Multiple Sequence Alignment
Sum-of-Pairs and Clustal-W

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This Lecture

- Multiple Sequence Alignment
  - The problem
  - Theoretical approach: Sum-of-Pairs scores
  - Practical approach: Clustal-W

Multiple Sequence Alignment

- We now align multiple (k>2) sequences
  - Note: Also BLAST aligns only two sequences
- Why?
  - Imagine k sequences of the promoter region of genes, all regulated by the same transcription factor f. Which subsequence within the k sequences is recognized by f?
  - Imagine k sequences of proteins that bind to DNA. Which subsequence of the k sequences code for the part of the proteins that performs the binding?
- General
  - We want to know the common part(s) in k sequences
  - “common” does not mean identical
  - This part can be anywhere within the sequences

Definition ??? Max für l dazu

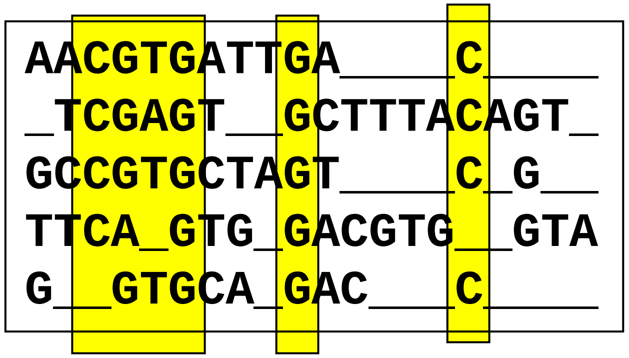
- Definition
  - *A multiple sequence alignment (MSA) of k Strings s_i, $1 \le i \le k$, is a table of k rows and l columns ($l \ge \max(|s_i|)$), such that*
    - *Row i contains the sequence of s_i, with an arbitrary number of blanks being inserted at arbitrary positions*
    - *Every symbol of every s_i stands in exactly one column*
    - *No column contains only blanks*

```
AACGTGATTGAC
TCGAGTGCTTTACAGT
GCCGTGCTAGTCG
TTCAGTGGACGTGGTA
GGTGCAGACC
```

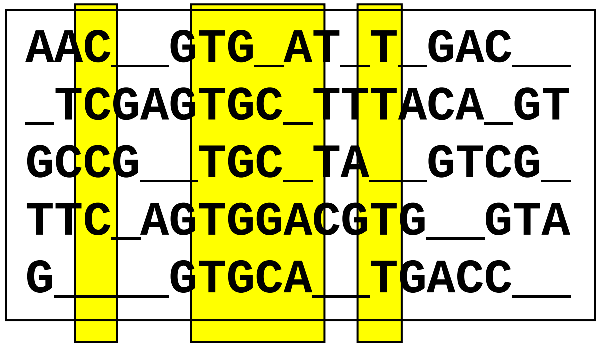
```
AACGTGATTGA____C____
_TCGAGT__GCTTTACAGT_
GCCGTGCTAGT____C_G__
TTCA_GTG_GACGTG__GTA
G__GTGCA_GAC___C____
```

Good MSA

- We are searching for good (optimal) MSAs
- Defining „optimal“ here is not as simple as in the k=2 case
- Intuition
  - All sequences had a common ancestor and evolved by evolution
  - We want to assume as few evolutionary events as possible
  - Thus, we want few columns (~ few INSDELs)
  - Thus, we want homogeneous columns (~ few replacements)



```
AACGTGATTGA____C____
_TCGAGT__GCTTTACAGT_
GCCGTGCTAGT____C_G__
TTCA_GTG_GACGTG__GTA
G__GTGCA_GAC___C____
```



```
AAC__GTG_AT_T_GAC__
_TCGAGTGC_TTTACA_GT
GCCG__TGC_TA__GTCG_
TTC_AGTGGACGTG__GTA
G____GTGCA__TGACC__
```

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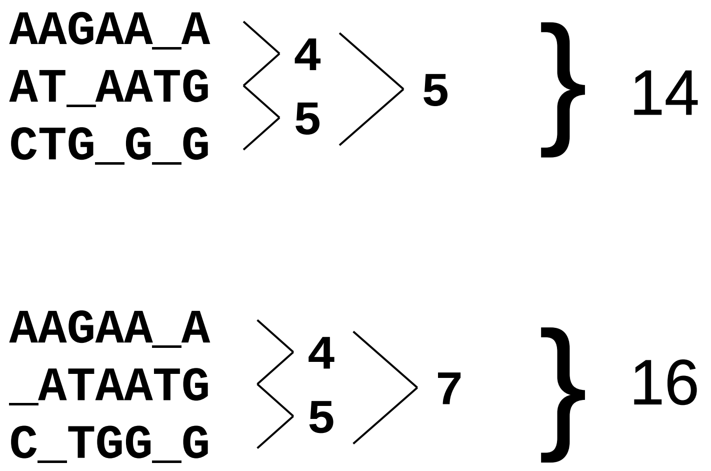
What Should we Count?

- For two sequences
  - We scored each column using a scoring matrix
  - Find the alignment such that the total score is maximal
- But – how do we score a column with 5*T, 3*A, 1*_?
  - We would need an exponentially large scoring matrix
- Alternative: Sum-of-Pairs Score
  - We score an entire MSA
  - We score the alignment of each pair of sequences in the usual way
  - We aggregate over all pairs to score the MSA
  - We need a clever algorithm to find the MSA with the best score

Formally

- Definition
  - *Let M be a MSA for the set S of k sequences $S=\{s_1,\ldots,s_k\}$*
  - *The alignment of s_i with s_j induced by M is generated as follows*
    - *Remove from M all rows except i and j*
    - *Remove all columns that contain only blanks*
  - *The sum-of-pairs score (sop) of M is the sum of all pair-wise induced alignment scores*
  - *The optimal MSA for S wrt. to sop is the MSA with the lowest sop-score over all possible MSA for S*

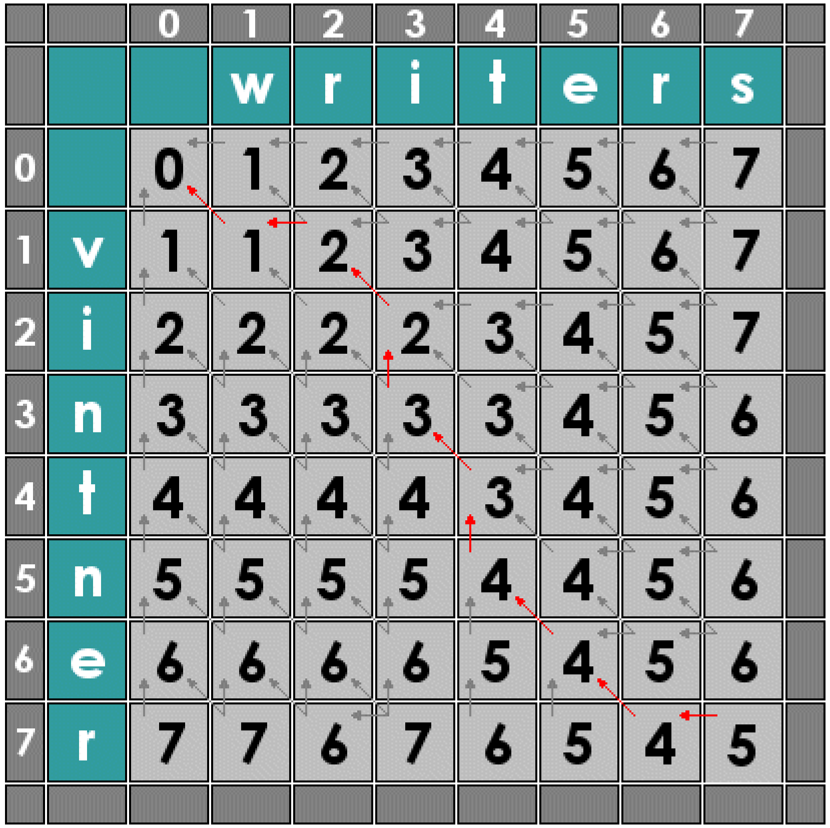
Example



```
d/i = 1
r   = 1
m   = 0
```

- Given a MSA over k sequences of length l – how complex is it to compute its sop-score?
- How do we find the best MSA?

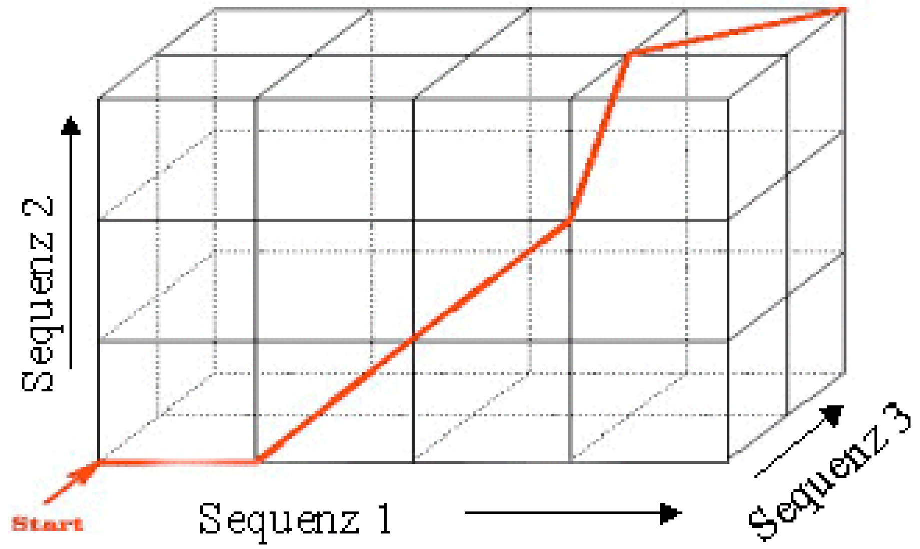
Analogy



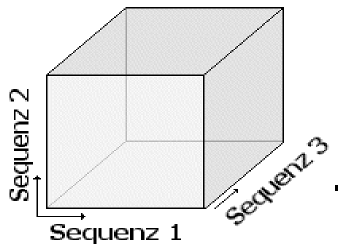
		0	1	2	3	4	5	6	7
			w	r	i	t	e	r	s
0		0	1	2	3	4	5	6	7
1	v	1	1	2	3	4	5	6	7
2	i	2	2	2	2	3	4	5	7
3	n	3	3	3	3	3	4	5	6
4	t	4	4	4	4	3	4	5	6
5	n	5	5	5	5	4	4	5	6
6	e	6	6	6	6	5	4	5	6
7	r	7	7	6	7	6	5	4	5

- Think of the k=2 case
- Every alignment is a path through the matrix
- The three possible directions (down, right, down-right) conform to the three possible constellations in a column (XX, X_, _X)
- With growing paths, we align growing prefixes of both sequences

Analogy

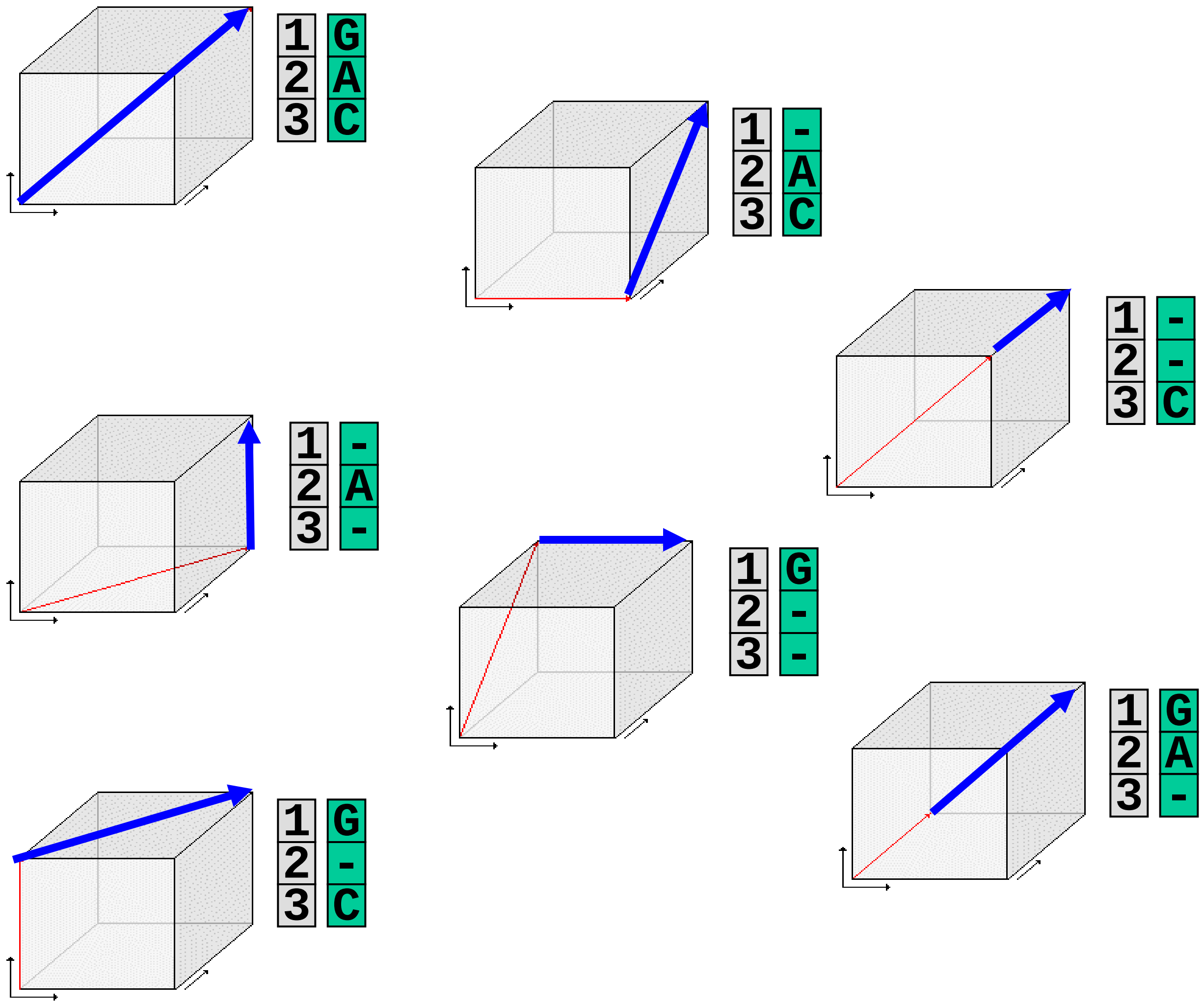


- Assume k=3
- Think of a 3-dimensional cube with the three sequences giving the values in each dimension
- Now, we have paths aligning growing prefixes of three sequences
- Every column has seven possible constellations (XXX, XX_, X_X, _XX, X__, _X_, __X)



All Possible Steps

- d(i-1,j-1,k-1)
- d(i,j-1,k-1)
- d(i,j,k-1)
- d(i,j-1,k)
- d(i-1,j,k)
- d(i-1,j-1,k)
- d(i-1,j,k-1)



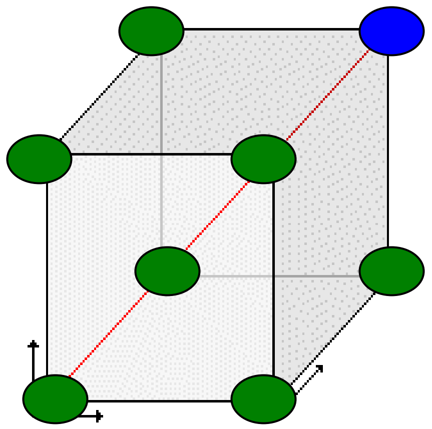
Dynamic Programming in three Dimensions

- We compute the best possible alignment d(i,j,k) for every triple of prefixes (lengths i,j,k) using the following formula
  - We assume the usual edit costs: I/D/R=+1, M=0

$$d(i,j,k) = \min \begin{cases} d(i-1,j-1,k-1) + c_{ij} + c_{ik} + c_{jk} \\ d(i-1,j-1,k) + c_{ij} + 2 \\ d(i-1,j,k-1) + c_{ik} + 2 \\ d(i,j-1,k-1) + c_{jk} + 2 \\ d(i-1,j,k) + 2 \\ d(i,j-1,k) + 2 \\ d(i,j,k-1) + 2 \end{cases}$$

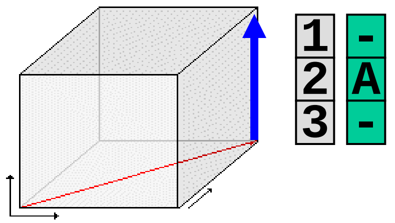
Three (mis)matches
One (mis)match, two ins
...

Let $c_{ij} = 0$, if $S_1(i) = S_2(j)$, else 1
Let $c_{ik} = 0$, if $S_1(i) = S_3(k)$, else 1
Let $c_{jk} = 0$, if $S_2(j) = S_3(k)$, else 1



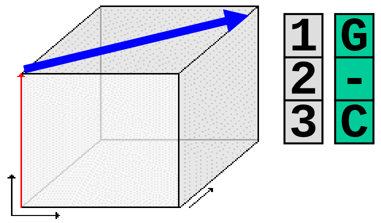
Concrete Examples

`d(i,j-1,k)`



- Best sop-score for d(i,j-1,k) is known
- We want to compute d(i,j,k)
- This requires to align one symbol with two blanks (blank/blank does not count)
- d(i,j,k) = d(i,j-1,k) + 2

`d(i-1,j,k-1)`



- Best sop-score for d(i-1, j,k-1) is known
- We want to compute d(i,j,k)
- This requires aligning a blank with s_1[i-1] and with s_3[k-1] and to align s_1[i-1] and s_3[k-1]
- d(i,j,k) = d(i-1,j,k-1) + 2 + c_{ik}

Initialization

- Of course, we have $d(0,0,0)=0$
- Aligning in one dimension: $d(i,0,0)=2*i$
  - Same for $d(0,j,0)$, $d(0,0,k)$
- Aligning in two dimensions: $d(i,j,0)=$ ...
  - Let $d_{a,b}(i,j)$ be the alignment score for $S_a[1..i]$ with $S_b[1..j]$
  - $d(i, j, 0) = d_{1,2}(i, j) + (i+j)$
  - $d(i, 0, k) = d_{1,3}(i, k) + (i+k)$
  - $d(0, j, k) = d_{2,3}(j, k) + (j+k)$

Algorithm

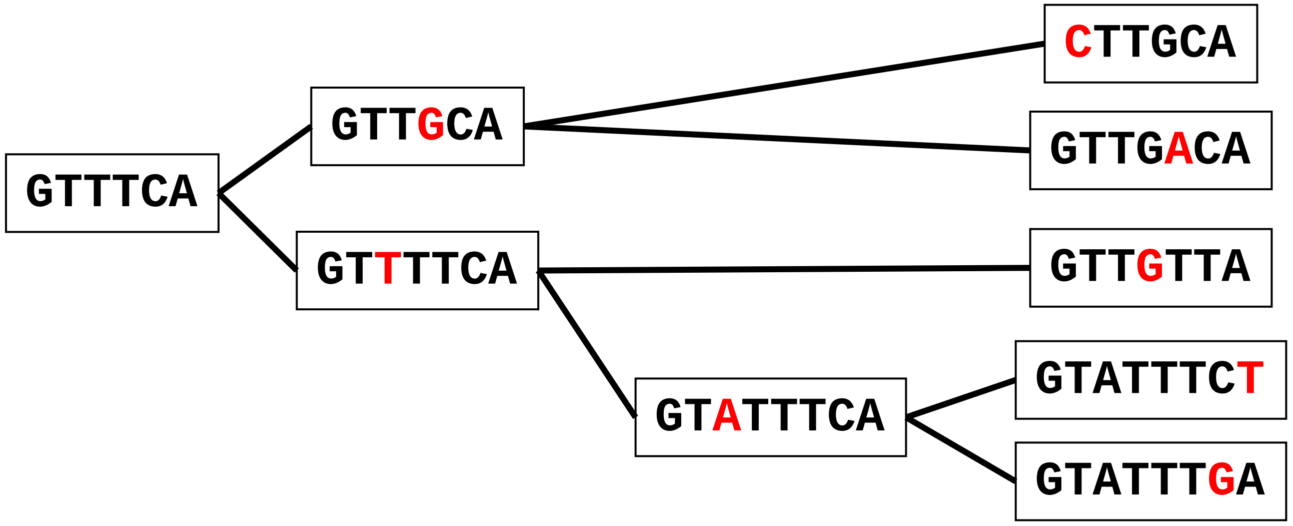
```
initialize matrix d;
for i := 1 to |$S_1$|
    for j := 1 to |$S_2$|
        for k := 1 to |$S_3$|
            if ($S_1$(i) = $S_2$(j)) then $c_{ij}$ := 0; else $c_{ij}$ := 1;
            if ($S_1$(i) = $S_3$(k)) then $c_{ik}$ := 0; else $c_{ik}$ := 1;
            if ($S_2$(j) = $S_3$(k)) then $c_{jk}$ := 0; else $c_{jk}$ := 1;
            $d_1$ := d[i – 1,j – 1,k - 1] + $c_{ij}$ + $c_{ik}$ + $c_{jk}$;
            $d_2$ := d[i – 1,j – 1,k] + $c_{ij}$ + 2;
            $d_3$ := d[i – 1,j,k - 1] + $c_{ik}$ + 2;
            $d_4$ := d[i,j – 1,k - 1] + $c_{jk}$ + 2;
            $d_5$ := d[i – 1,j,k) + 2;
            $d_6$ := d[i,j – 1,k) + 2;
            $d_7$ := d[i,j,k - 1) + 2;
            d[i,j,k] := min($d_1$, $d_2$, $d_3$, $d_4$, $d_5$, $d_6$, $d_7$);
        end for;
    end for;
end for;
```

Bad News: Complexity

- For 3 sequences of length n
  - There are n^3 cells in the cube
  - For each cell (top-left-front corner), we need to look at 7 corners
  - Together: $O(7*n^3)$ operations
- For k sequences of length n
  - There are n^k cell corners in the cube
  - For each corner, we need to look at 2^k-1 other corners
  - Together: $O(2^k * n^k)$ operations

Bad News: Biological Meaningfulness

- Let‘s take one step back
- What happened during evolution?



- Real number of events: 8
- sop-score: 2+3+6+6+2+...
  - Single mutations are counted multiple times

```
CT_TGC_A
GT_TGACA
GT_TGTTA
GTATTTCT
GTATTTGA
```

???

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Different Scoring Function

- If we knew the phylogenetic tree of the k sequences
  - Align every parent node pairwise with its children
  - Aggregate all alignment scores
  - This gives the “real” number of evolutionary operations
    - But not yet the best MSA
- But: Finding the true phylogenetic tree requires a MSA
  - Not covered in this lecture
- Use a heuristic: ClustalW
  - Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994). "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." *Nucleic Acids Res* **22**(22): 4673-80.

Clustal-W

- Main idea
  - Compute a “good enough” phylogeny – the guide tree
  - Use the guide tree to iteratively align small MSA to larger MSA
    - “Progressive” MSA
    - Starting from single sequences
    - Add more and more sequences and smaller MSA to ever bigger MSA
    - Does not necessarily find the optimal solution
    - Needs a fast method to align two MSAs
- Standard method for a long time
- Many newer (better) proposals
  - DAlign, T-Coffee, HMMT, PRRT, MULTALIGN, ...

Step 1: Compute the Guide Tree

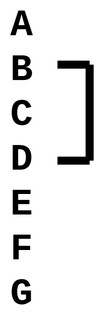
- Compute all pair-wise alignments and store in distance matrix M
  - $M[i,j] = sim(s_i, s_j)$
- Compute the guide tree using hierarchical clustering
  - Choose the smallest M[i,j]
  - Let s_i and s_j form a new (next) branch of the tree
  - Compute the distance from the ancestor of s_i and s_j to all other sequences as the average of the distances to s_i and s_j
    - Set M' = M
    - Delete rows and columns i and j
    - Add a new column and row (ij)
    - For all $k \neq ij$: $M'[ij,k] = (M[i,k] + M[j,k]) / 2$
  - Iterate until M' has only one column / row

Sketch

```
A
B
C
D
E
F
G
```

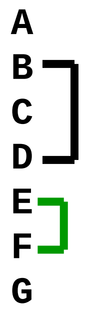
```
 ABCDEFG
A
B.
C..
D...
E....
F.....
G......
```

$\boxed{(B,D)\rightarrow a}$



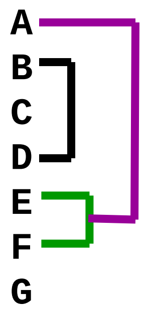
```
 ACEFGa
A
C.
E..
F...
G....
a.....
```

$\boxed{(E,F)\rightarrow b}$



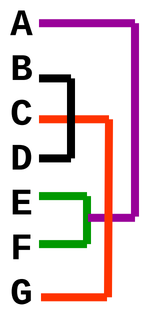
```
 ACGab
A
C.
G..
a...
b....
```

$\boxed{(A,b)\rightarrow c}$



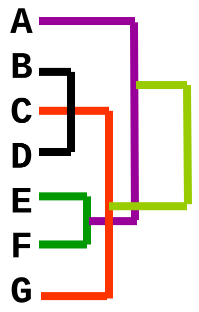
```
 CGac
C
G.
a..
c...
```

$\boxed{(C,G)\rightarrow d}$



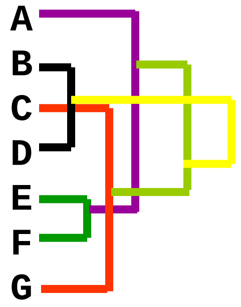
```
 acd
a
c.
d..
```

$\boxed{(d,c)\rightarrow e}$



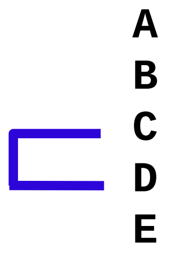
```
 ae
a
e.
```

$\boxed{(a,e)\rightarrow f}$

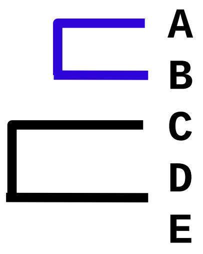


Example

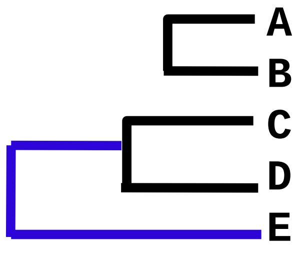
	A	B	C	D	E
A		17	59	59	77
B			37	61	53
C				13	41
D					21

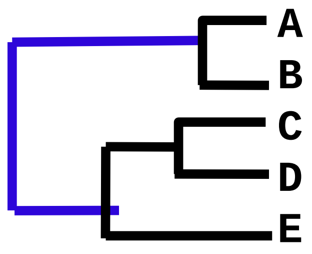


	A	B	E	CD
A		17	77	59
B			53	49
E				31

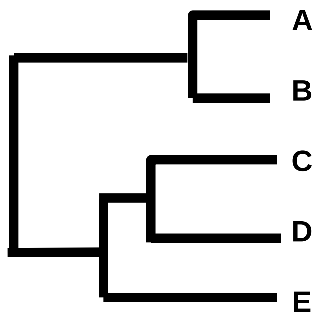


	E	CD	AB
E		31	65
CD			54





Example



```
C   PADKTNVKAAWGKVGAHAGEYGA
D   AADKTNVKAAWSKVGGHAGEYGA

A   PEEKSAVTALWGKVNVDEYGG
B   GEEKAAVLALWDKVNEEEYGG

C   PADKTNVKAAWG_KVGAHAGEYGA
D   AADKTNVKAAWS_KVGGHAGEYGA
E   AA__TNVKTAWSSKVGGHAPA__A

A   PEEKSAV_TALWG_KVN__VDEYGG
B   GEEKAAV_LALWD_KVN__EEEYGG
C   PADKTNVKAA_WG_KVGAHAGEYGA
D   AADKTNVKAA_WS_KVGGHAGEYGA
E   AA__TNVKTA_WSSKVGGHAPA__A
```

Once a gap, always a gap

Step 2: Progressive MSA

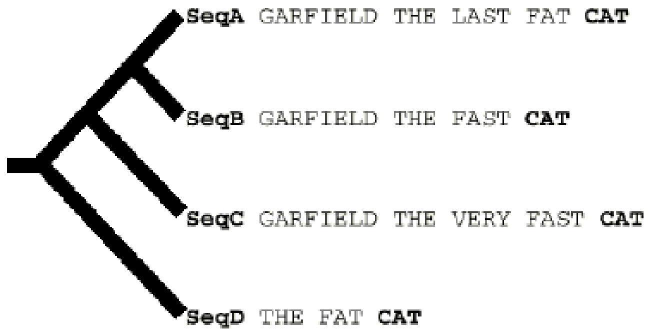
- Pair-wise alignment of MSAs in the order of the guide tree
- Aligning a MSA M_1 with a MSA M_2
  - Use the usual (global) alignment algorithm
  - To score a column, compute the average score over all pairs of symbols in these columns
- Example

```
A   …P…
B   …G…        Score of this column
C   …P…            (2*s(P,A)+s(P,Y)+
                    2*s(G,A)+s(G,Y)+
D   …A…             2*s(P,A)+s(P,Y) ) / 9
E   …A…
F   …Y…
```

Issues

- There is a lot to say about whether hierarchical clustering actually computes the “correct” tree
- Clustal-W actually uses a different, more accurate phylogenetic algorithm called “neighbor-joining”
- Clustal-W is fast: $O(k^2*n^2+k^2*\log(k))$
  - For k sequences; plus cost for computing pairwise alignments
- Idea behind progressive alignment
  - Find strong signals (highly conserved blocks) first
  - Outliers are added last
  - Increases the chances that conserved blocks survive
  - Several improvements to this scheme are known

Problems with progressive MSA


SeqA GARFIELD THE LAST FAT CAT
SeqB GARFIELD THE FAST CAT
SeqC GARFIELD THE VERY FAST CAT
SeqD THE FAT CAT

```
CLUSTALW (Score=20, Gop=-1, Gep=0, M=1)

  SeqA GARFIELD THE LAST FA-T CAT
  SeqB GARFIELD THE FAST CA-T ---
  SeqC GARFIELD THE VERY FAST CAT
  SeqD -------- THE ---- FA-T CAT
```

```
CORRECT (Score=24)

  SeqA GARFIELD THE LAST FA-T CAT
  SeqB GARFIELD THE FAST ---- CAT
  SeqC GARFIELD THE VERY FAST CAT
  SeqD -------- THE ---- FA-T CAT
```

Source: Cedric Notredame, 2001

Further Reading

- Merkl & Waack, chapter 13
- Böckenhauer & Bongartz, chapter 5.3