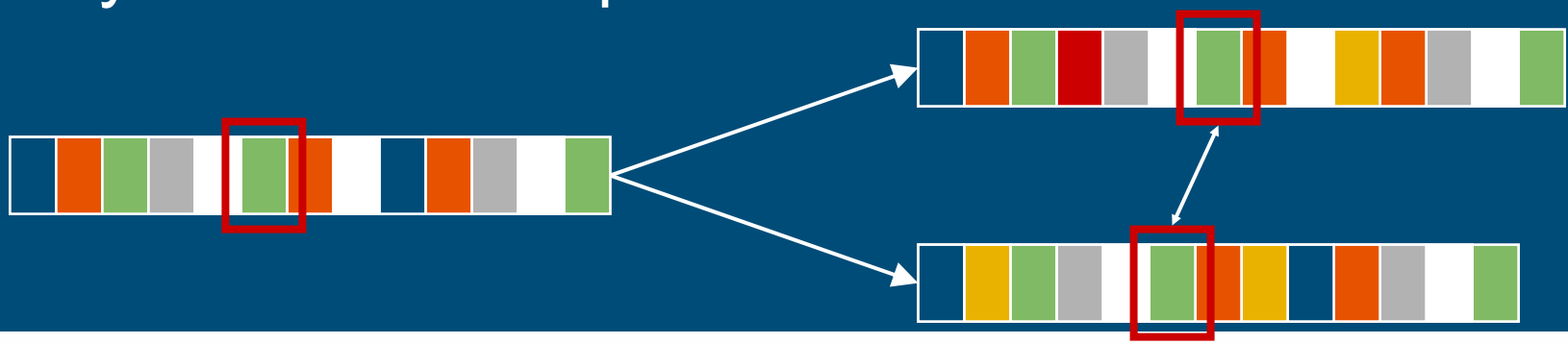
Multiple Alignment

- The purpose of a multiple alignment is to line up all residues that were derived from the same residue position in the ancestral gene or protein in any number of sequences



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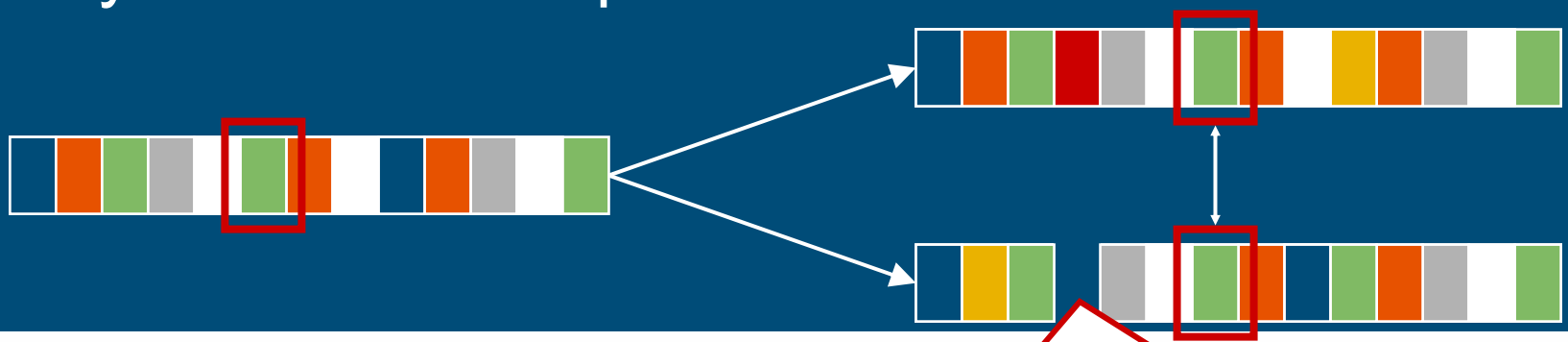
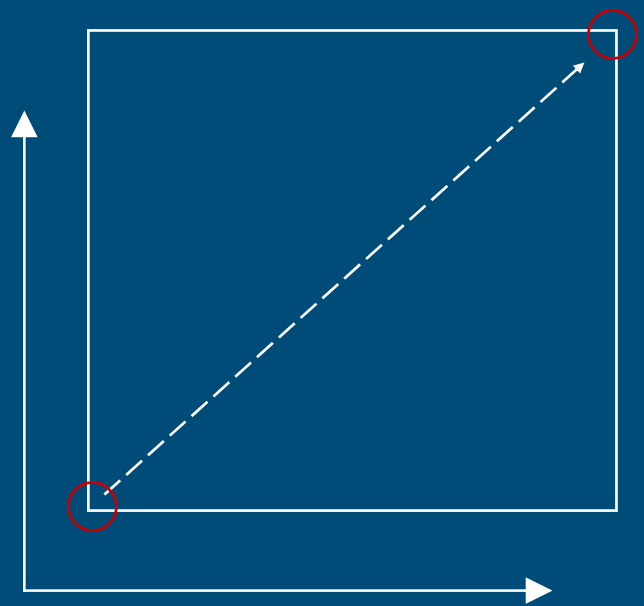
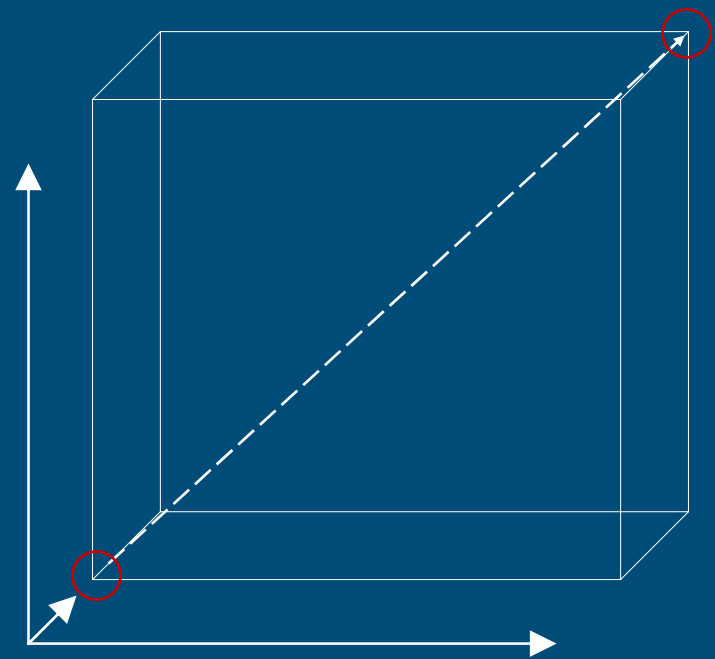


From Pairwise To Multiple

Two sequences



Three sequences



And Beyond ...

- Assuming that it takes 1 kilobyte (1kb) to store one single sequence, then ...
- To do simultaneous alignment it takes for
  - 2 sequences : 1 megabyte of memory
  - 3 sequences : 1 gigabyte of memory
  - 4 sequences : 1 terabyte of memory
  - 5 sequences : 1 petabyte of memory
  - 6 sequences : 1 exabyte of memory

Iterative Approach

Or: the way we did multiple alignment in the middle ages

- First do all the easy alignments
- Then gradually add all the difficult ones

- But how do we know what alignments are easy or difficult?

Iterative Algorithm

as applied in ClustalW and similar programs

- Do a pairwise comparison of all sequences
- From this, calculate how sequences are related to each other (the more similar are easier to align)
- Perform multiple alignment in order; the most similar are aligned first, the others are saved for later

1: Pairwise Comparison

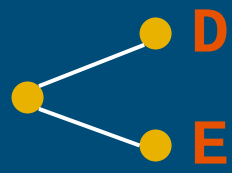
- Compare every single sequence to every other sequence, using pairwise sequence alignment
  - seq 1 ↔ seq 2 ⇒ 0.91
  - seq 1 ↔ seq 3 ⇒ 0.23
  - …
  - seq 8 ↔ seq 9 ⇒ 0.87
- Record the resulting similarity scores
  - You can in fact use either similarities or differences between sequence

2: Calculate The Guide Tree

- Construct a guide tree from the matrix containing the pairwise comparison values, using a (relatively simple) clustering algorithm
  - UPGMA (PileUp & Clustal V)
  - Neighbor-Joining (Clustal W, Clustal X)

UPGMA - Step 1

	A	B	C	D	E
A	0	6	9	11	9
B	6	0	7	9	7
C	9	7	0	8	6
D	11	9	8	0	4
E	9	7	6	4	0

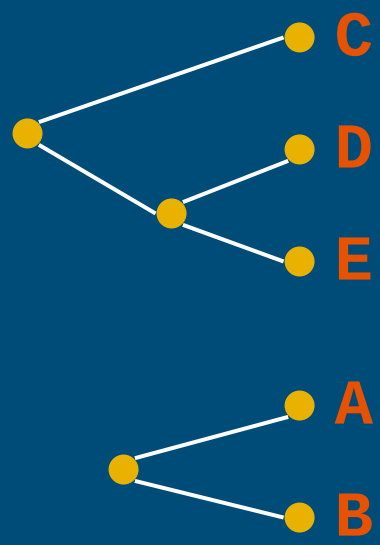


UPGMA - Step 2

	A	B	C	DE
A	0	6	9	10
B	6	0	7	8
C	9	7	0	7
DE	10	8	7	0

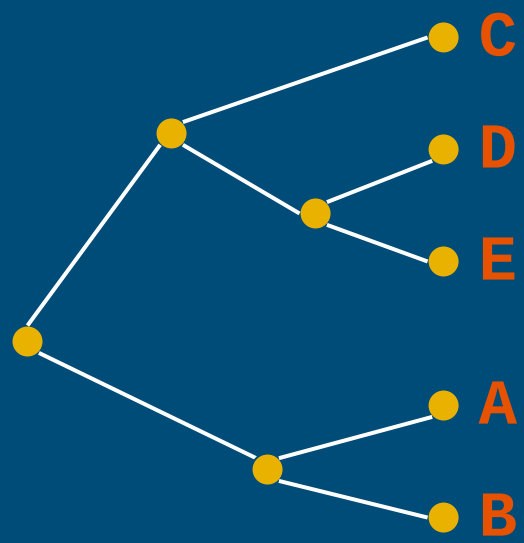
UPGMA - Step 3

	AB	C	DE
AB	0	8	9
C	8	0	7
DE	9	7	0



UPGMA - Step 4

	AB	CDE
AB	0	8.5
CDE	8.5	0



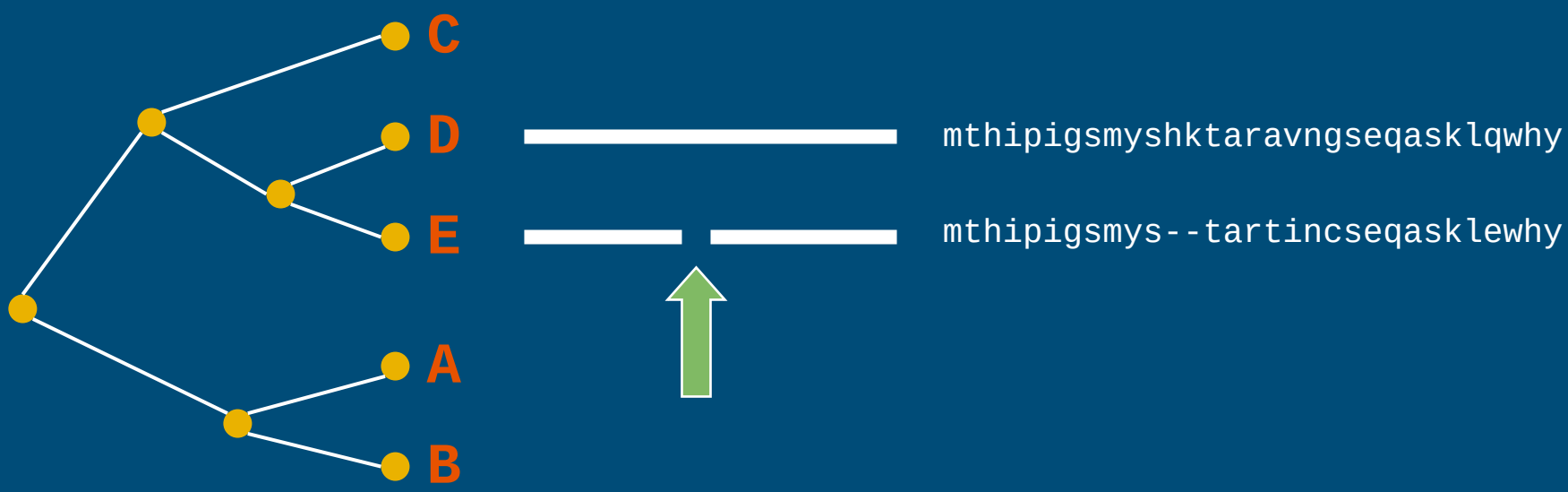
3: Multiple Alignment

- Using the guide tree, we start aligning groups of sequences
- The purpose of the guide tree is to know which sequences are most alike; so we can align the “easy” ones first, and postpone the tricky ones to later in the procedure!

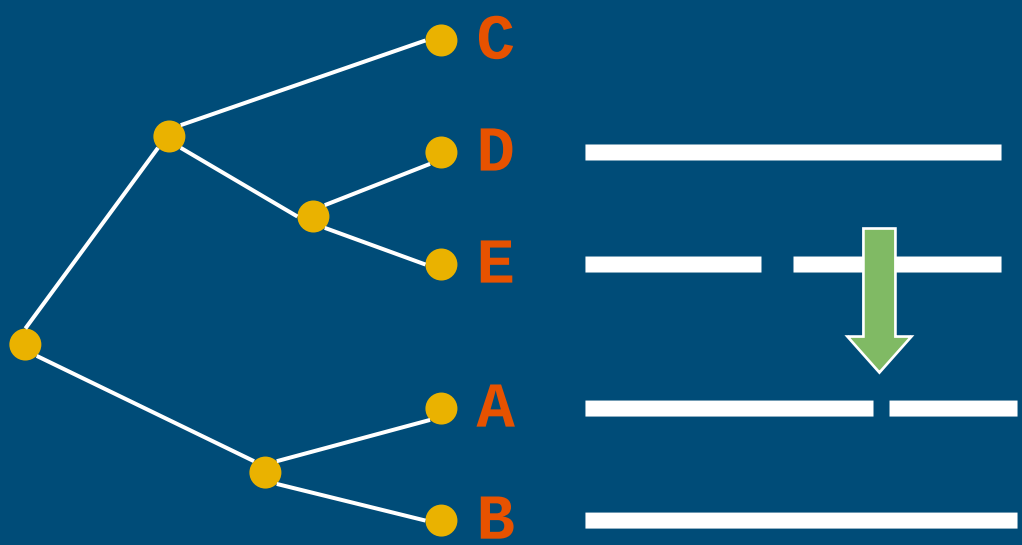
Input: Unaligned Sequences

```
A  mthislgslyshktaktingsdeaskmewhf
B  mthvslgsmyshktgrtingsdqaskkmewhy
C  mshisitmyshktartidgseqaskmewhy
D  mthipigsmyshktaravngseqasklqwhy
E  mthipigsmystartincseqasklewhy
```

Multiple Alignment



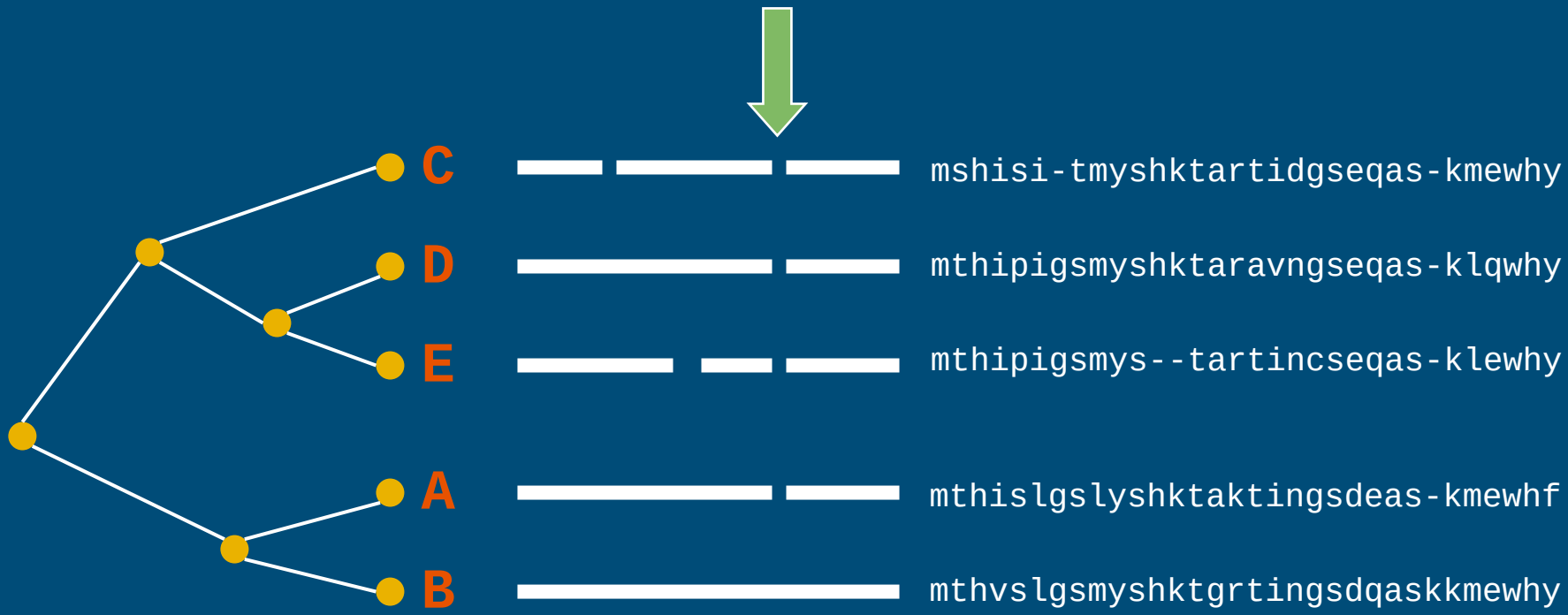
Multiple Alignment




```
mthipigsmyshktaravngseqasklqwhy
mthipigsmys--tartincseqasklewhy
mthislgslyshktaktingsdeas-kmewhf
mthvslgsmyshktgrtingsdqaskkmewhy
```

Multiple Alignment

Multiple Alignment



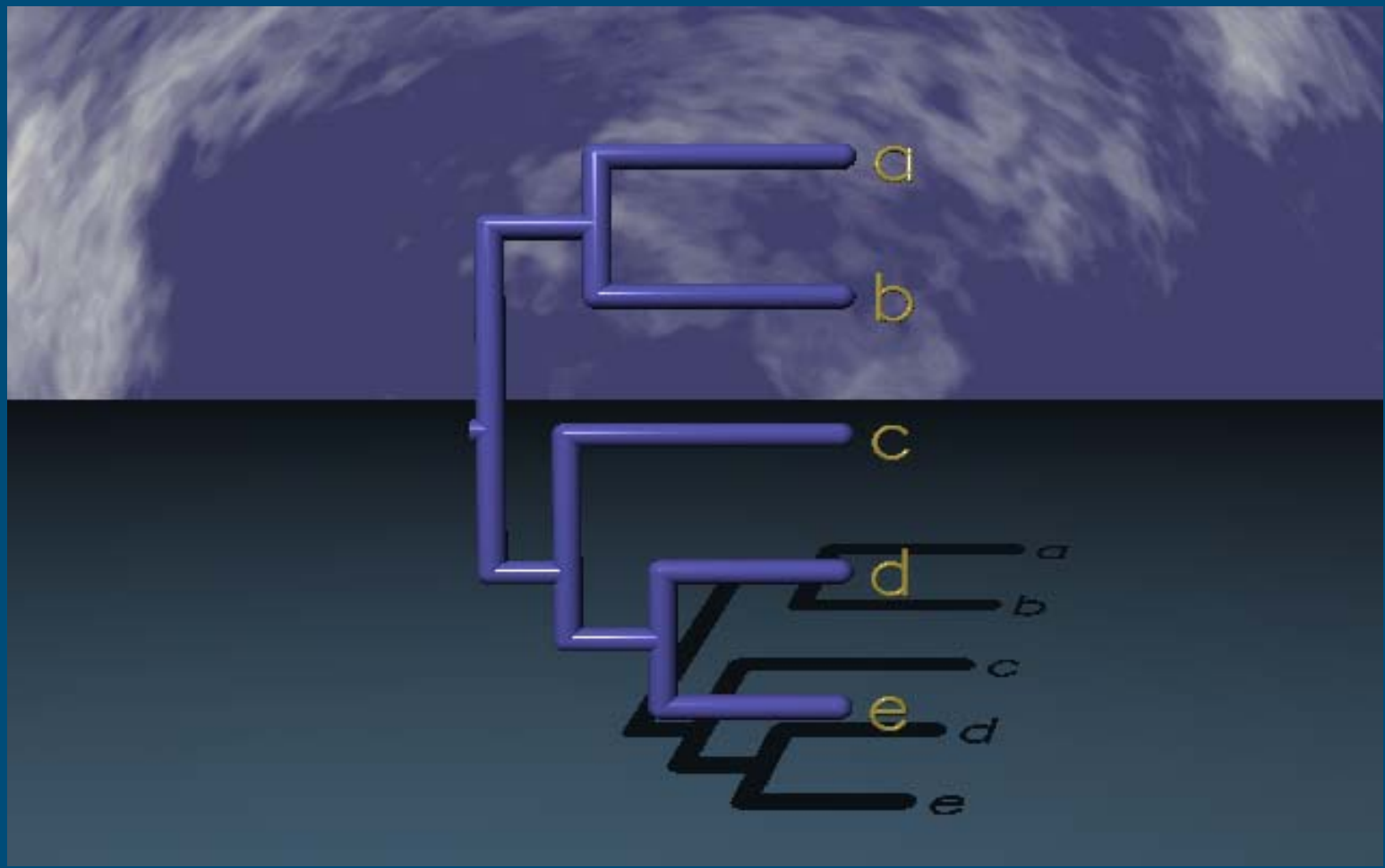
Output: Aligned Sequences

```
A  mthislgslyshktaktingsdeas-kmewhf
B  mthvslgsmyshktgrtingsdqaskkmewhy
C  mshisi-tmyshktartidgseqas-kmewhy
D  mthipigsmyshktaravngseqas-klqwhy
E  mthipigsmys--tartincseqas-klewhy
```

Things To Remember ...

- Most multiple alignment programs are GLOBAL alignment programs
- The guide tree is NOT the phylogenetic tree

… no matter how beautiful it looks!



Things To Remember ...

- Most multiple alignment programs are GLOBAL alignment programs
- The guide tree is NOT the phylogenetic tree
- A multiple alignment program is the *starting point*, not the end point of producing a good, meaningful alignment

Dependencies

Pairwise Alignment Step

- Order:
  - independent
- Memory:
  - depend on longest pair
- Time:
  - quadratic

Multiple Alignment Step

- Order:
  - order in guide tree
- Memory:
  - depends on alignment
- Time:
  - linear

Progressive approaches

- Global: clustalw
- Partially ordered graphs: poa
- Local, whole segments: dialign
- Extension of matching triplets: t-coffee

Program Performance

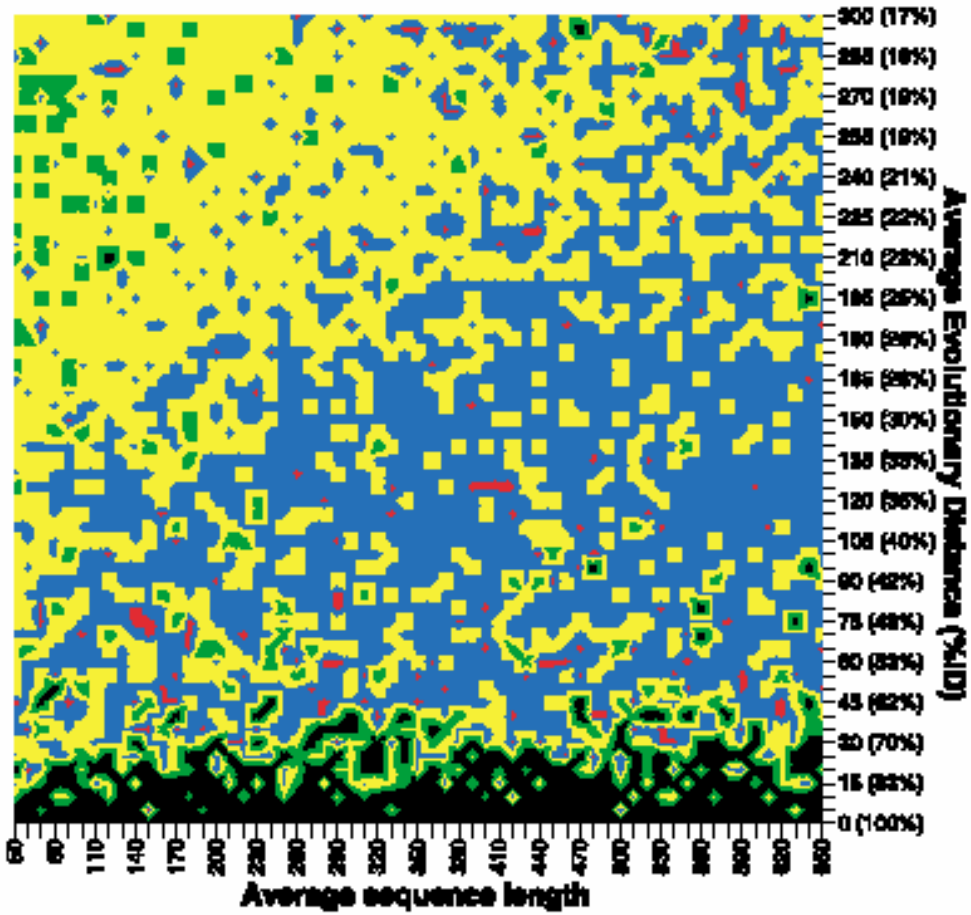
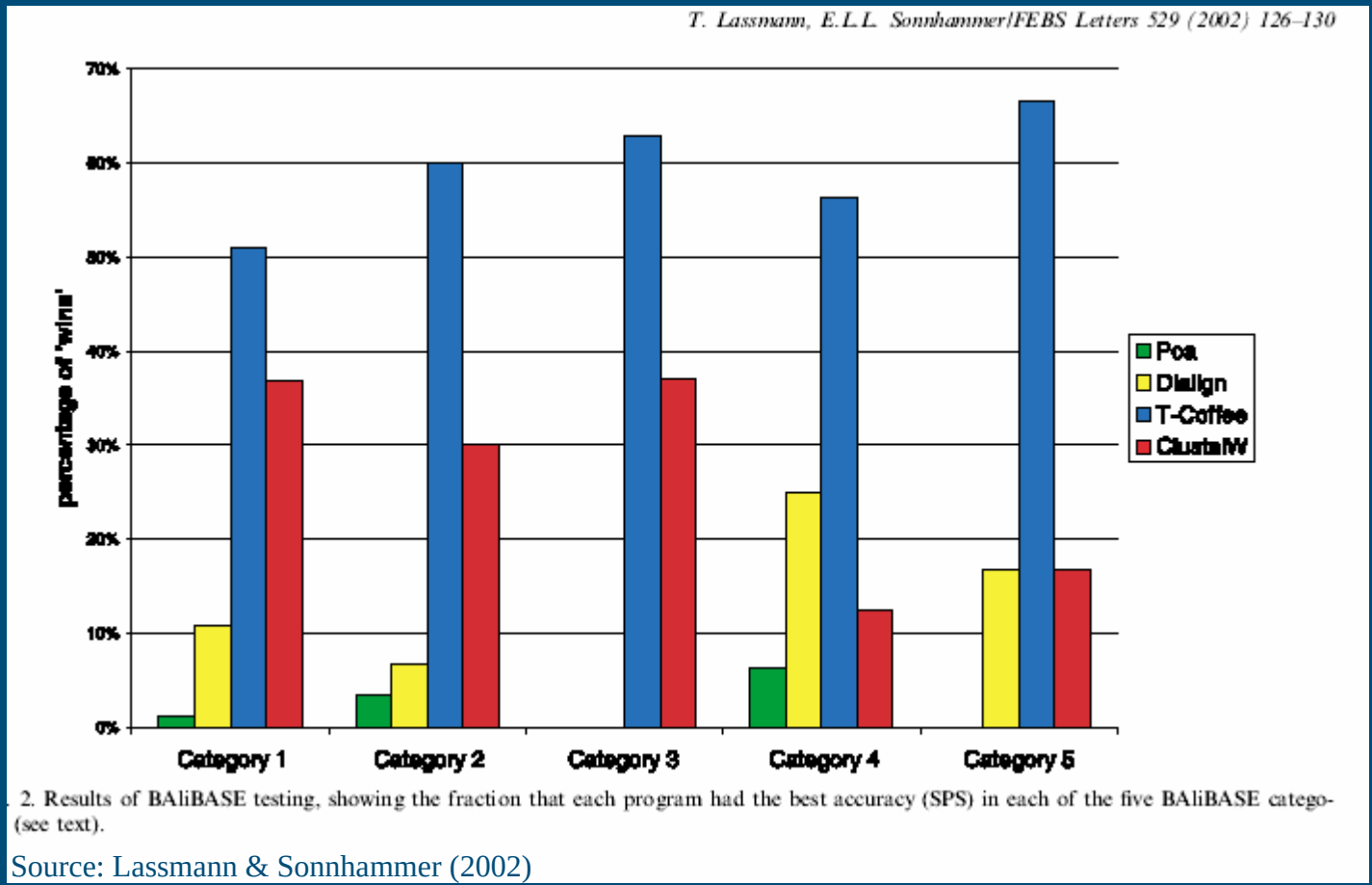


Fig. 1. Color coded matrix showing which method performed best for each pair-combination of conditions: average sequence length (x-axis) and average evolutionary distance (y-axis). The methods are Poa (green), Dialign (yellow), T-Coffee (blue) and ClustalW (red).

from: Lassmann & Sonnhammer (2002)

Alignment Accuracy



Source: Lassmann & Sonnhammer (2002)