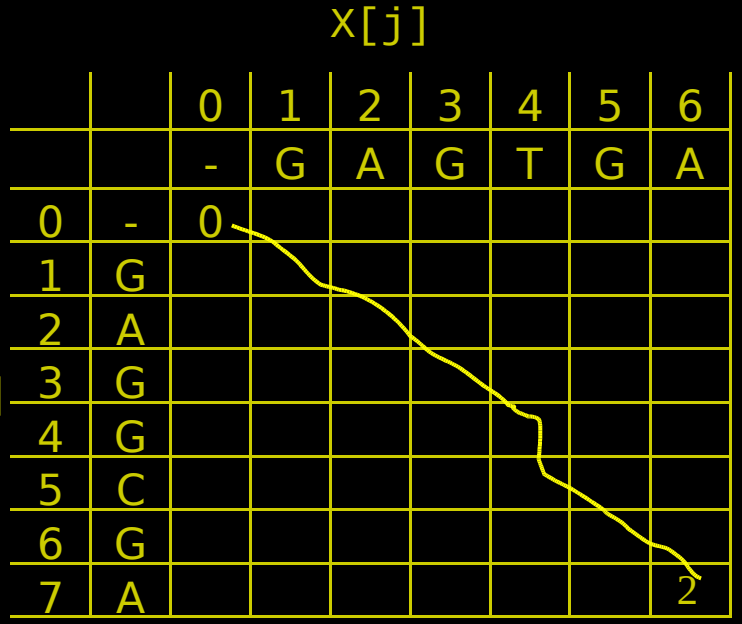
Global alignments – review

- Take two sequences: X[j] and Y[j]
- The best alignment for X[1…i] and Y[1…j] is called M[i, j]
- Initiation: M[0,0]=0
- Apply the equation
- Find the alignment with backtracing

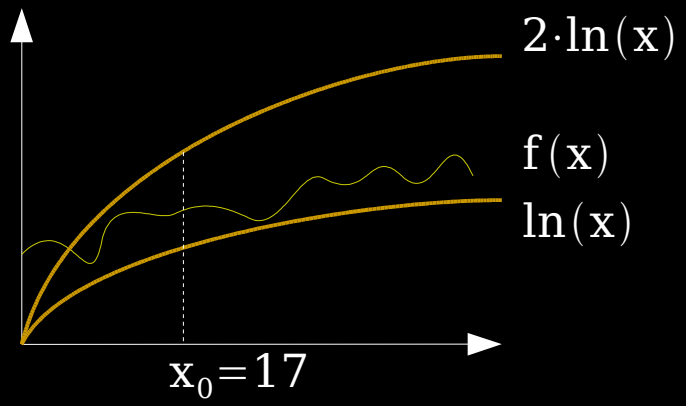
$$M[i, j] = \max \begin{cases} M[i-1, j-1] \pm 1 \\ M[i, j-1] - 2 \\ M[i-1, j] - 2 \end{cases}$$



Algorithm time/space complexity – Big–O Notation

- a simple description of complexity:
  - constant O(1), linear O(n), quadratic $O(n^2)$, cubic $O(n^3)$...
- asymptotic upper bound
- read: “order of”

$$f(n)=O(\underbrace{g(n)}_{\text{simple, e.g. } n^2}) \text{ iff. } \underset{x_0, c}{\exists} \underset{x \geq x_0}{\forall} f(x) \leq c g(x)$$



Big–O Notation Example

- Time complexity of global alignment:

$$M[i, j] = \max \begin{cases} M[i\text{-}1, j\text{-}1] \pm 1 \\ M[i, j\text{-}1] - 2 \\ M[i\text{-}1, j] - 2 \end{cases}$$

$$\underbrace{n + m - 1}_{\text{init}} + \underbrace{10nm}_{\text{calc M}} + \underbrace{1}_{\text{print}} = O(nm)$$

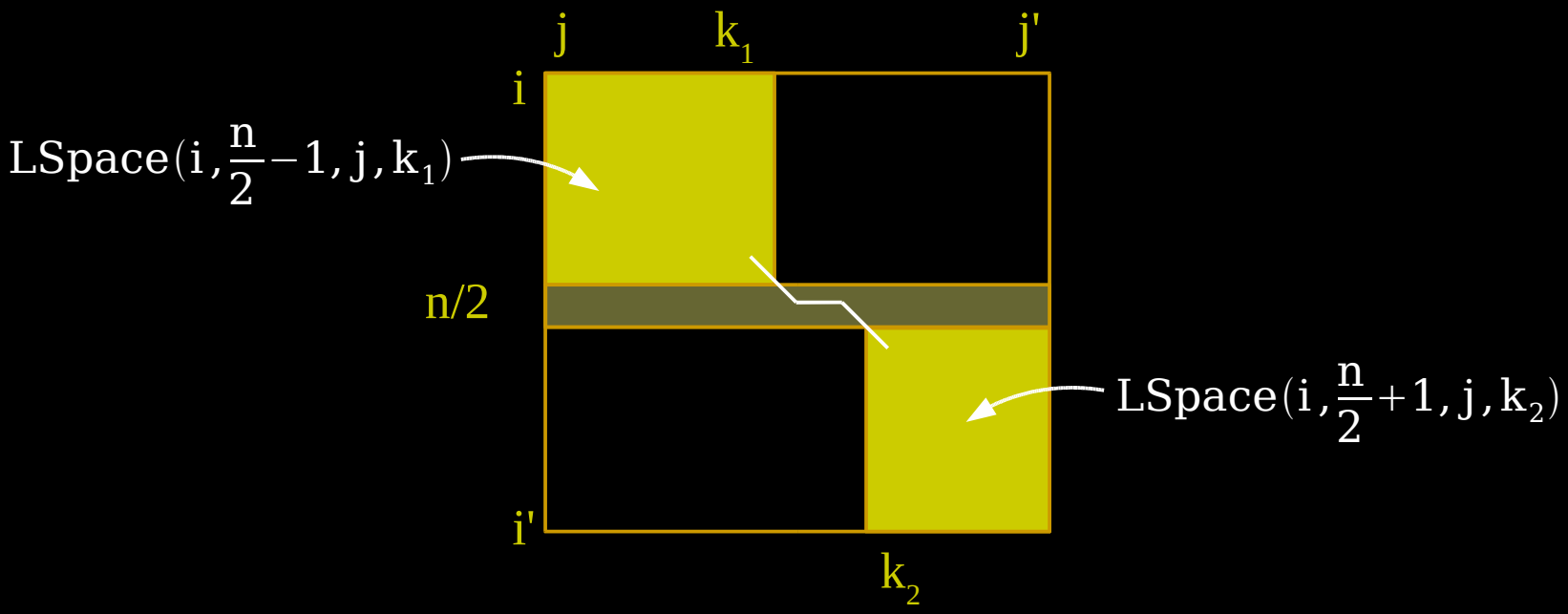
Global alignment – linear space

$$M[i, j] = \max \begin{cases} M[i-1, j-1] \pm 1 \\ M[i, j-1] - 2 \\ M[i-1, j] - 2 \end{cases}$$

- We need O(nm) time, but only O(m) space
  - how?
- problem with backtracking

Global alignment – linear space, recursion

LSpace(i,i',j,j'):



- space complexity: O(m)

Global alignment – linear space, algorithm

LSpace(i, i', j, j'):

return if area(i, i', j, j') empty

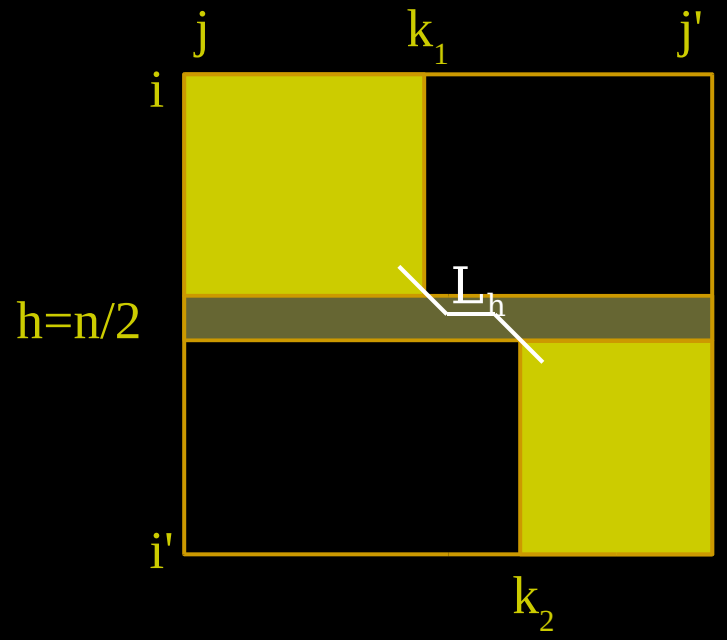
$h := \frac{i' - i}{2}$

calc. M using O(m) memory
plus find path L_h
crossing the row h

LSpace(i, h − 1, k_1, j')

print L_h

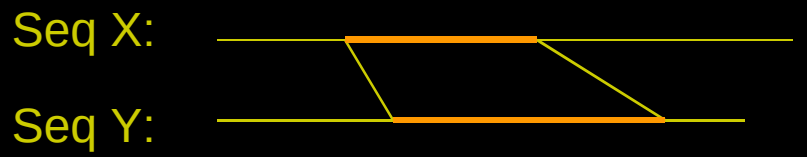
LSpace(i, h + 1, k_2, j')



- time complexity: $\sum_{i=0}^{\log_2 n} \frac{nm}{2^i} \leq 2nm$

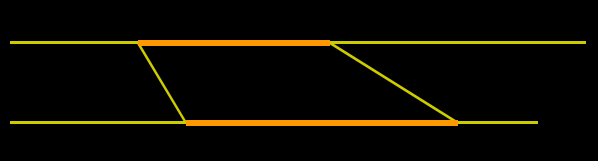
Alignments: Local alignment

Local alignment: Smith–Waterman algorithm

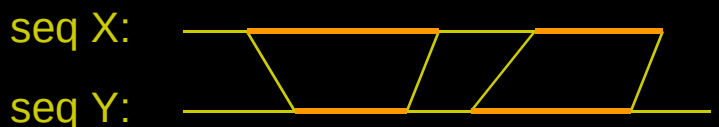


- What's local?
  - Allow only parts of the sequence to match
  - Locally maximal: can not make it better by trimming/extending the alignment

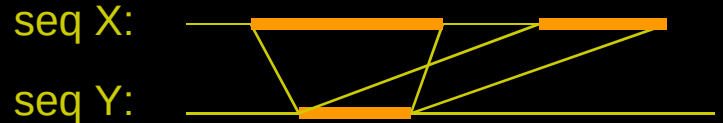
Local alignment



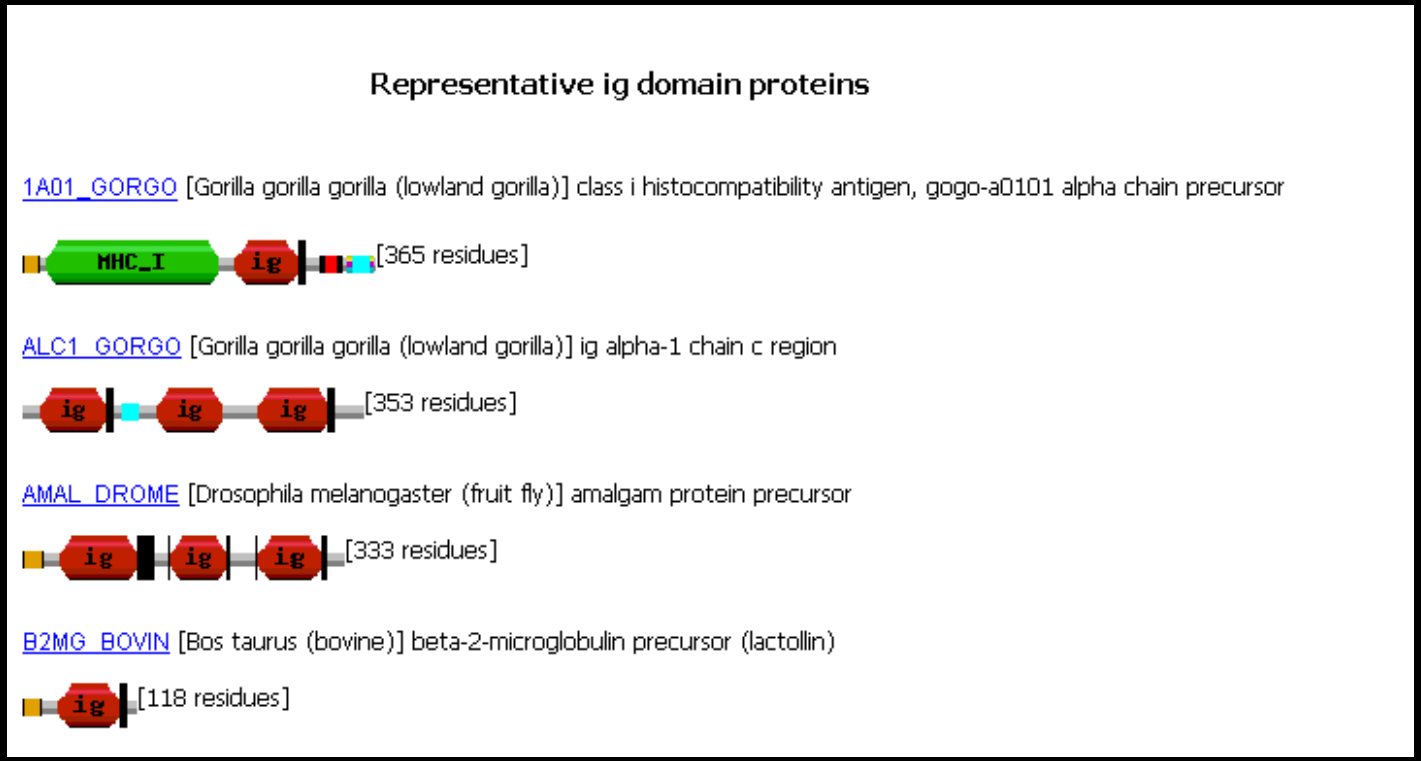
- Why local?
  - Parts of sequence diverge faster
    evolutionary pressure does not put constraints on *the whole* sequence



  - Proteins have modular construction
    sharing domains between sequences



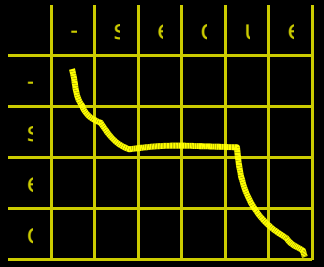
Domains – example
Immunoglobulin domain



Global → local alignment

- Take the *global* equation
- Look at the result of the *global* alignment

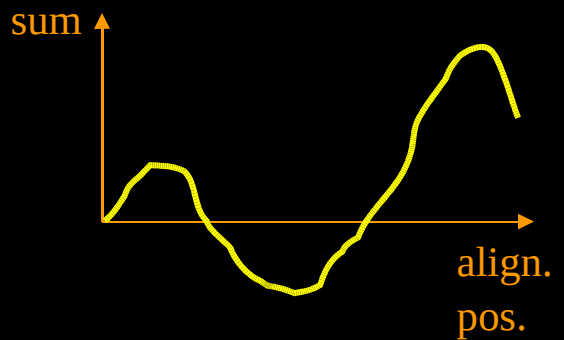
a) global align



b) retrieve the result

```
CAGCACTTGGATTCTCG-
CA-C-----GATTCGT-G
```

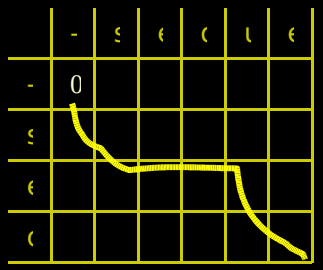
c) sum score along the result

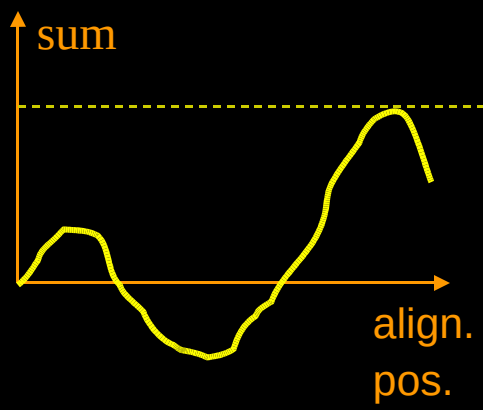


Local alignment – breaking the alignment

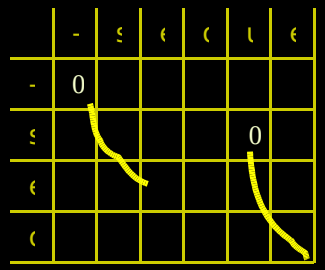
- A recipe
  - Just don't let the score go below 0
  - Start the new alignment when it happens
  - Where is the result in the matrix?

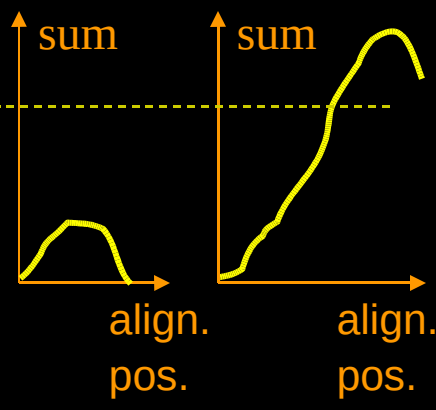
Before:





After:



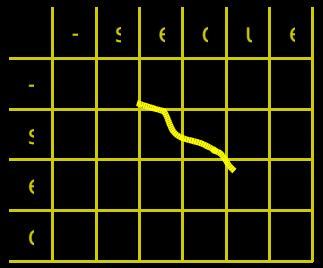


Local alignment – the equation

$$M[i, j] = \max \begin{cases} M[i-1, j-1] + score(X[i], Y[j]) \\ M[i, j-1] - g \\ M[i-1, j] - g \\ 0 \end{cases}$$

Great contribution to science!

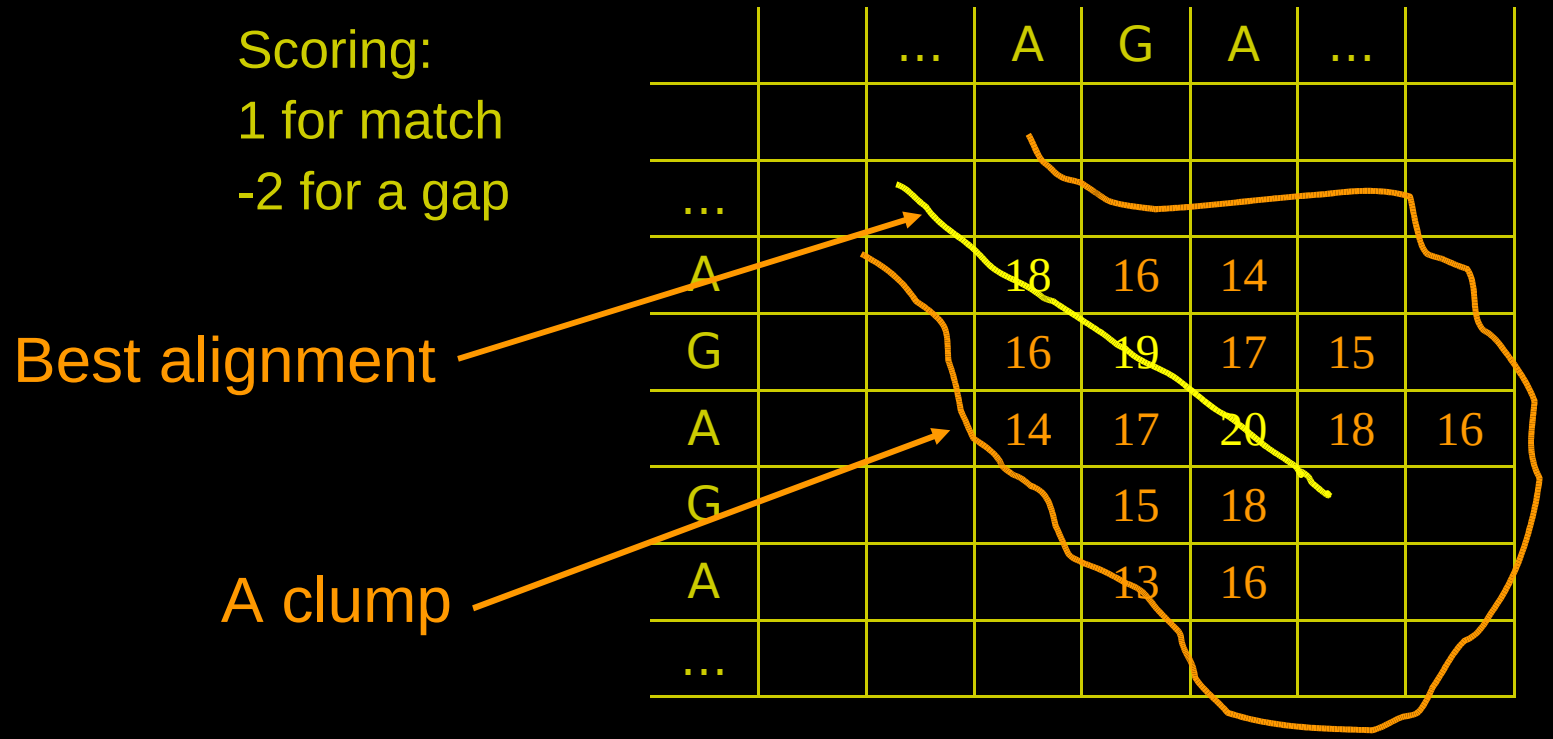
- Init the boundaries with 0's
- Run the algorithm
- Read the maximal value from anywhere in the matrix
- Find the result with backtracking



Finding second best alignment

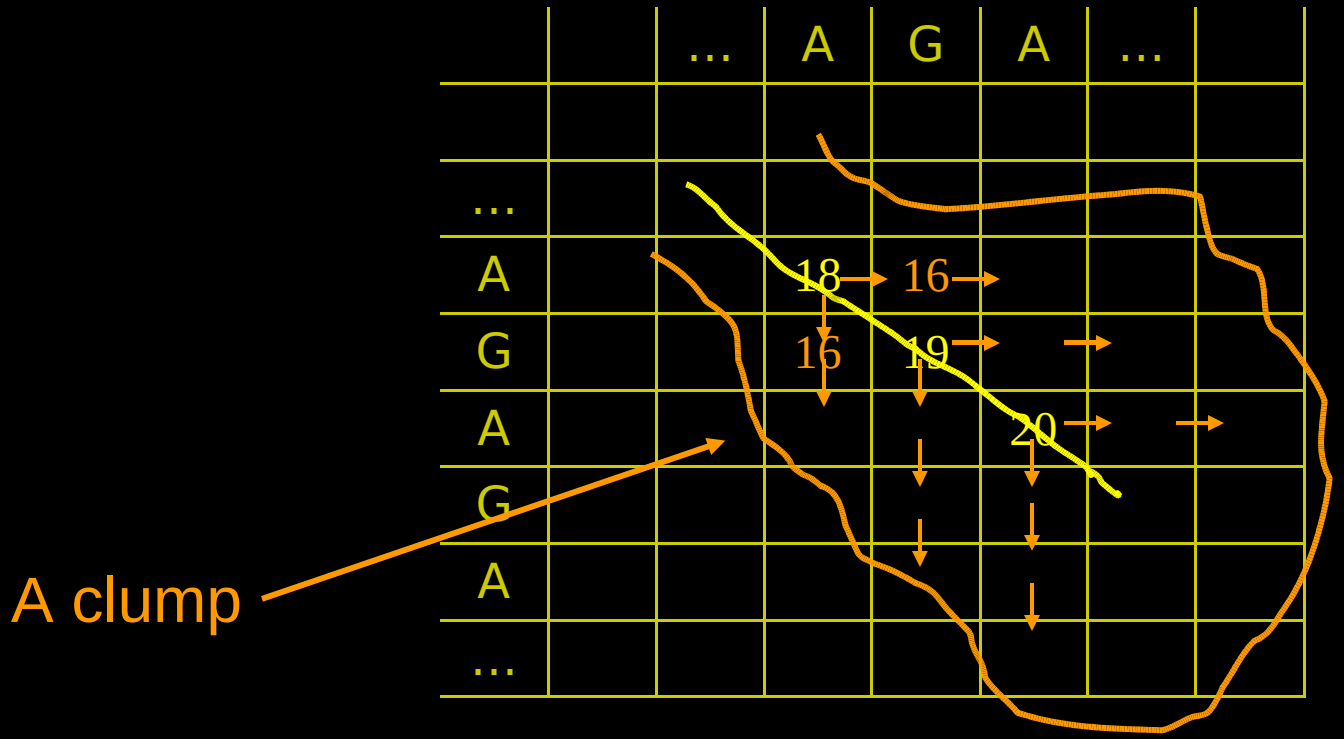
- We can find the best *local* alignment in the sequence
- But where is the second best one?

Scoring:
1 for match
-2 for a gap



Clump of an alignment

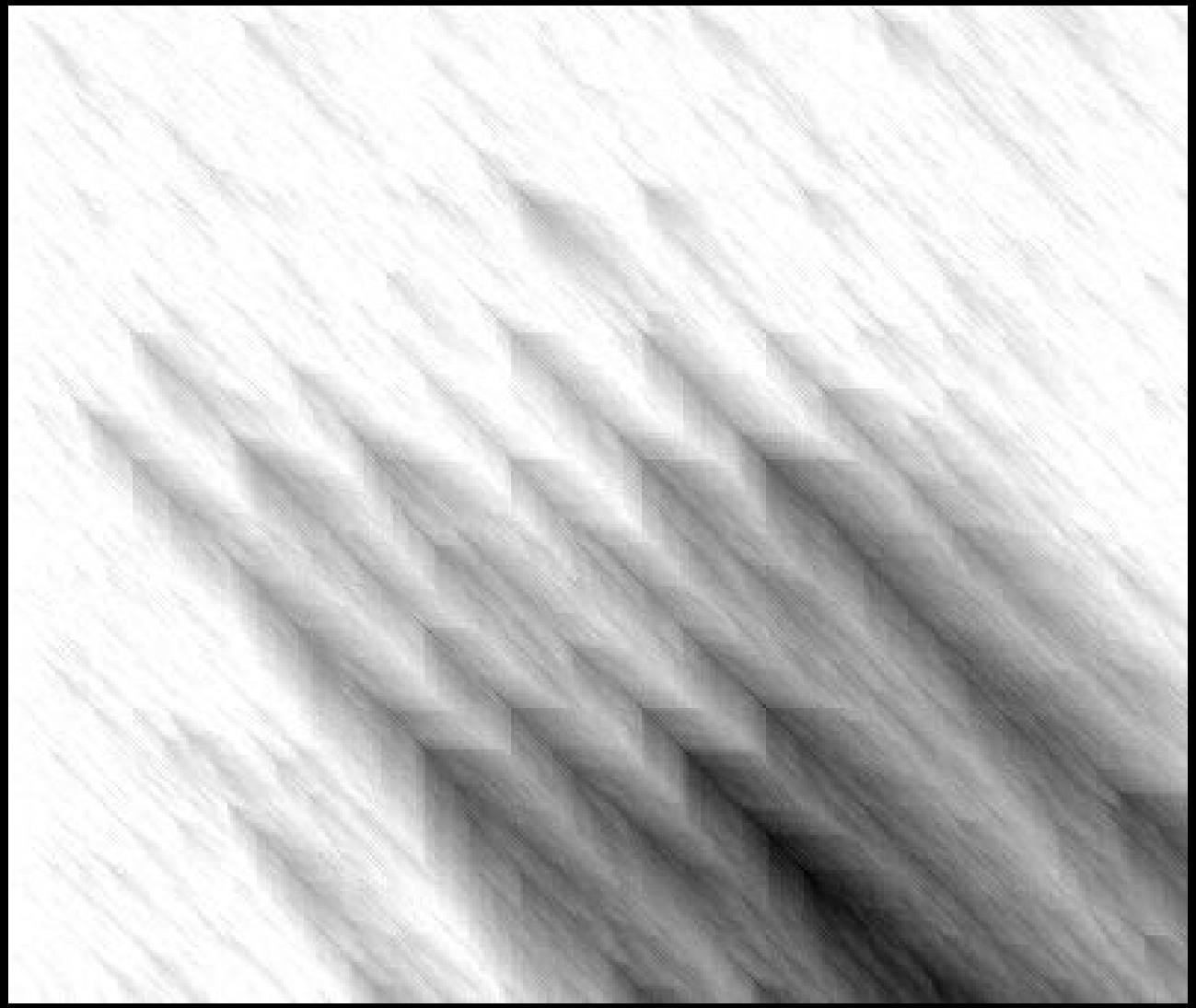
- Alignments sharing at least one aligned pair



Clumps

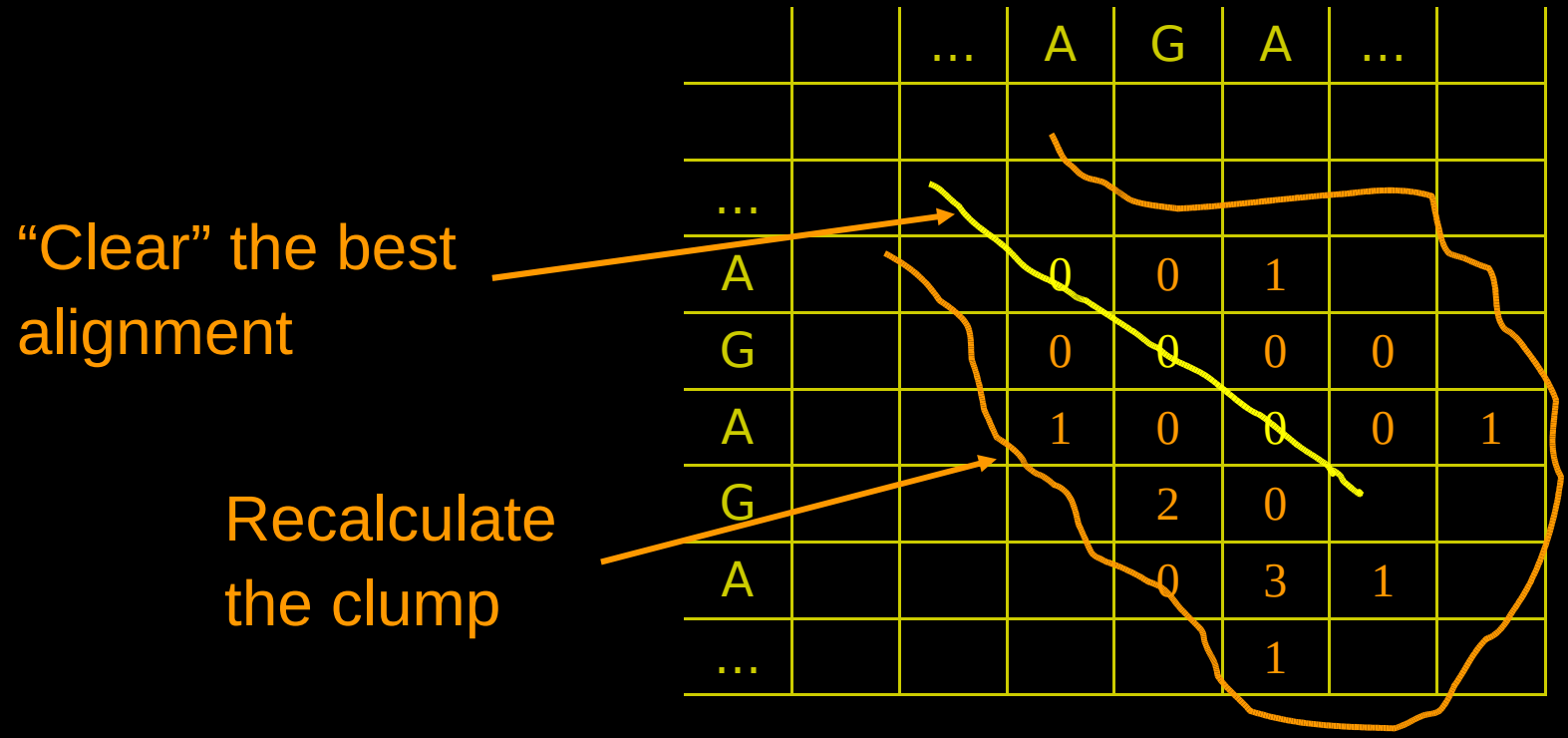
gene X

gene Y



Finding second best alignment

- Don’t let any matched pair to contribute to the next alignment



Extraction of local alignments – Waterman–Eggert algorithm

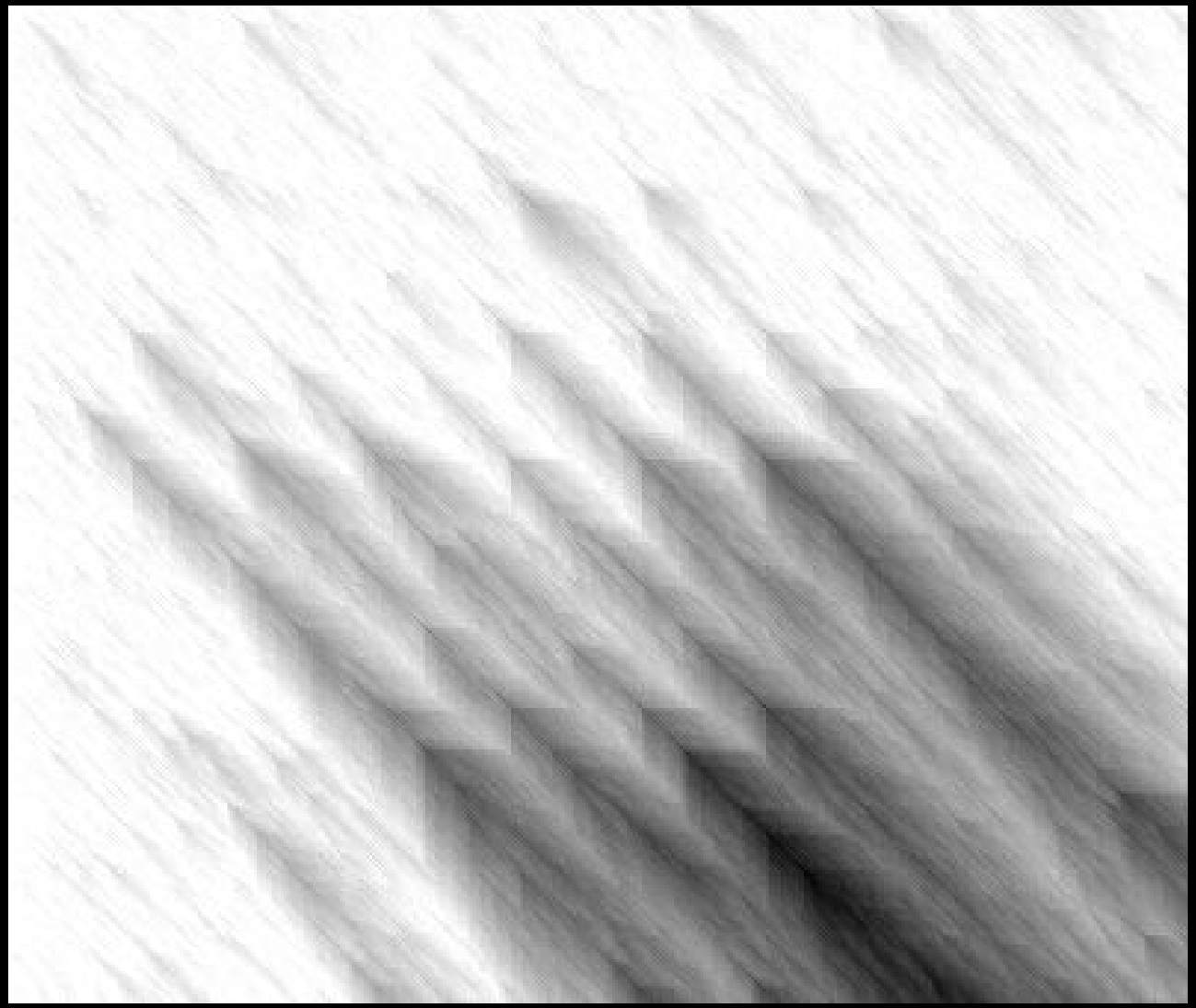
1. Repeat
   a. Calc M without taking ☠ cells into account
   b. Retrieve the highest scoring alignment
   c. Set it's trace to ☠

Clumps

Clumps

gene X

gene Y

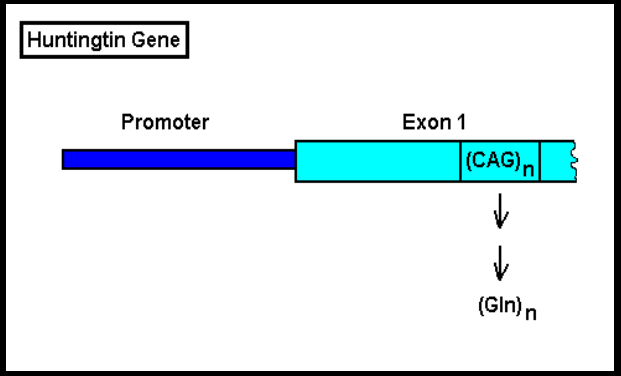


Low complexity regions

- Local composition bias
  - Replication slippage: e.g. triplet repeats
- Results in spurious hits
  - Breaks down statistical models
  - Different proteins reported as hits due to similar composition
  - Up to ¼ of the sequence can be biased

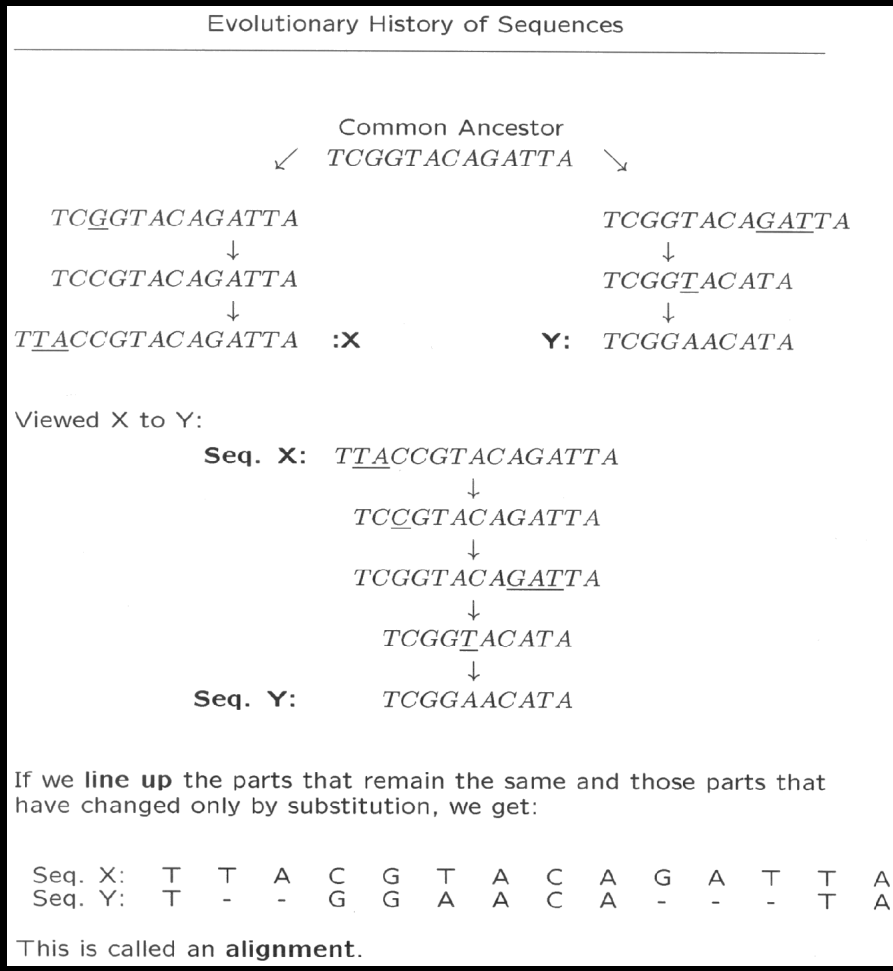
Huntington's disease

- *Huntingtin* gene of unknown (!) function
- Repeats #: 6-35: normal; 36-120: disease



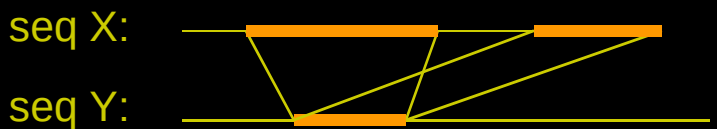
Pitfalls of alignments

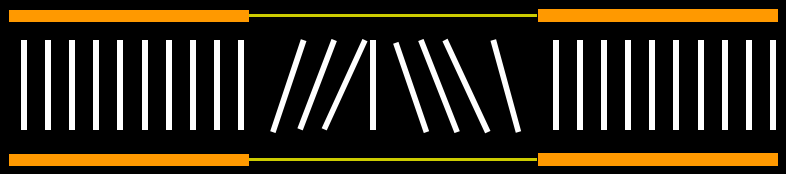
Alignment is not a reconstruction of the evolution
(common ancestor is *extinct* by the time of alignment)



Pitfalls of alignments

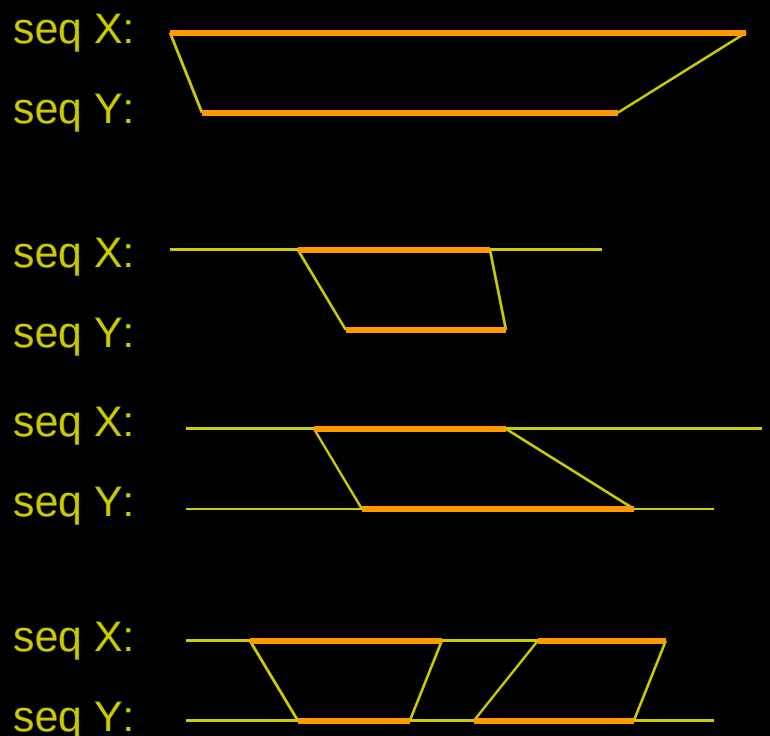
- Matches to the same fragment
- Arbitrary poor alignment regions





Summary

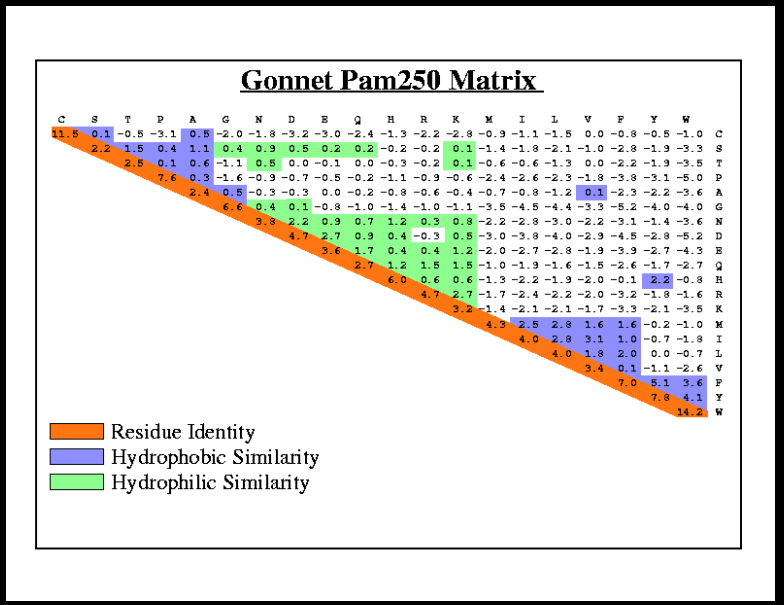
1. Global
   a.k.a. Needleman-Wunsch algorithm
2. Global-local
3. Local
   a.k.a. Smith-Waterman algorithm
4. Many local alignments
   a.k.a. Waterman-Eggert algorithm

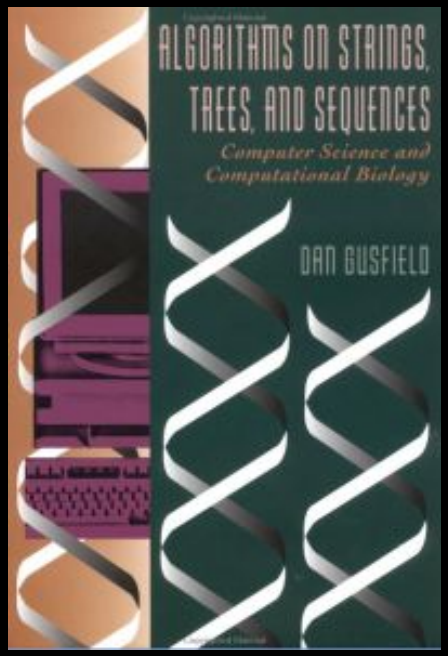


What's the number of steps in these algorithms?

How much memory is used?

Amino acid substitution matrices





Percent Accepted Mutations distance *and* matrices

- Accepted by natural selection
  - not lethal
  - not silent
- *Def.:* S_1 and S_2 are PAM 1 distant if on avg. there was one mutation per 100 aa
- *Q.:* If the seqs are PAM 8 distant, how many residues may be diffent?

PAM matrix

- Created from “easy”alignments
  - pairwise
  - gapless
  - 85% id

$f(\text{proline})$ – frequency of occurence of proline

$f(\text{proline},\text{valine})$ – frequency of substitution proline with valine, for PAM 1

- i.e. $f(a,b)=\dfrac{\sum_{\text{aligns}} \text{count}(a,b)}{\sum_{\text{aligns}} \sum_{c,d\,!=a,b} \text{count}(c,d)}$

M – symmetric matrix,

i.e. $M=\begin{bmatrix} f(a,a) & f(a,b) \\ f(b,a) & f(b,b) \end{bmatrix}$

PAM matrix

- How to calculte M for PAM 2 distance?
  - Take more distant seqs
  - or extrapolate...

$$M^2 = \begin{bmatrix} f(a,a) & f(a,b) \\ f(b,a) & f(b,b) \end{bmatrix}^2 = \begin{bmatrix} f(a,a)f(a,a)+f(a,b)f(b,a) & f(a,a)f(a,b)+f(a,b)f(b,b) \\ \cdots & \cdots \end{bmatrix}$$

PAM *log odds* matrix

- Making of the PAM N matrix

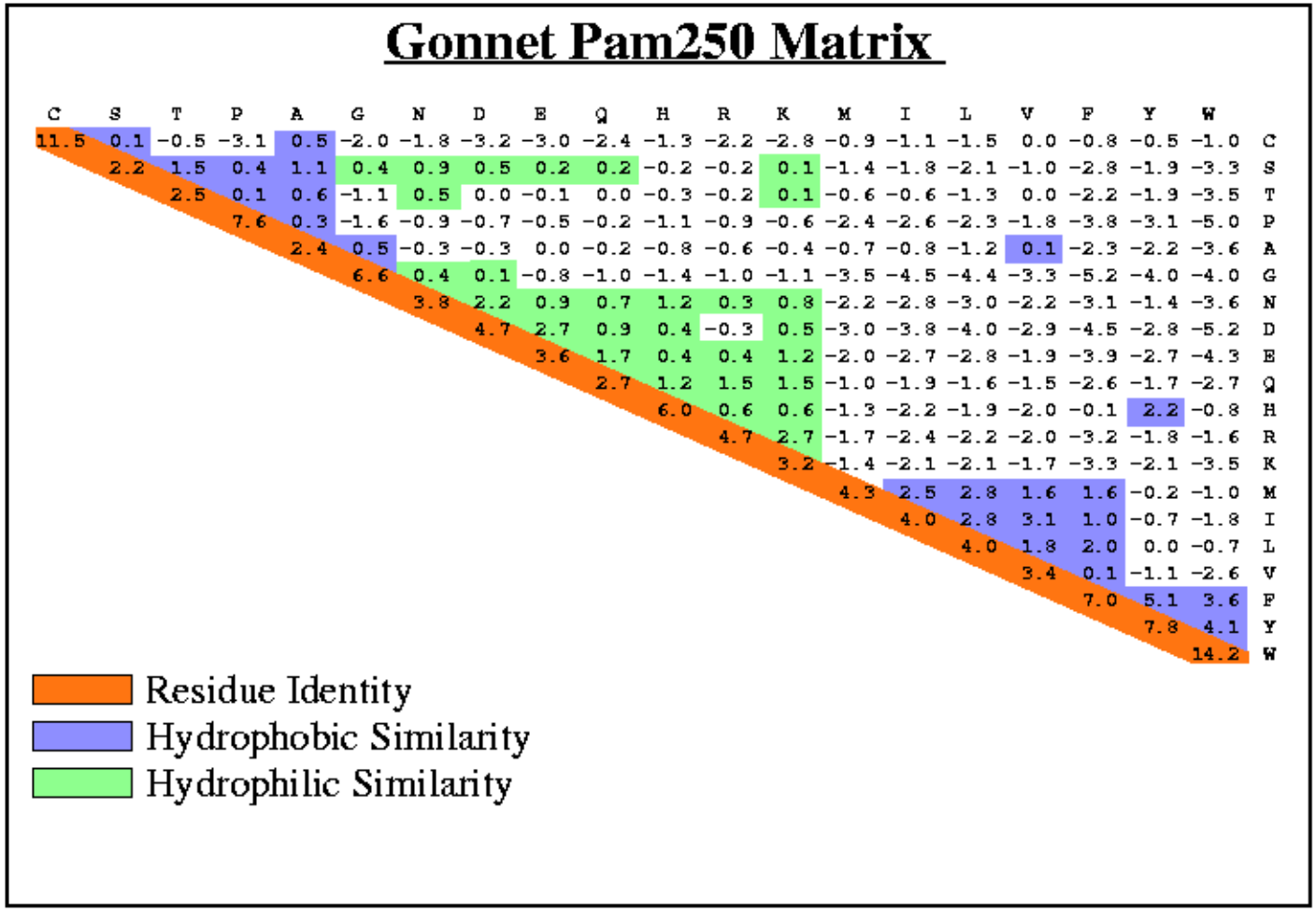
observed

$$\mathrm{PAMN}[a,b] = \log_2 \frac{f(a)M^N[a,b]}{f(a)f(b)} = \underbrace{\log_2 \underbrace{\frac{M^N[a,b]}{f(b)}}_{\text{odds}}}_{\log \text{odds}}$$

By chance alone

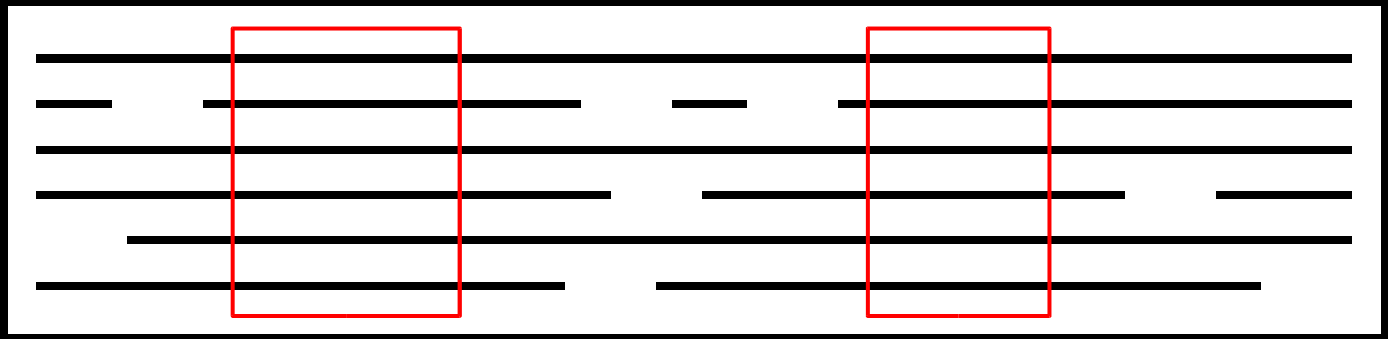
- Why log?
- Mutations and chance:
  - More freq: PAM N[a,b] > 0
  - Less freq: PAM N[a,b] < 0

PAM 250 matrix



BLOSUM matrix

- BLOcks SUbstitution Matrix
- Based on gapless alignments
- More often used than PAM



BLOSUM N matrix

- Cluster together sequences N% identity
  - f(a,b) frequency of occurrence a and b in the same column

$$f(a) = f(a,a) + \sum_{a \neq b} \frac{f(a,b)}{2}$$

  - e(a,b) – chance alone $\sum_{a \leq b} e(a,b) = 1$

$$e(a,a) = f(a)^2$$

$$e(a,b) = 2\, f(a) f(b) \quad \text{for } a \neq b$$

$$\text{BLOSUM}_N[a,b] = \underbrace{\log_2 \frac{f(a,b)}{e(a,b)}}_{\text{log odds}}$$

BLOSUM 62 matrix

```
#  BLOSUM Clustered Scoring Matrix in 1/2 Bit Units
#  Blocks Database = /data/blocks_5.0/blocks.dat
#  Cluster Percentage: >= 62
#  Entropy =   0.6979, Expected =  -0.5209
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4
```

PAM vs BLOSUM

http://www.ncbi.nih.gov/Education/BLASTinfo/Scoring2.html

- PAM is extrapolation from closely related seqs
- We are interested more distant relationships