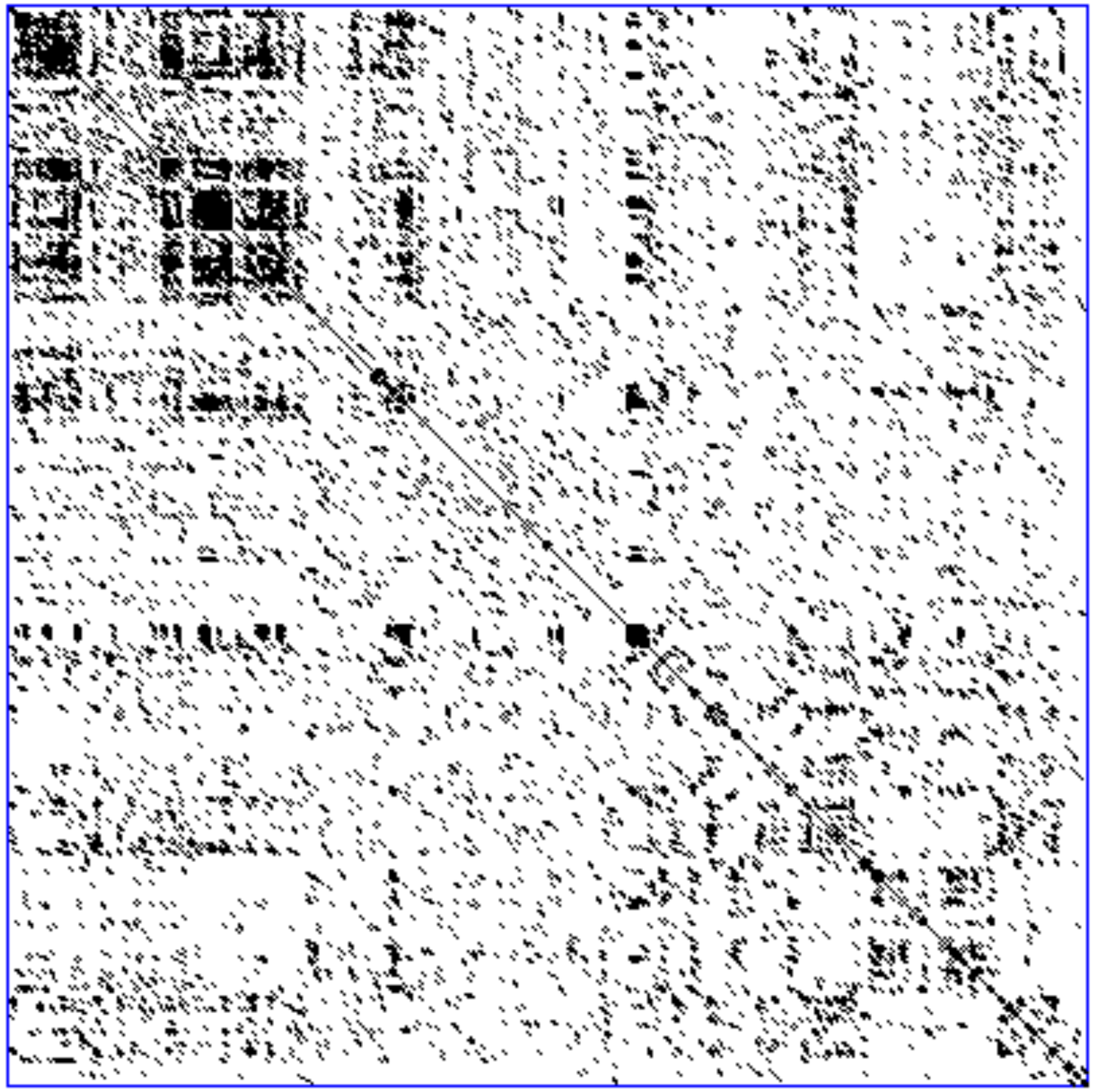


Dotplots

PAPA_CARPA / STPA_STAAU



Dotplots



Types of alignment

Types of alignment

(A) local

```
PI3-kinase  DRHNSNIMVKDDGQLFHIDFG
cAMP PK     DLKPENLLIDQQGYIQVTDFG
```

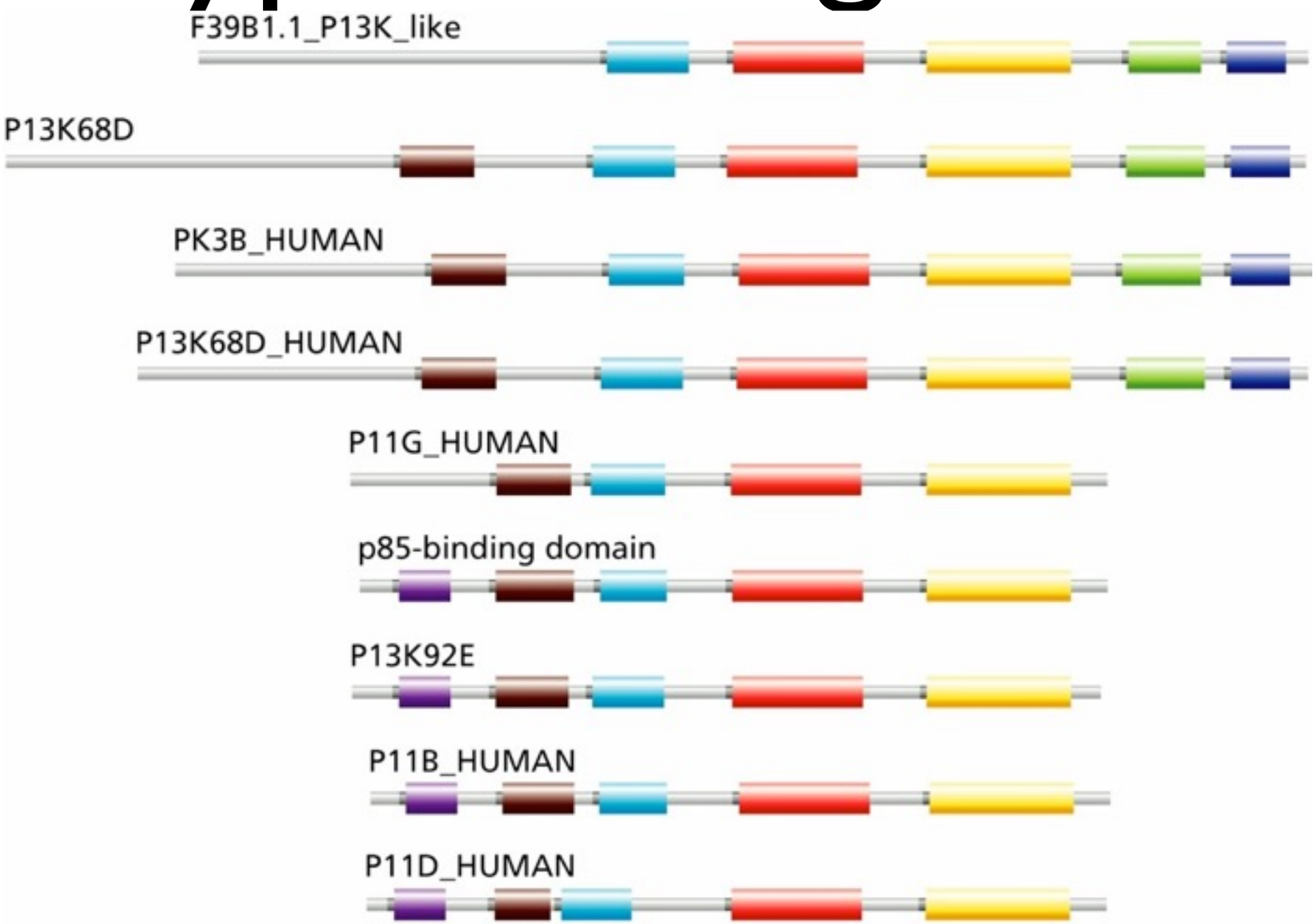
(B) global

```
                        10        20        30        40        50
PI3-kinase  HQLGNLR--LEECRI---MSSAKRPLWLNWENPDIMSELLFQNNEIIFKNGDDLRQDMLT
cAMP PK     GNAAAAKKGXEQESVKEFLAKAKEDFLKKWENPAQNTAHLDQFERIKTLGTGSFGRVML-
                      10        20        30        40        50

                 60        70        80        90       100       110
PI3-kinase  LQIIRIME--NIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQ-IQCKGGLKGAL
cAMP PK     ---VKHMETGNHYAMKILDKQKVVK--------LKQIEHTLNEKRILQAVNFPFLVKLEF
                60        70        80                  90       100

                      120       130       140       150       160
PI3-kinase  QFNSHT-LHQWLKDKNKGEIYDAA--IDLFTRSCAGYCVATFILGIGDRHNSNIMVKD-D
cAMP PK     SFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLK
           110       120       130       140       150       160

           170       180       190       200       210       220
PI3-kinase  GQLFHIDFGHFLDHKKKKFGYKRERVP-----FVLTQDFL---IVISKGAQECTKTREFE
cAMP PK     PENLLIDQQGYI--QVTDFGFAK-RVKGRTWXLCGTPEYLAPEIILSKGYNKAVDWWALG
           170       180       190       200       210       220

                 230       240       250       260       270
PI3-kinase  RF-QEMC--YKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEA
cAMP PK     VLIYEMAAGYPPFFA-DQPIQIYEKIVSGKVR--FPSHFSSDLKDLLRNLLQVDLTKR--
                230       240       250       260       270       280

            280       290       300                                 310
PI3-kinase  LEYFMKQMNDAHHGGWTTKMDWI-----------------------FHTIKQHALN----
cAMP PK     FGNLKNGVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVXIN
                     290       300       310       320       330       340
```

Types of alignment

```
Global FTFTALILLLAVAV
       F--TAL-LLA-AV

Local  FTFTALILL-AVAV
       --FTAL-LLAAV--
```

Types of alignment



Inserting gaps

(A)

```
Bovine PI-3Kinase p110a        LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQIQCKGGLKGAL
cAMP-dependent protein kinase --WENPAQNTAHLDQFERIKTLGTGSFGRVMLVKHMETGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVKLEFSFKDNSNLY

Bovine PI-3Kinase p110a        QFNSHTLHQWLKDKNKGEIYDAAIDLFTRSCAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLTQDF
cAMP-dependent protein kinase MVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWXLCGTPEYLAP

Bovine PI-3Kinase p110a        LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDAHHGG
cAMP-dependent protein kinase EIILSKGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEKIVSGKVRFPSHFSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKNHKWF

Bovine PI-3Kinase p110a        WTTKMDWIFHTIKQHALN-----------------------------------
cAMP-dependent protein kinase ATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVXINEKCGKEFSEF
```

(B)

```
Bovine PI-3Kinase p110a        LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQIQCKGGLKGAL
cAMP-dependent protein kinase ?-WENPAQNTAHLDQFERIKTLGTGSFGRVMLVKHM--ETGNHYAMKILDKQKV-VKLKQIEHTLNEKRILQAVNFPFLVKLEFSFKDN-

Bovine PI-3Kinase p110a        QFNSHTLHQWLKDKNKGEIYDAAIDLFTRSCAGYCVATFILGIGDRHNSNIMVKD-DGQLFHIDFGHFLDHKKKKFGYKRERVPFVL--T
cAMP-dependent protein kinase -SNLYMVMEYVPGGEMFSHLRR-IGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWXLCGT

Bovine PI-3Kinase p110a        QDFL---IVISKGAQECTKTREFERF-QEMC--YKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMK
cAMP-dependent protein kinase PEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFA-DQPIQIYEKIVSGKVRF--PSHFSSDLKDLLRNLLQVDLTKR--FGNLKN

Bovine PI-3Kinase p110a        QMNDAHHGGWTTKMDWI------------------------FHTIKQHAL----N----------
cAMP-dependent protein kinase GVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVXINEKCGKEFSEF
```

What is an optimal alignment ?

```
T H I S S E Q U E N C E
| |     | | | | | | | |        10/12 Identical
T H A T S E Q U E N C E

T H A T S E Q U E N C E
| |             |     |        4/12 Identical
T H I S I S A S E Q U E N C E

T H I S I S A - S E Q U E N C E
| |         |   | | | | | | | |    11/12 Identical
T H - - - - A T S E Q U E N C E
```

Different scoring

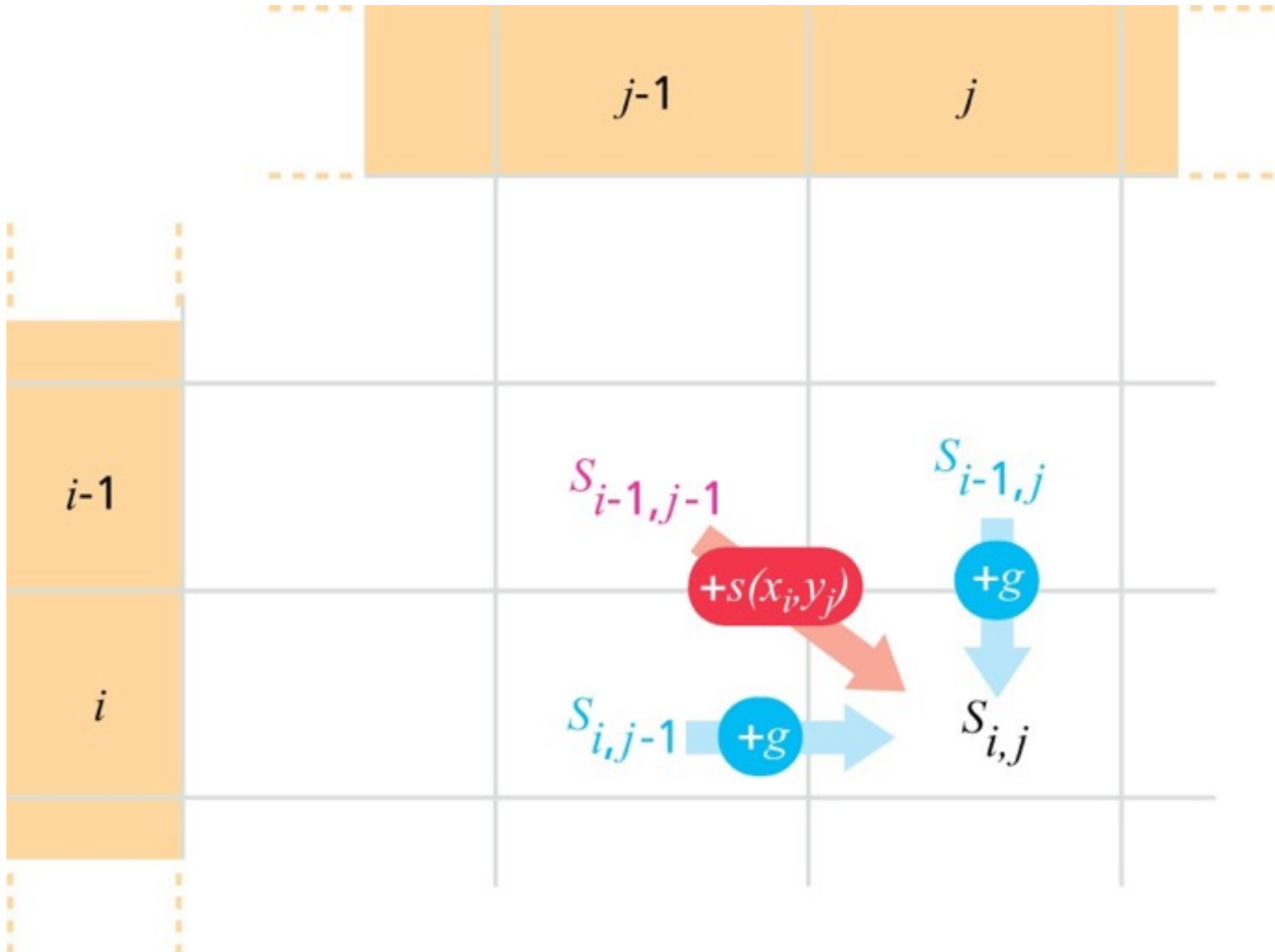
```
T H I S S E Q U E N C E
5 8-1 1 4 5 6 0 5 6 9 5        Score = 52
T H A T S E Q U E N C E

T H A T S E Q U E N C E
5 8-1-1-2 0-1 0 5 0 0 5        Score = 18
T H I S I S A S E Q U E N C E

T H I S I S A - S E Q U E N C E
5 8 0 0 0 0 4 0 4 5 6 0 5 6 9 5        Score = 56
T H - - - - A T S E Q U E N C E
```

With Gap cost

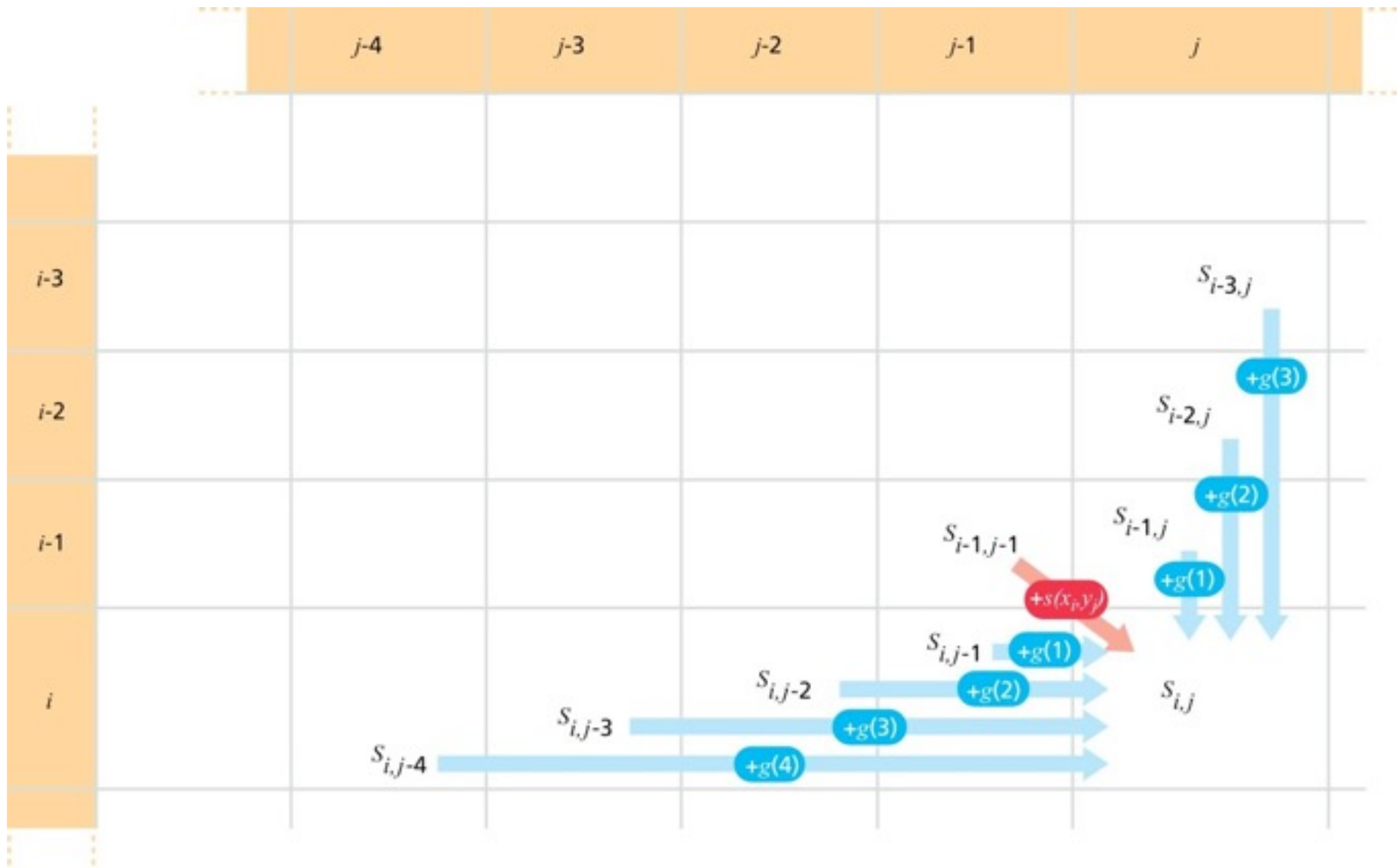
```
T H I S S E Q U E N C E
5 8-1 1 4 5 6 0 5 6 9 5        Score = 52
T H A T S E Q U E N C E

T H A T S E Q U E N C E
5 8-1-1-2 0-1 0 5 0 0 5        Score = 18
T H I S I S A S E Q U E N C E

T H I S I S A - S E Q U E N C E
5 8-1-1-1-1 4-1 4 5 6 0 5 6 9 5        Score = 51
T H - - - A T S E Q U E N C E
```

Dynamic programming

Dynamic programming



Dynamic programming



Dynamic programming

Initialisation step: Create Matrix with M + 1 columns and N + 1 rows. First row and column filled with 0.

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0											
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

Matrix fill step: Each position $M_{i,j}$ is defined to be the MAXIMUM score at position i,j

$M_{i,j}$ = MAXIMUM [

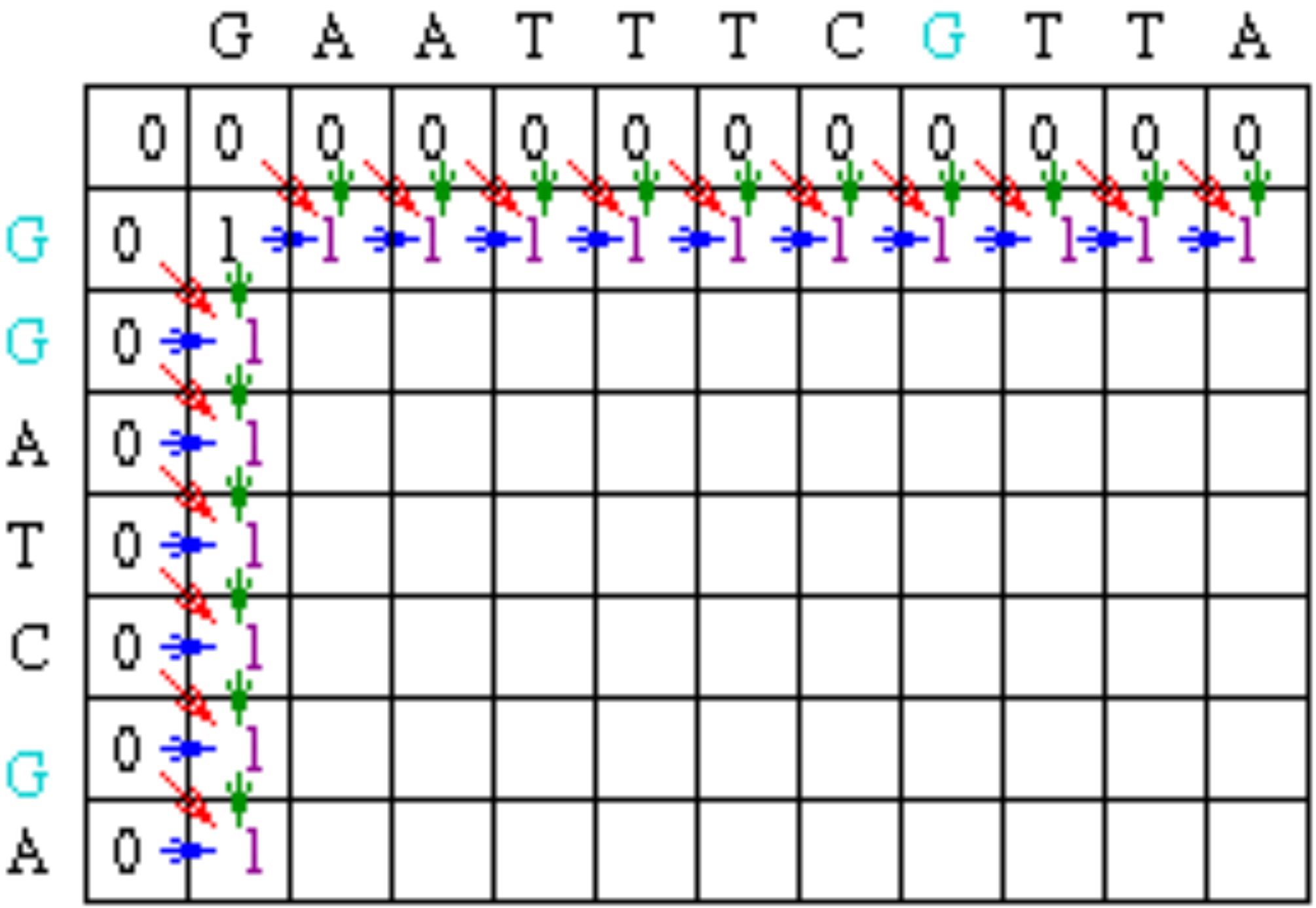
$M_{i-1,\,j-1} + s_{i,,j}$ (match or mismatch in the diagonal)

$M_{i,\,j-1} + w$ (gap in sequence #1)

$M_{i-1,\,j} + w$ (gap in sequence #2)]

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1										
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

Fill in rest of row 1 and column 1



Fill in column 2

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1	1	1	1	1	1	1	1	1	1	1
G	0	1	1									
A	0	1	2									
T	0	1	2									
C	0	1	2									
G	0	1	2									
A	0	1	2									

Fill in column 3

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1	1	1	1	1	1	1	1	1	1	1
G	0	1	1									
A	0	1	2									
T	0	1	2									
C	0	1	2									
G	0	1	2									
A	0	1	2									

Column 3 with answers

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1	1	1	1	1	1	1	1	1	1	1
G	0	1	1	1								
A	0	1	2	2								
T	0	1	2	2								
C	0	1	2	2								
G	0	1	2	2								
A	0	1	2	3								

Fill in rest of matrix with answers

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1	1	1	1	1	1	1	1	1	1	1
G	0	1	1	1	1	1	1	1	2	2	2	2
A	0	1	2	2	2	2	2	2	2	2	2	3
T	0	1	2	2	3	3	3	3	3	3	3	3
C	0	1	2	2	3	3	3	4	4	4	4	4
G	0	1	2	2	3	3	3	4	4	5	5	5
A	0	1	2	3	3	3	3	4	5	5	5	**6**

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	0
G	0	1	1	1	1	1	1	1	1	1	1	1
G	0	1	1	1	1	1	1	1	2	2	2	2
A	0	1	1	2	2	2	2	2	2	2	2	3
T	0	1	2	2	3	3	3	3	3	3	3	3
C	0	1	2	2	3	3	4	4	4	4	4	4
G	0	1	2	2	3	3	4	4	5	5	5	5
A	0	1	2	3	3	3	4	5	5	5	5	6

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	
G	0	1	1	1	1	1	1	1	1	1	1	
G	0	1	1	1	1	1	1	1	2	2	2	
A	0	1	1	2	2	2	2	2	2	2	2	
T	0	1	2	2	3	3	3	3	3	3	3	
C	0	1	2	2	3	3	4	4	4	4	4	
G	0	1	2	2	3	3	4	4	5	5	5	
A												6

```
Seq#1 A
      |
Seq#2 A
```

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0	0	
G	0	1	1	1	1	1	1	1	1	1	1	
G	0	1	1	1	1	1	1	1	2	2	2	
A	0	1	1	2	2	2	2	2	2	2	2	
T	0	1	2	2	3	3	3	3	3	3	3	
C	0	1	2	2	3	3	4	4	4	4	4	
G	0	1	2	2	3	3	4	4	5	5	5	
A												6

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0	0		
G	0	1	1	1	1	1	1	1	1	1		
G	0	1	1	1	1	1	1	1	2	2		
A	0	1	1	2	2	2	2	2	2	2		
T	0	1	2	2	3	3	3	3	3	3		
C	0	1	2	2	3	3	4	4	4	4		
G	0	1	2	2	3	3	4	4	5	5	5	
A												6

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0	0			
G	0	1	1	1	1	1	1	1	1			
G	0	1	1	1	1	1	1	1	2			
A	0	1	1	2	2	2	2	2	2			
T	0	1	2	2	3	3	3	3	3			
C	0	1	2	2	3	3	4	4	4			
G	0	1	2	2	3	3	4	4	5	5	5	
A												6

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0	0				
G	0	1	1	1	1	1	1	1				
G	0	1	1	1	1	1	1	1				
A	0	1	1	2	2	2	2	2				
T	0	1	2	2	3	3	3	3				
C	0	1	2	2	3	3	4	4				
G									5	5	5	
A												6

Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0	0					
G	0	1	1	1	1	1	1					
G	0	1	1	1	1	1	1					
A	0	1	1	2	2	2	2					
T	0	1	2	2	3	3	3					
C	0	1	2	2	3	3	4	4				
G									5	5	5	
A												6

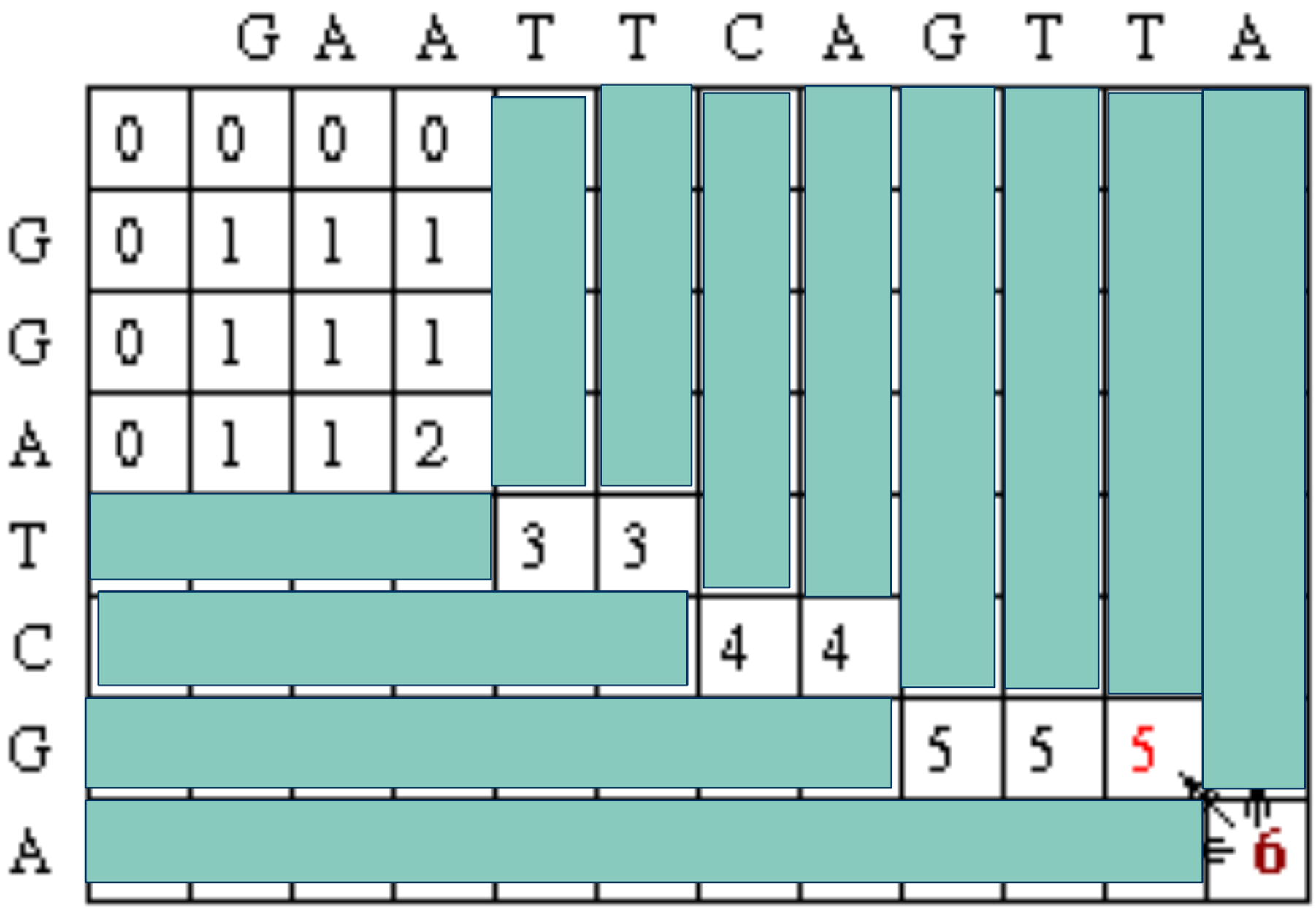
Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0	0						
G	0	1	1	1	1	1						
G	0	1	1	1	1	1						
A	0	1	1	2	2	2						
T	0	1	2	2	3	3						
C							4	4				
G									5	5	5	
A												6

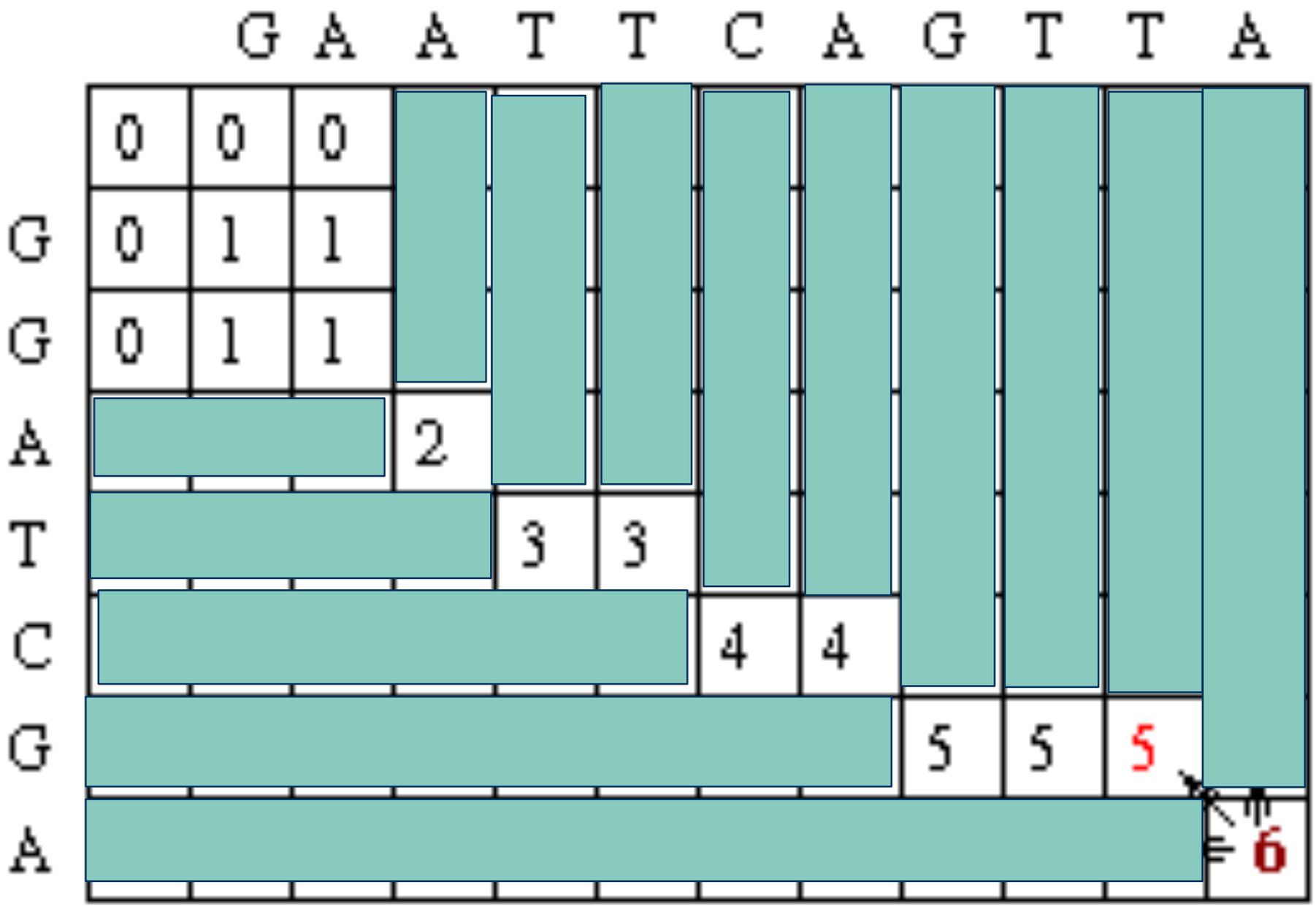
Traceback step:
Position at current cell and look at direct predecessors

		G	A	A	T	T	C	A	G	T	T	A
	0	0	0	0	0							
G	0	1	1	1	1							
G	0	1	1	1	1							
A	0	1	1	2	2							
T	0	1	2	2	3	3						
C							4	4				
G									5	5	5	
A												6

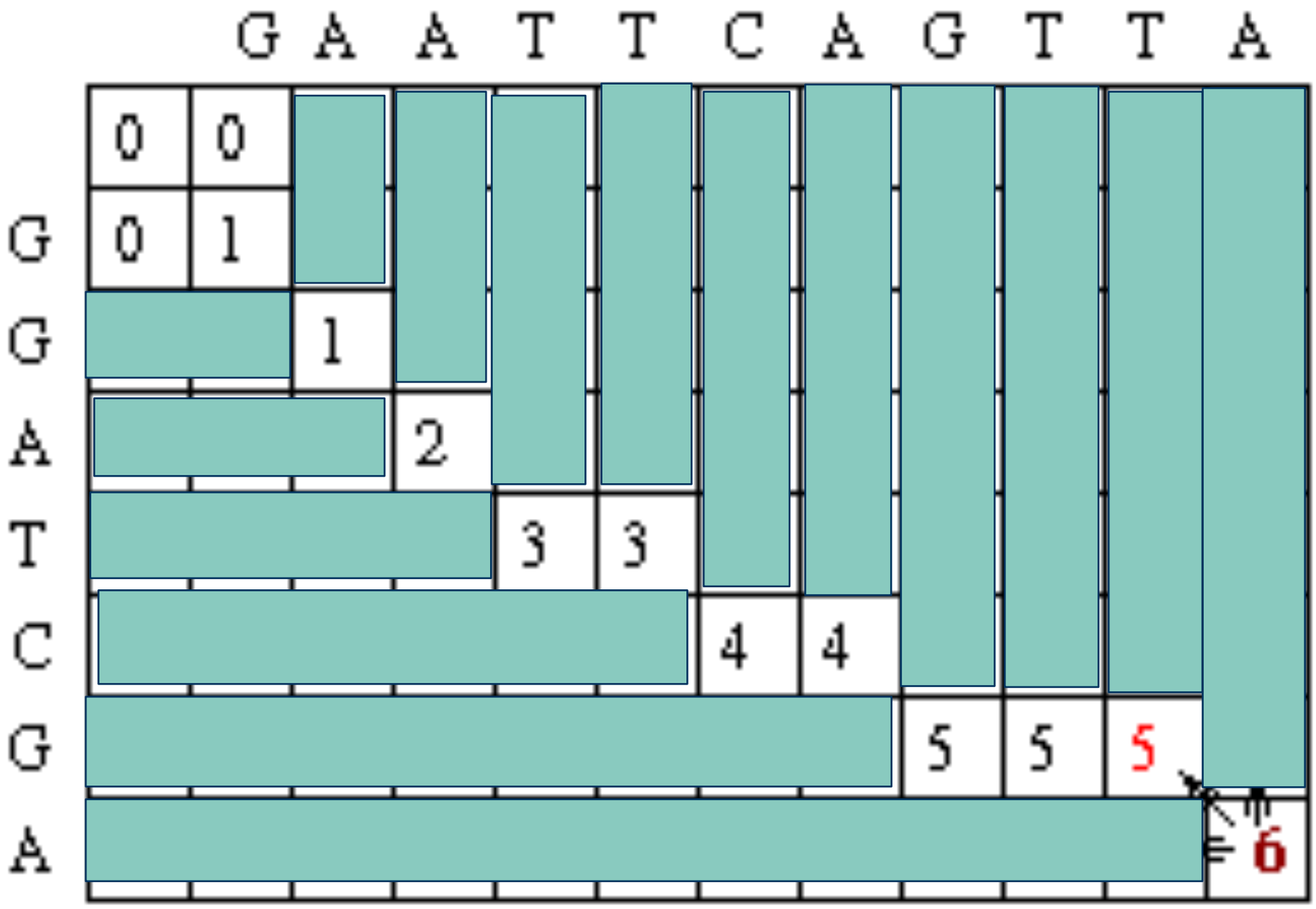
Traceback step:
Position at current cell and look at direct predecessors



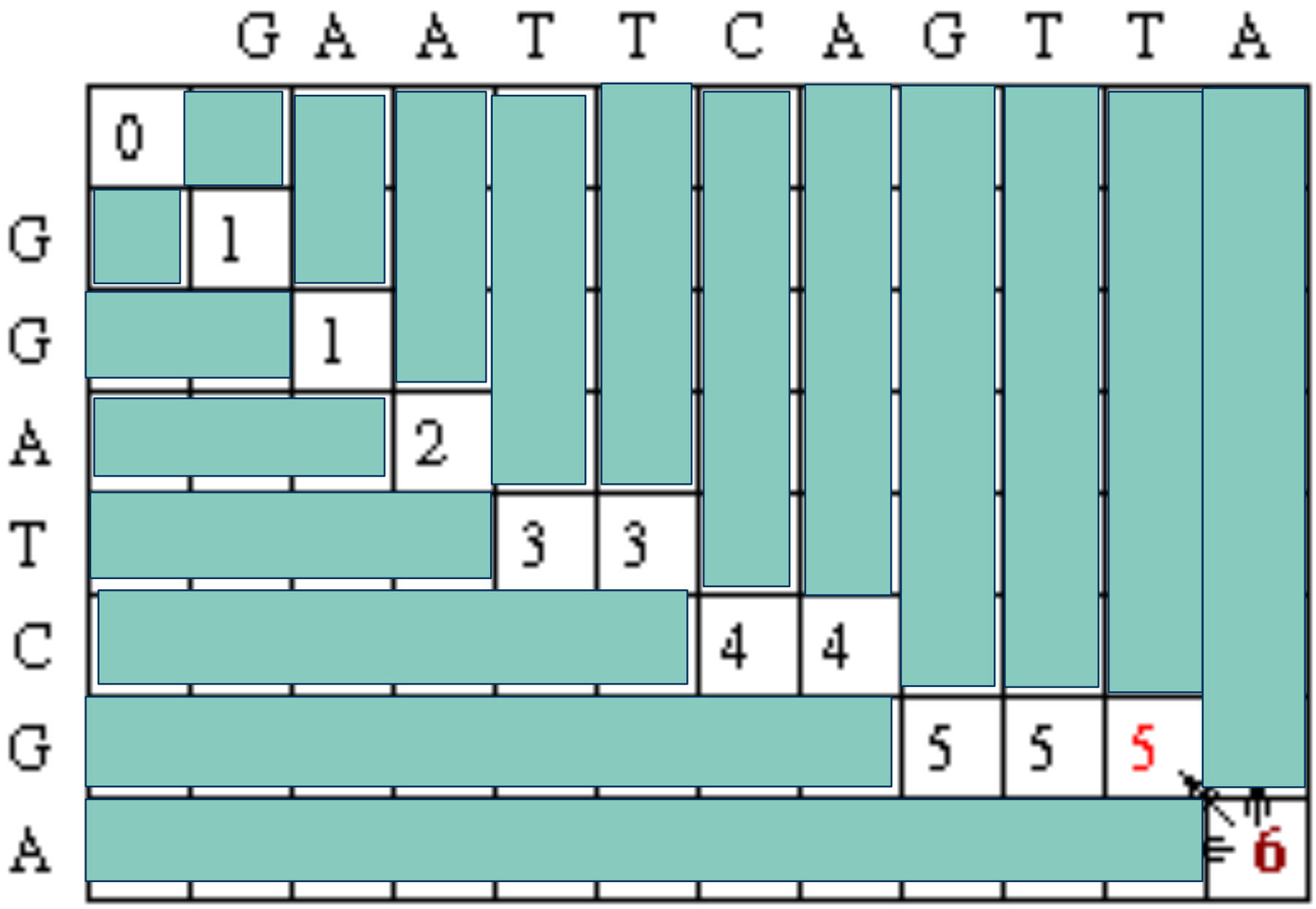
Traceback step:
Position at current cell and look at direct predecessors



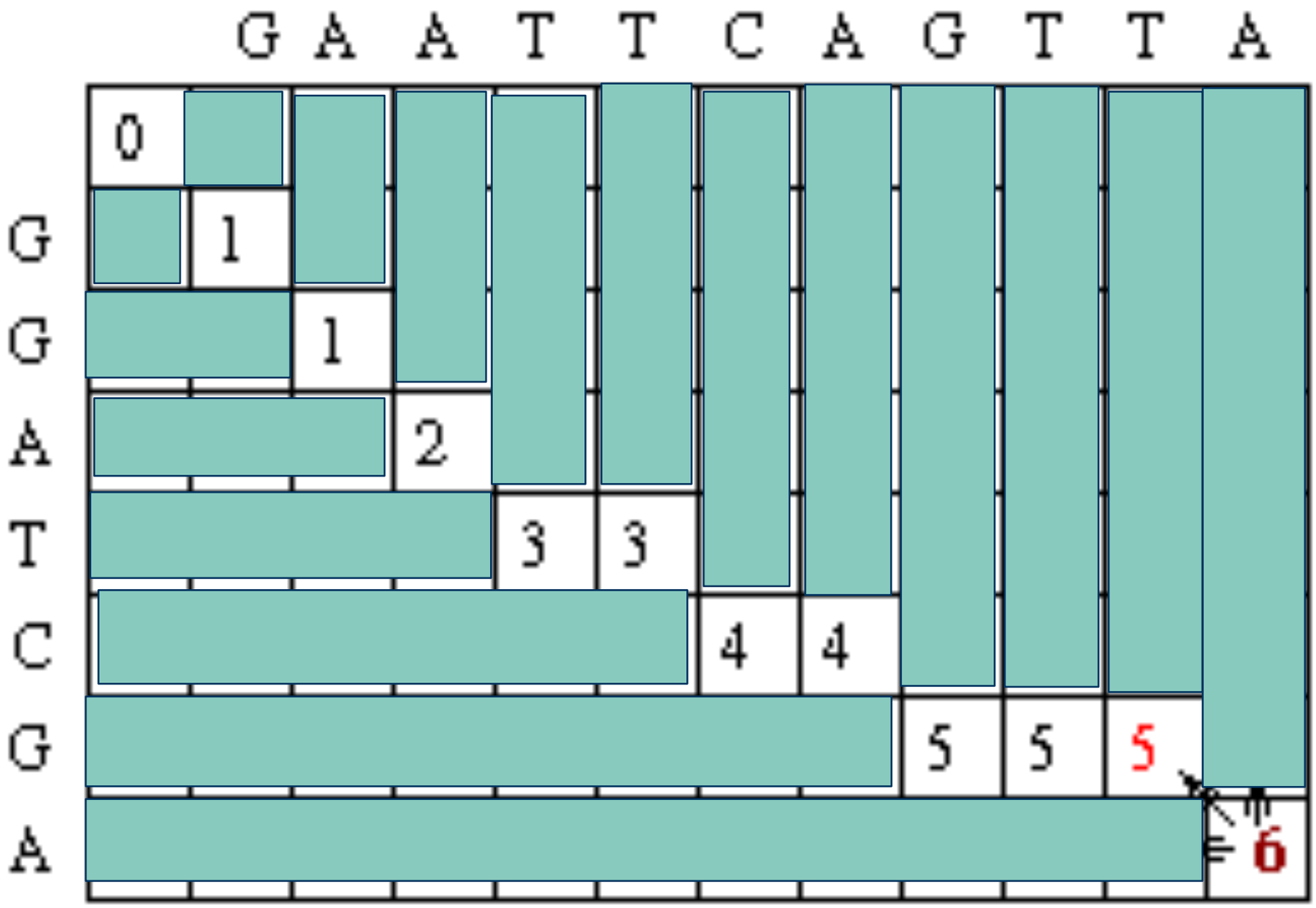
Traceback step:
Position at current cell and look at direct predecessors



Traceback step:
Position at current cell and look at direct predecessors



Traceback step:
Position at current cell and look at direct predecessors




```
Seq#1   G A A T T C A G T T A
        |   | |   |   |     |
Seq#2   G G A T - C - G - - A
```

Pseudocode

```
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(Ai, Bj)
    Delete ← F(i-1, j) + d
    Insert ← F(i, j-1) + d
    F(i,j) ← max(Match, Insert,
Delete)
  }
```

Traceback

```
AlignmentA ← ""
AlignmentB ← ""
 i ← length(A)
 j ← length(B)
while (i > 0 or j > 0)
{
if (i > 0 and j > 0 and F(i,j) == F(i-1,j-1) + S(Ai, Bj))
{
AlignmentA ← Ai + AlignmentA
AlignmentB ← Bj + AlignmentB
i ← i - 1
j ← j - 1
}
else if (i > 0 and F(i,j) == F(i-1,j) + d)
{
AlignmentA ← Ai + AlignmentA
AlignmentB ← "-" + AlignmentB
i ← i - 1
}
else (j > 0 and F(i,j) == F(i,j-1) + d)
{
AlignmentA ← "-" + AlignmentA
AlignmentB ← Bj + AlignmentB
j ← j - 1
}
}
```

Substitution matrices

Substitution matrices

Scoring Matrices

S = [s_{ij}] gives score of aligning character i with character j for every pair i, j.

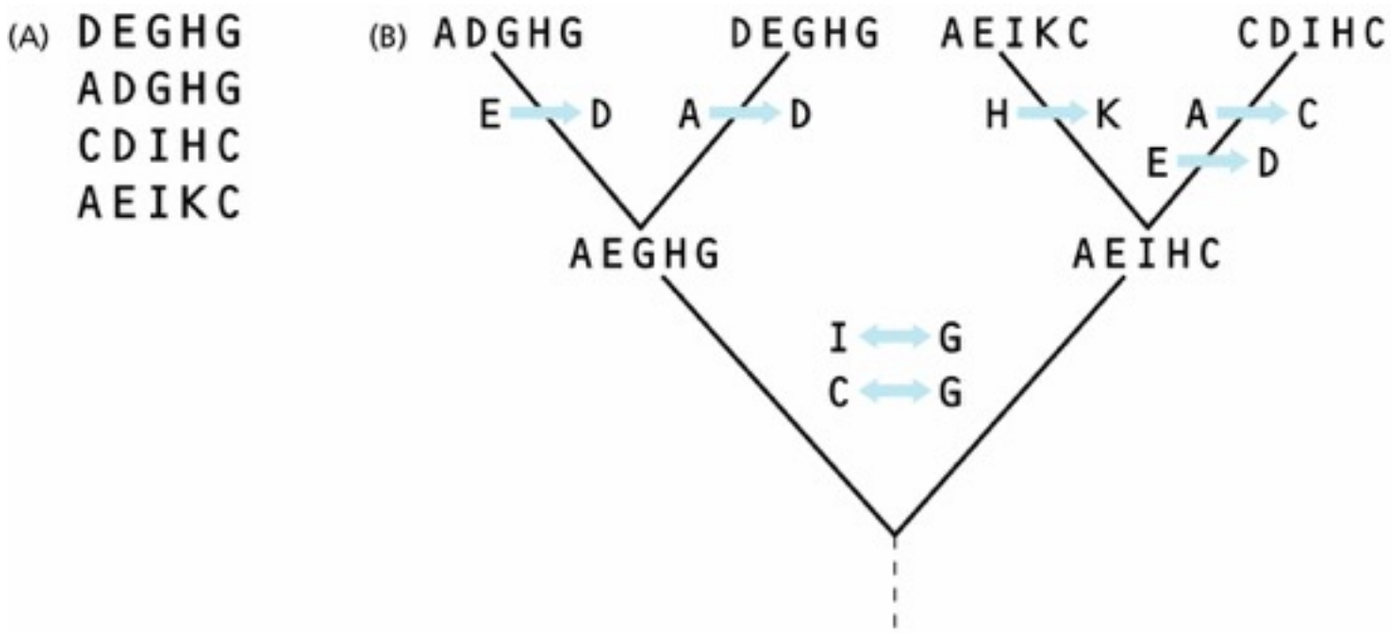
	C	S	T	P	A
C	12				
S	0	2			
T	-2	1	3		
P	-3	1	0	6	
A	-2	1	1	1	2

STPP
CTCA

0 + 3 + (-3) + 1

= 1

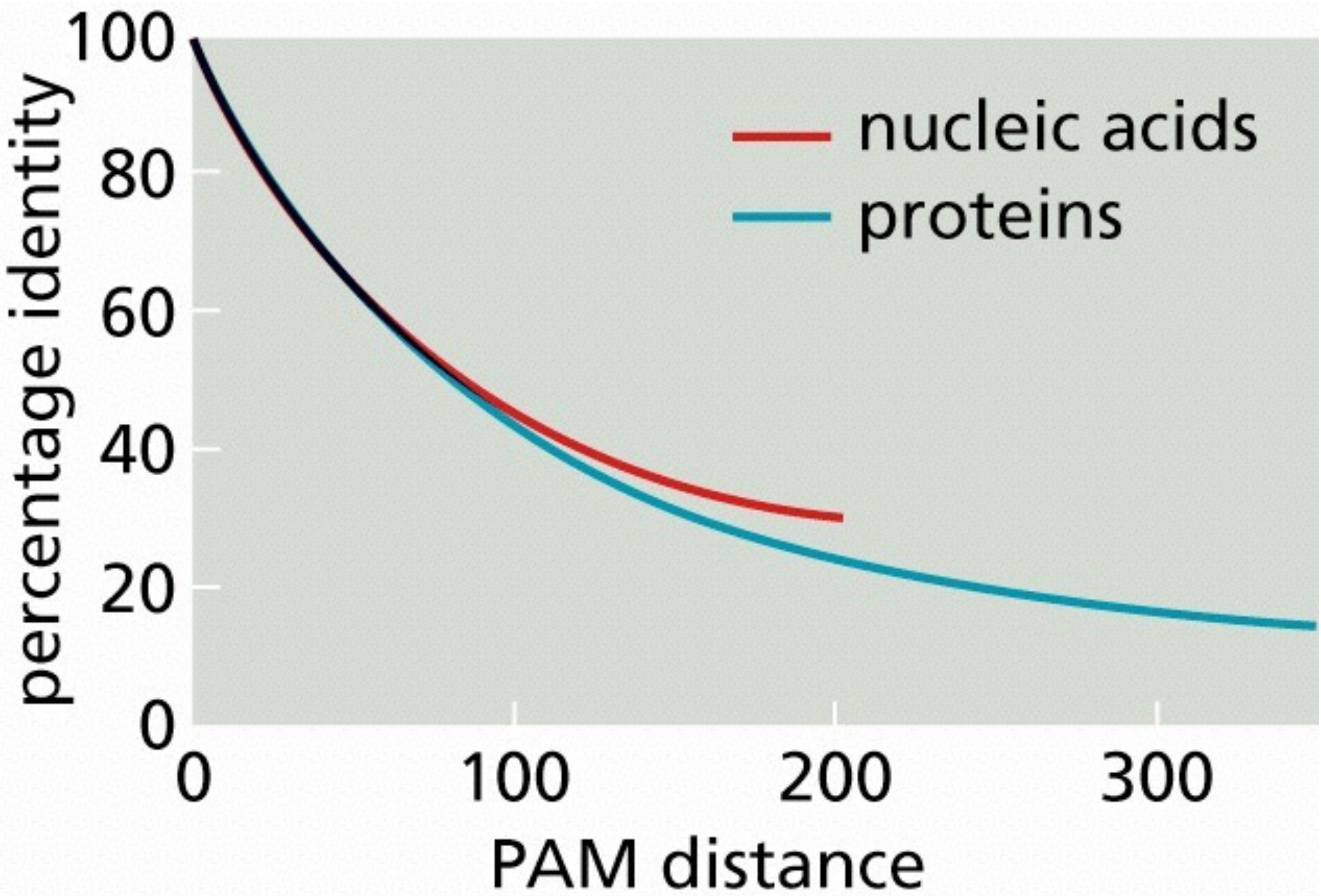
Substitution matrices



(C)

	A	C	D	E	G	H	I	K
A		1	1					
C	1				1			
D	1			2				
E			2					
G		1					1	
H								1
I					1			
K						1		

Substitution matrices



Log Odds Ratios

$$S_{i,j} = \log \frac{p_i \cdot M_{i,j}}{p_i \cdot p_j} = \log \frac{M_{i,j}}{p_j} = \log \frac{observed\ frequency}{expected\ frequency}$$

Point Accepted Mutations (PAM)

$$\mathrm{PAM}_n(i,j) = log\frac{f(i)M^n(i,j)}{f(i)f(j)} = log\frac{M^n(i,j)}{f(j)}$$

Point Accepted Mutations (PAM)

$$\mathrm{PAM}_n(i,j) = log\frac{f(i)M^n(i,j)}{f(i)f(j)} = log\frac{M^n(i,j)}{f(j)}$$

The PAM Family

Define a ***family*** of substitution matrices — PAM 1, PAM 2, etc. — where PAM n is used to compare sequences at distance n PAM.

$$\text{PAM } n = (\text{PAM } 1)^n$$

Do not confuse with scoring matrices!

Scoring matrices are derived from PAM matrices to yield log-odds scores.

Point Accepted Mutations (PAM)

$$\mathrm{PAM}_n(i,j) = log\frac{f(i)M^n(i,j)}{f(i)f(j)} = log\frac{M^n(i,j)}{f(j)}$$

PAM matrices

- Let M be a PAM 1 matrix. Then,

$$\sum_i p_i(1 - M_{ii}) = 0.01$$

- **Reason:** M_{ii}s are the probabilities that a given amino acid does not change, so (1-M_{ii}) is the probability of mutating away from i.

Point Accepted Mutations (PAM)

$$\mathrm{PAM}_n(i,j) = log\frac{f(i)M^n(i,j)}{f(i)f(j)} = log\frac{M^n(i,j)}{f(j)}$$

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

Blosum

Blosum

(A)

	1	2	3	4	5
1	A	T	C	K	Q
2	A	T	C	R	N
3	A	S	C	K	N
4	S	S	C	R	N
5	S	D	C	E	Q
6	S	E	C	E	N
7	T	E	C	R	Q

(B)

	q_{QN}	q_{NN}	q_{QQ}	p_N	p_Q
C=62%	0.114	0.057	0.029	0.114	0.086
C=50%	0.117	0.025	0.058	0.084	0.117
C=40%	–	–	–	–	–

Blosum

Equivalent PAM and Blossum matrices (according to *H*)

- PAM100 ==> Blosum90
- PAM120 ==> Blosum80
- PAM160 ==> Blosum60
- PAM200 ==> Blosum52
- PAM250 ==> Blosum45

Blosum

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	11																			
S	1	2																		
T	-1	1	2																	
P	-2	1	1	6																
A	-1	1	2	1	2															
G	-1	1	-1	-1	1	5														
N	-1	1	1	-1	0	0	3													
D	-3	0	-1	-2	0	1	2	5												
E	-4	-1	-1	-2	-1	0	1	4	5											
Q	-3	-1	-1	0	-1	-1	0	1	2	5										
H	0	-1	-1	0	-2	-2	1	0	0	2	6									
R	-1	-1	-1	-1	-1	0	0	-1	0	2	2	5								
K	-3	-1	-1	-2	-1	-1	1	0	1	2	1	4	5							
M	-2	-1	0	-2	-1	-3	-2	-3	-3	-2	-2	-2	-2	6						
I	-2	-1	1	-2	0	-3	-2	-3	-3	-3	-3	-3	-3	3	4					
L	-3	-2	-1	0	-1	-4	-3	-4	-4	-2	-2	-3	-3	3	2	5				
V	-2	-1	0	-1	1	-2	-2	-2	-2	-3	-3	-3	-3	2	4	2	4			
F	0	-2	-2	-3	-3	-5	-3	-5	-5	-4	0	-4	-5	0	0	2	0	8		
Y	2	-1	-3	-3	-3	-4	-1	-2	-4	-2	4	-2	-3	-2	-2	-1	-3	5	9	
W	1	-3	-4	-4	-4	-2	-5	-5	-5	-3	-3	0	-3	-3	-4	-2	-3	-1	0	15

Difference between Pam and Blosum

- PAM matrices are based on an explicit evolutionary model (i.e. replacements are counted on the branches of a phylogenetic tree), whereas the BLOSUM matrices are based on an implicit model of evolution.
- The PAM matrices are based on mutations observed throughout a global alignment, this includes both highly conserved and highly mutable regions. The BLOSUM matrices are based only on highly conserved regions in series of alignments forbidden to contain gaps.
- The method used to count the replacements is different: unlike the PAM matrix, the BLOSUM procedure uses groups of sequences within which not all mutations are counted the same.
- Higher numbers in the PAM matrix naming scheme denote larger evolutionary distance, while larger numbers in the BLOSUM matrix naming scheme denote higher sequence similarity and therefore smaller evolutionary distance. Example: PAM150 is used for more distant sequences than PAM100; BLOSUM62 is used for closer sequences than BLOSUM50.

Nucleotide Matrices

Dayhoff's PAM matrix

	A	R	N	D	C
A	9867	2	9	10	3
R	1	9913	1	0	1
N	4	1	9822	36	0
D	6	0	42	9859	0
C	1	1	0	0	9973

All entries × 10^4

(A)

	A	C	G	T
A	67	-96	-20	-117
C	-96	100	-79	-20
G	-20	-79	100	-96
T	-117	-20	-96	67

(B)

	A	C	G	T
A	91	-114	-31	-123
C	-114	100	-125	-31
G	-31	-125	100	-114
T	-123	-31	-114	91

(C)

	A	C	G	T
A	100	-123	-28	-109
C	-123	91	-140	-28
G	-28	-140	91	-123
T	-109	-28	-123	100

Gap models

- Gap-extension
- Gap opening cost

Local and global

(A) local

```
PI3-kinase  DRHNSNIMVKDDGQLFHIDFG
cAMP PK     DLKPENLLIDQQGYIQVTDFG
```

(B) global

```
                        10                  20                  30                  40                  50
PI3-kinase  HQLGNLR--LEECRI---MSSAKRPLWLNWENPDIMSELLFQNNEIIFKNGDDLRQDMLT
cAMP PK     GNAAAAKKGXEQESVKEFLAKAKEDFLKKWENPAQNTAHLDQFERIKTLGTGSFGRVML-
                        10                  20                  30                  40                  50

                    60                  70                  80                  90                  100                 110
PI3-kinase  LQIIRIME--NIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQ-IQCKGGLKGAL
cAMP PK     ---VKHMETGNHYAMKILDKQKVVK--------LKQIEHTLNEKRILQAVNFPFLVKLEF
                    60                  70                  80                  90                  100

                            120                 130                 140                 150                 160
PI3-kinase  QFNSHT-LHQWLKDKNKGEIYDAA--IDLFTRSCAGYCVATFILGIGDRHNSNIMVKD-D
cAMP PK     SFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLDLIYRDLK
                    110                 120                 130                 140                 150                 160

                170                 180                 190                 200                 210                 220
PI3-kinase  GQLFHIDFGHFLDHKKKKFGYKRERVP-----FVLTQDFL---IVISKGAQECTKTREFE
cAMP PK     PENLLIDQQGYI--QVTDFGFAK-RVKGRTWXLCGTPEYLAPEIILSKGYNKAVDWWALG
                    170                 180                 190                 200                 210                 220

                        230                 240                 250                 260                 270
PI3-kinase  RF-QEMC--YKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEA
cAMP PK     VLIYEMAAGYPPFFA-DQPIQIYEKIVSGKVR--FPSHFSSDLKDLLRNLLQVDLTKR--
```

Global alignment
Needleman-Wunch

	GAP	M	N	A	L	S	D	R	T
GAP	0	-12	-16	-20	-24	-28	-32	-36	-40
M	-12	6 (6)	-6 (-2)	-10	-14	-18	-22	-26	-30
G	-16	-6 (-3)	6 (0)	-5	-10	-13	-17	-22	-26
S	-20	-10	-5	7	-5	-8	-13	-17	-21
D	-24	-14	-8	-5	3	-5	-4	-14	-17
R	-28	-18	-14	-9	-8	3	-6	2	-10
T	-32	-22	-18	-13	-11	-7	3	-7	5
T	-36	-26	-22	-17	-15	-10	-7	2	-4
E	-40	-30	-25	-21	-20	-15	-7	-8	2
T	-44	-34	-30	-24	-23	-19	-15	-8	-5

Local alignment
Smith Waterman

	GAP	M	N	A	L	S	D	R	T
GAP	0	0	0	0	0	0	0	0	0
M	0	6	0	0	4	0	0	0	0
G	0	0	6	1	0	5	1	0	0
S	0	0	1	7	0	2	5	1	1
D	0	0	2	1	3	0	6	4	1
R	0	0	0	0	0	3	0	12	3
T	0	0	0	1	0	1	3	0	15
T	0	0	0	1	0	1	1	2	3
E	0	0	1	0	0	0	4	0	2
T	0	0	0	2	0	1	0	3	3

Different alignments

Different alignments

(A)

		I	S	A	L	I	G	N	E	D
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72
T	-8	-1	-7	-15	-23	-31	-39	-47	-55	-63
H	-16	-9	-2	-9	-17	-25	-33	-40	-47	-55
I	-24	-12	-10	-3	-7	-13	-21	-29	-37	-45
S	-32	-20	-8	-9	-5	-9	-13	-20	-28	-35
L	-40	-28	-16	-9	-5	-3	-11	-16	-23	-31
I	-48	-36	-24	-17	-7	-1	-7	-14	-19	-26
N	-56	-44	-32	-25	-15	-9	-1	-1	-9	-17
E	-64	-52	-40	-33	-23	-17	-9	-1	4	-4

(B)

```
THISLINE-
  ||
ISALIGNED
```

Different alignments

(A)

		I	S	A	L	I	G	N	E	D
	0	-4	-8	-12	-16	-20	-24	-28	-32	-36
T	-4	-1	-3	-7	-11	-15	-19	-23	-27	-31
H	-8	-5	-2	-5	-9	-13	-17	-18	-22	-26
I	-12	-4	-6	-3	-3	-5	-9	-13	-17	-21
S	-16	-8	0	-4	-5	-5	-5	-8	-12	-16
L	-20	-12	-4	-1	0	-3	-7	-8	-11	-15
I	-24	-16	-8	-5	1	4	0	-4	-8	-12
N	-28	-20	-12	-9	-3	0	4	6	2	-2
E	-32	-24	-16	-13	-7	-4	0	4	11	7

(B)

```
THIS-LI-NE-
 || || || |
--ISALIGNED
```

Different alignments

(A)

		I	S	A	L	I	G	N	E	D
	0	0	0	0	0	0	0	0	0	0
T	0	0	1	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	1	0	0
I	0	4	0	0	2	4	0	0	0	0
S	0	0	8	1	0	0	4	1	0	0
L	0	2	0	7	5	2	0	1	0	0
I	0	4	0	0	9	9	1	0	0	0
N	0	0	5	0	1	6	9	7	0	1
E	0	0	0	4	0	0	4	9	12	4

(B)

```
SLI-NE
||  ||
ALIGNE
```

Different alignments

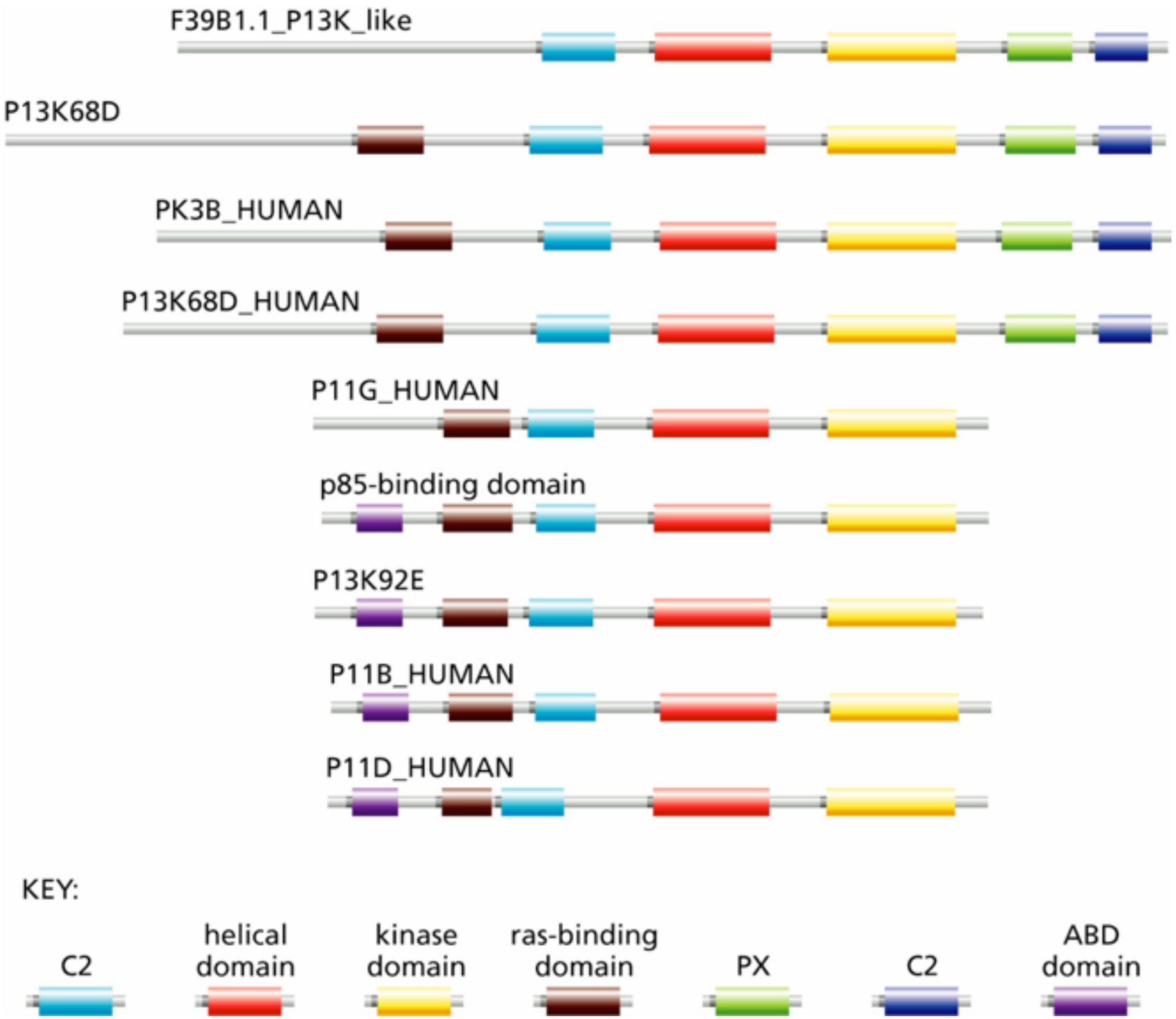
(A)

		I	S	A	L	I	G	N	E	D
	0	0	0	0	0	0	0	0	0	0
T	0	0	1	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	1	0	0
I	0	4	0	0	2	4	0	0	0	0
S	0	0	8	4	0	0	4	1	0	0
L	0	2	4	7	8	4	0	1	0	0
I	0	4	0	3	9	12	8	4	0	0
N	0	0	5	1	5	8	12	14	10	6
E	0	0	1	4	1	4	8	12	19	15

(B)

```
IS-LI-NE
||  ||  ||
ISALIGNE
```

Multidomain proteins



Next lecture

- O(nm) is too slow. How to speed up
- When is a “score” significant.