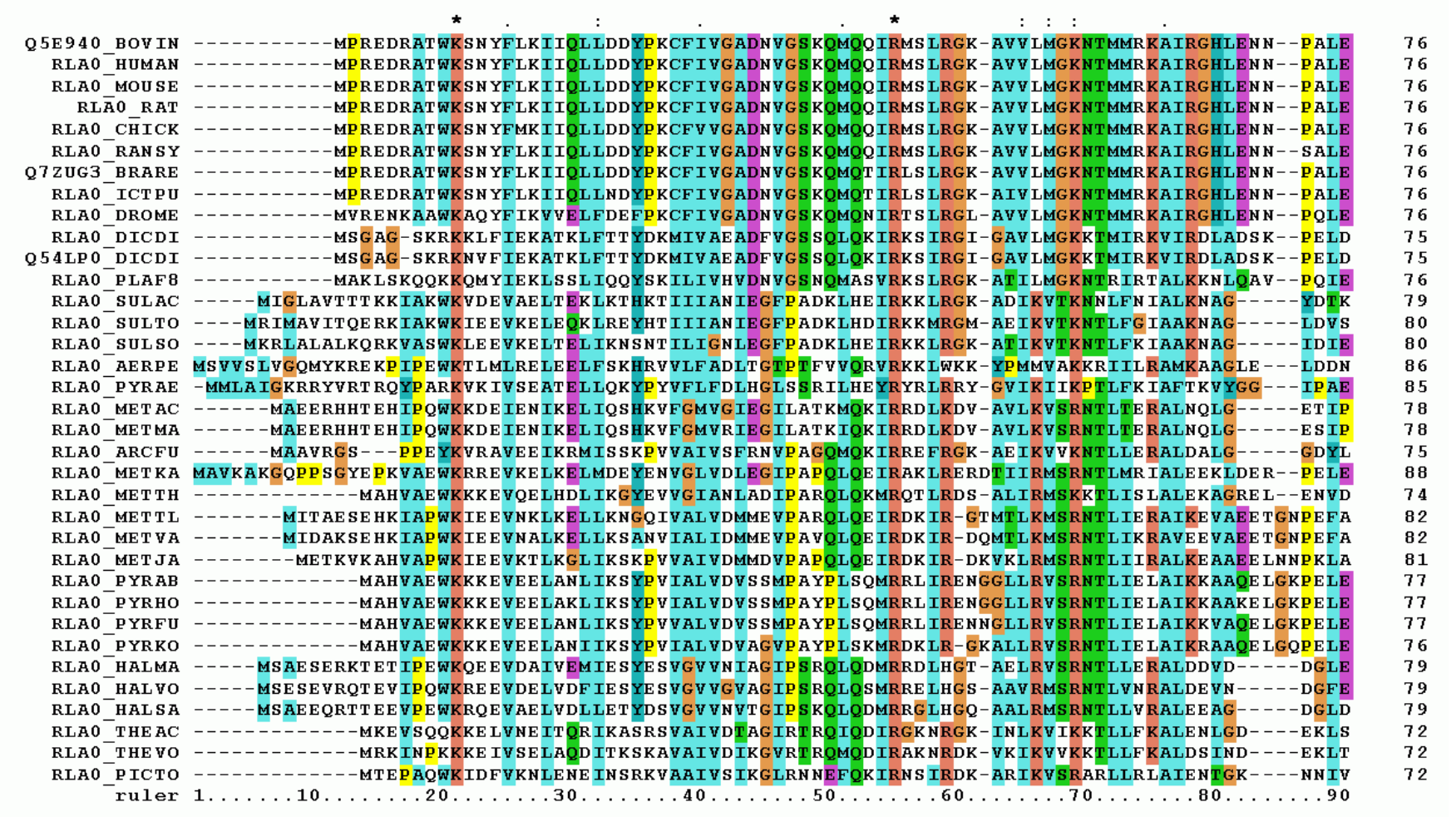
Chapter 3
Multiple Sequence Alignments



By Miguel Andrade at English Wikipedia, https://commons.wikimedia.org/w/index.php?curid=3930704

Multiple alignment algorithms

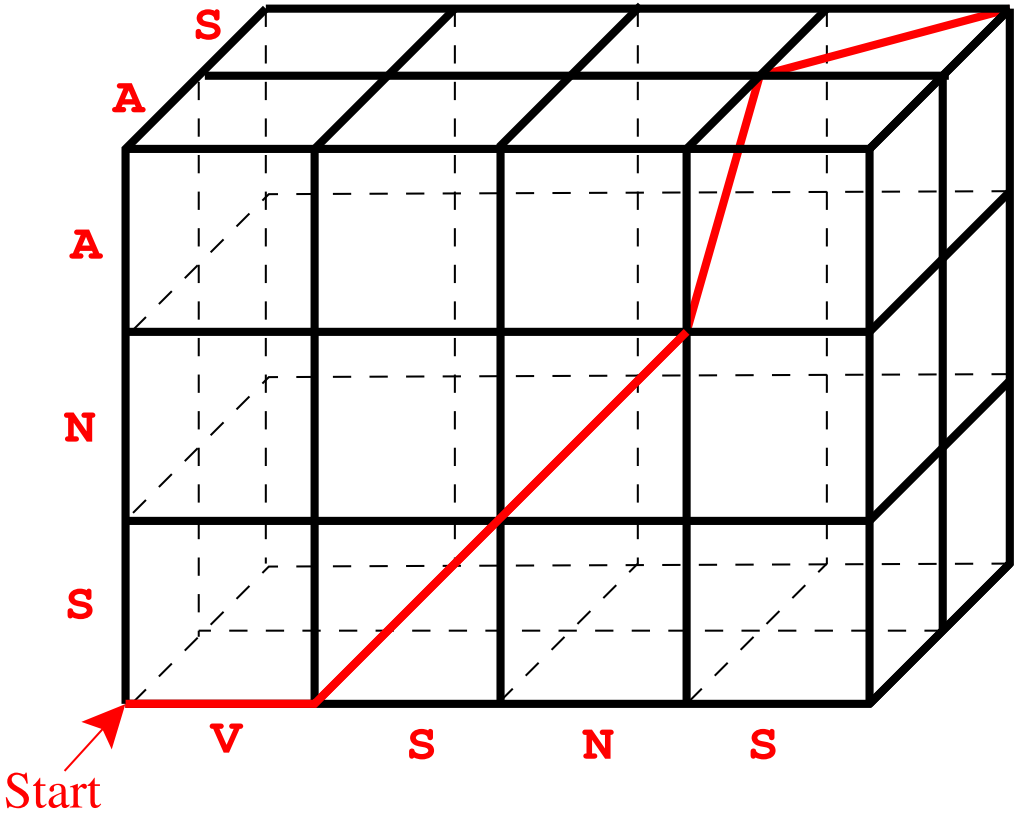
Definition. A multiple alignment of sequences $X^1, \ldots, X^n$ is a series of gapped sequences $\tilde{X}^1, \ldots, \tilde{X}^n$ such that
(i) $\tilde{X}^i$ is an extension of X^i obtained by insertions of spaces;
(ii) $|\tilde{X}^1| = |\tilde{X}^2| = \cdots = |\tilde{X}^n|$.

Why are we interested in multiple alignments?

- A multiple alignment **carries more information** than a pairwise one, as a protein can be matched against a family of proteins instead of only against another one.
- Multiple similarity of (protein) sequences suggests
  - a **common structure**,
  - a **common function**,
  - a **common evolutionary source**.

The alignment hyper-cube

Best multiple alignment of r sequences:
Best path through r**-dimensional hyper-cube** D.




```
V S N _ S
_ S N A _
_ _ _ A S

1 1 1 0 1   →  ε1
0 1 1 1 0   ↑  ε2
0 0 0 1 1   ↗  ε3
```

Alignment path for three example sequences.

Dynamic Programming Solution

- Best multiple alignment of r sequences:
  Best path through r**-dimensional hyper-cube**.

- Define $S(j_1, j_2, \ldots, j_r)$ as as the best score for aligning the prefixes of lengths $j_1, j_2, \ldots, j_r$ of the sequences $X^1, X^2, \ldots, X^r$.

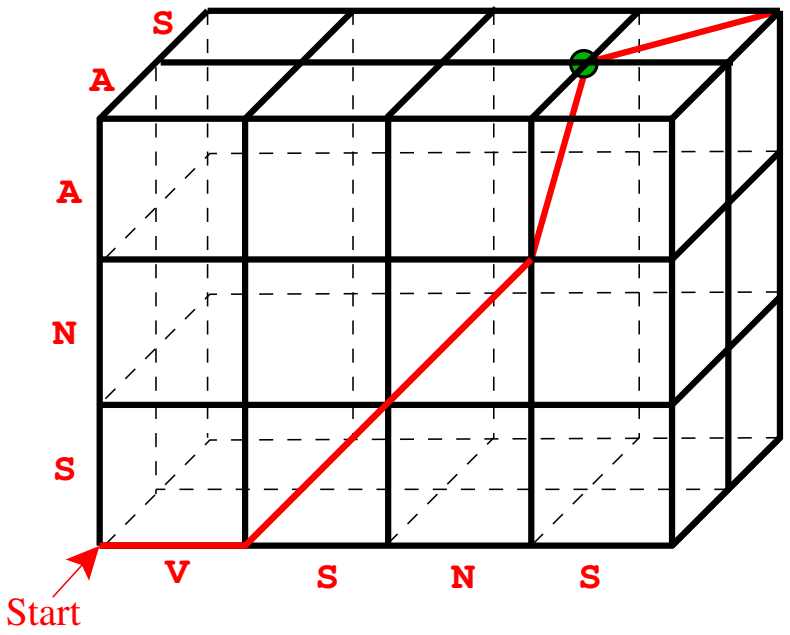
- We define $S(0, 0, \ldots, 0) = 0$, and we calculate

$$S(j_1, j_2, \ldots, j_r) = \max_{(\epsilon_1,\ldots,\epsilon_r):\ \epsilon_i \in \{0,1\}, \boldsymbol{\epsilon} \neq \mathbf{0}} \Big[S(j_1 - \epsilon_1, j_2 - \epsilon_2, \ldots, j_r - \epsilon_r) + s(\epsilon_1 x_{j_1}, \ldots, \epsilon_r x_{j_r}) \Big],$$

  where s is the **scoring function** (example $s(a, b, 0)$: joint score for aligning characters a, b and a gap) and
  $\boldsymbol{\epsilon} = (\epsilon_1, \ldots, \epsilon_r)$ is a binary vector that indicates the **directions** of the alignment progress in the hyper-cube.

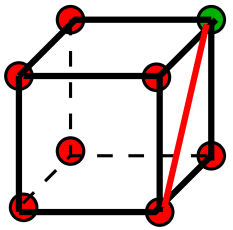
Dynamic Programming Solution: Complexity

- The size of the hyper-cube is $O(\prod_{j=1}^{r} n_j)$ (n_j = length of x_j).
- Computation of each entry considers $2^r - 1$ other entries. Example: $000, 001, 010, 011, 100, 101, 110, 111$
- If $n_1 = n_2 = \cdots = n_r = n$, the space complexity is of $O(n^r)$ and the time complexity is of $O(2^r n^r)$.



V S N _ S
_ S N A _
_ _ _ A S

1 1 1 0 1
0 1 1 1 0
0 0 0 1 1



S(3,3,0) + s(0N,0A,1A)
S(3,2,1) + s(0N,1A,0A)
S(3,2,0) + s(0N,1A,1A) = S(3,3,1)
S(2,3,1) + s(1N,0A,0A)
S(2,3,0) + s(1N,0A,1A)
S(2,2,1) + s(1N,1A,0A)
S(2,2,0) + s(1N,1A,1A)

Scoring Metrics

- A scoring scheme should take into account that...
  1. some positions are more conserved than others
     $\rightsquigarrow$ **position specific scoring**;
  2. the sequences are not independent, but are related by a **phylogenetic tree**.

- **Ideal scoring:** Complete probabilistic model of evolution
  $\rightsquigarrow$ Probability of a multiple alignment is composed of the probabilities of all evolutionary events necessary to produce the alignment.

- In practice, we do not have such a model
  $\rightsquigarrow$ **simplifying assumptions:** Two main concepts:
  1. Position specific, but ignoring the phylogenetic tree;
  2. explicit tree model, but position independent.

Multiple alignments by Profile HMM training

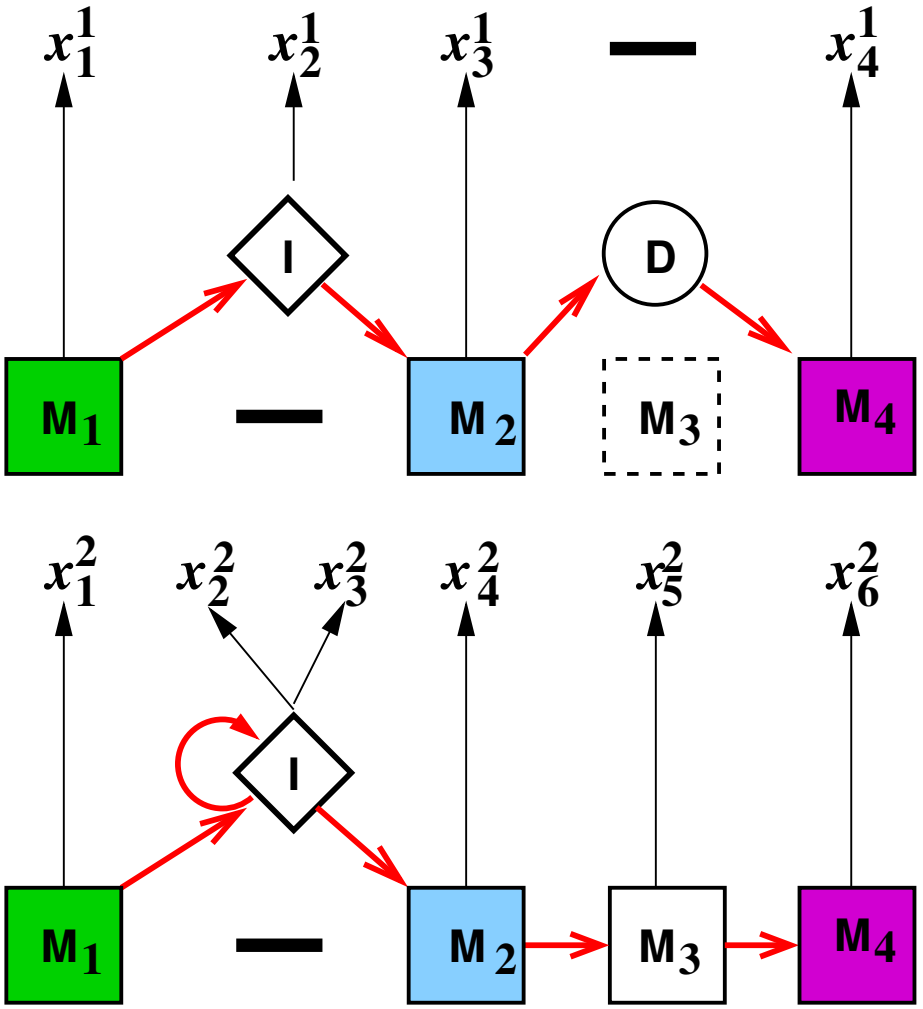
- Suppose we have successfully trained a **profile HMM** from a set of labeled sequences.
  How can we use this HMM to derive the multiple alignment of n sequences?

- **Answer:** align all n sequences to the profile using the Viterbi algorithm $\rightsquigarrow$ most probable state paths for all sequences.

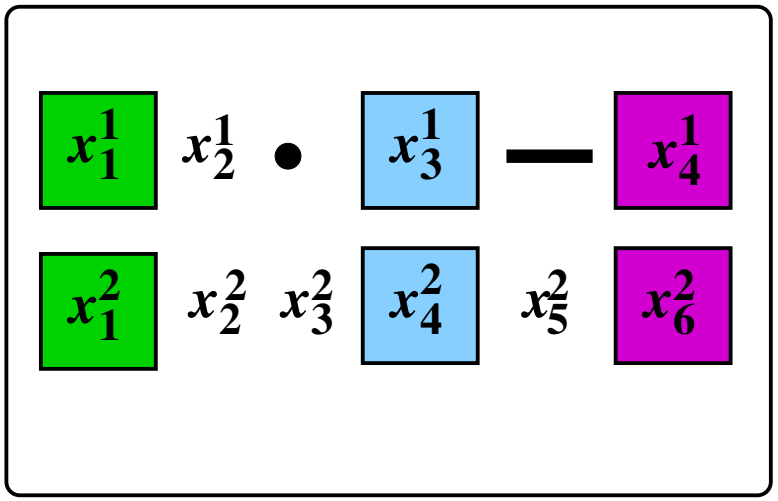
- Characters aligned to the same match state are aligned in columns.

- Multiple alignments from HMMs are approximations of type one:
  - Score is **position specific**,
  - but sequences are treated as **independent objects**.

Computing the multiple alignment: example



Multiple Alignment



Computing the multiple alignment: Real example

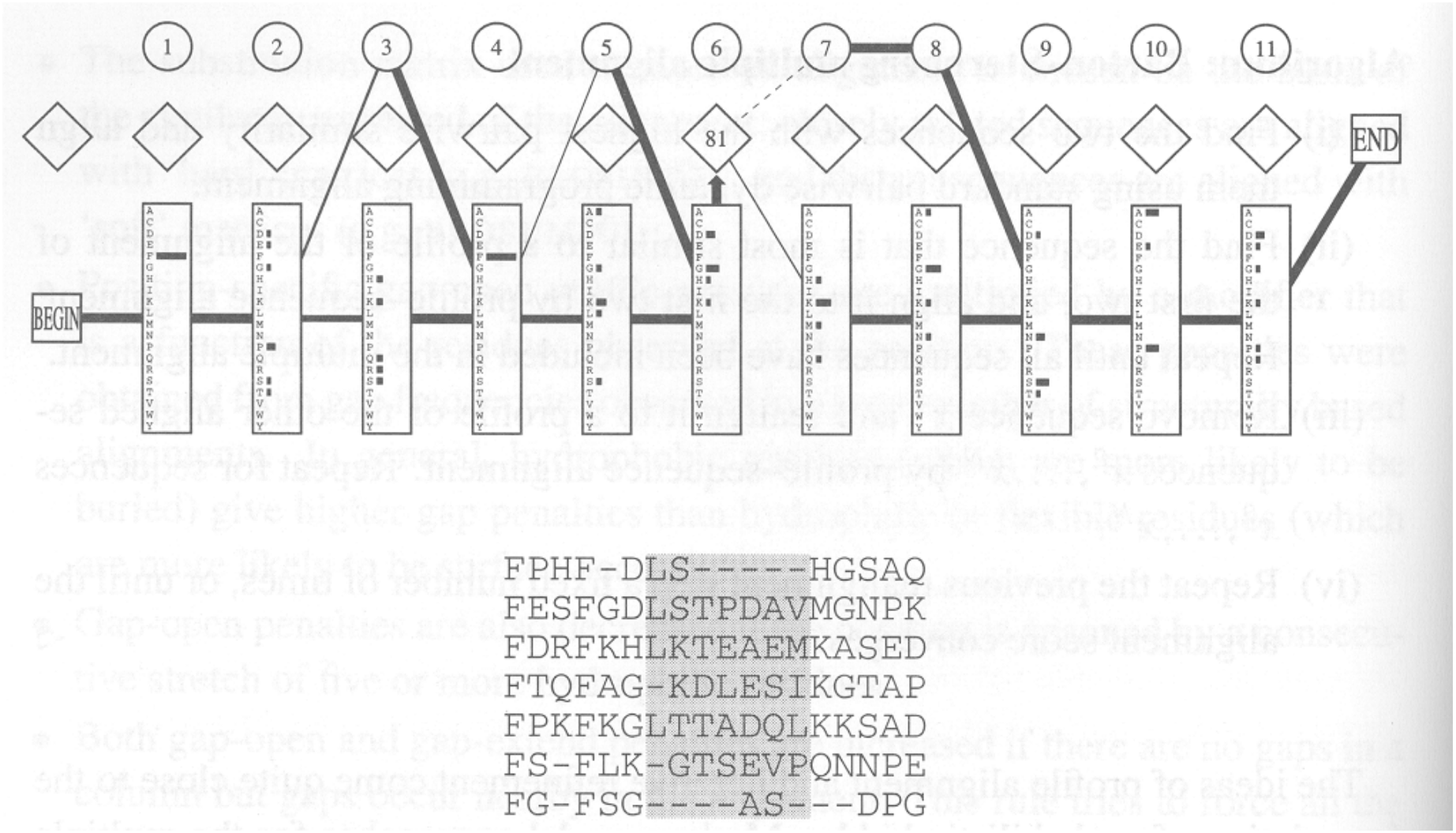


Figure 6.4 A model (top) estimated from an alignment (bottom). The characters in the shaded area of the alignment were treated as inserts.

Durbin et al., Cambridge University Press. https://doi.org/10.1017/CBO9780511790492.004

Computing the multiple alignment: Real example

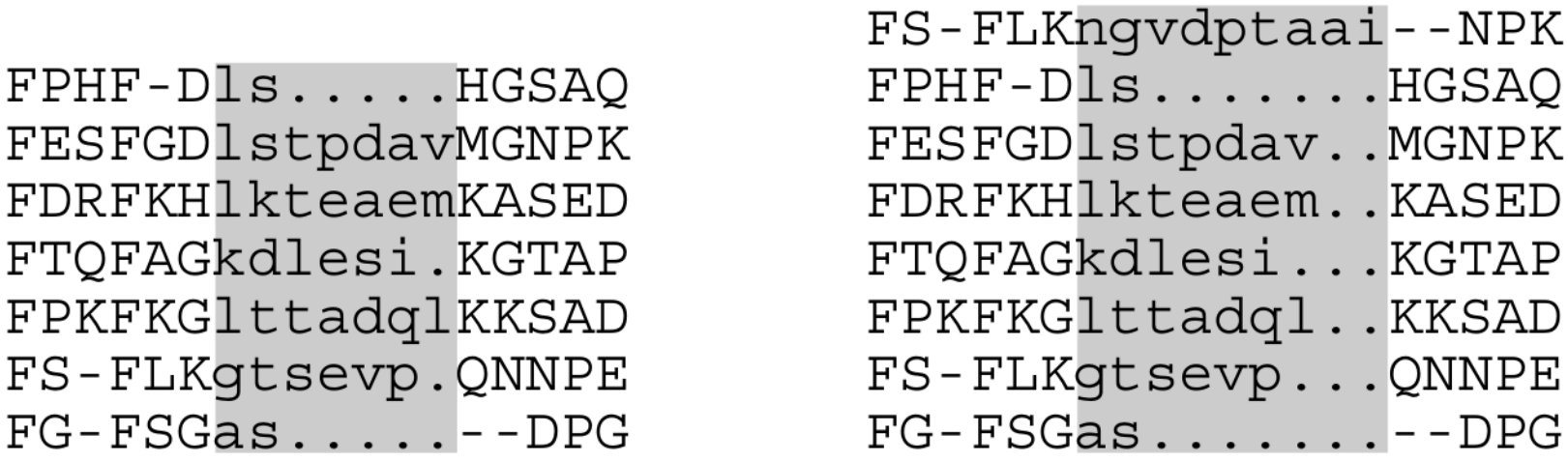


Figure 6.6 *Left: the alignment of the seven sequences is shown with lower-case letters meaning inserts. The dots are just space-filling characters to make the matches line up correctly. Right: the alignment is shown after a new sequence was added to the set. The new sequence is shown at the top, and because it has more inserts more space-filling dots were added.*

Durbin et al., Cambridge University Press. https://doi.org/10.1017/CBO9780511790492.004

Multiple Alignments by Profile HMM training

- For parameter estimation in Profile HMMs, aligned training sequences are often unavailable
  $\rightsquigarrow$ usually we only have a sample of unaligned sequences,
  the **state paths are unknown.**

- **Idea:** Use EM algorithm for iterative parameter optimization (Baum-Welch algorithm).

- Recall: for the EM algorithm, we need the forward and backward probabilities in the E-step for calculating
  - E_{bl} (the expected emission counts) and
  - $A_{l'l}$ (expected transition counts).

Simpler Multiple Alignment Algorithms

- Alternative to the probabilistic HMM formulation:
  Sum of Pairs score:
  Sum of scores between all pairs of sequences.
- The SP score for a column m_j of the multiple alignment is

$$S(m_j) = \sum_{k<l} \underbrace{s(m_j^k, m_j^l)}_{\text{from scoring matrix}}$$

- SP scores lack a probabilistic justification:
  Correct log-odds score for 3-way alignment would be

$$s(a,b,c) = \log \frac{p_{abc}}{q_a q_b q_c} \neq \underbrace{\log \frac{p_{ab}}{q_a q_b} + \log \frac{p_{bc}}{q_b q_c} + \log \frac{p_{ac}}{q_a q_c}}_{\text{SP score}}.$$

Approximation Algorithms for MSA

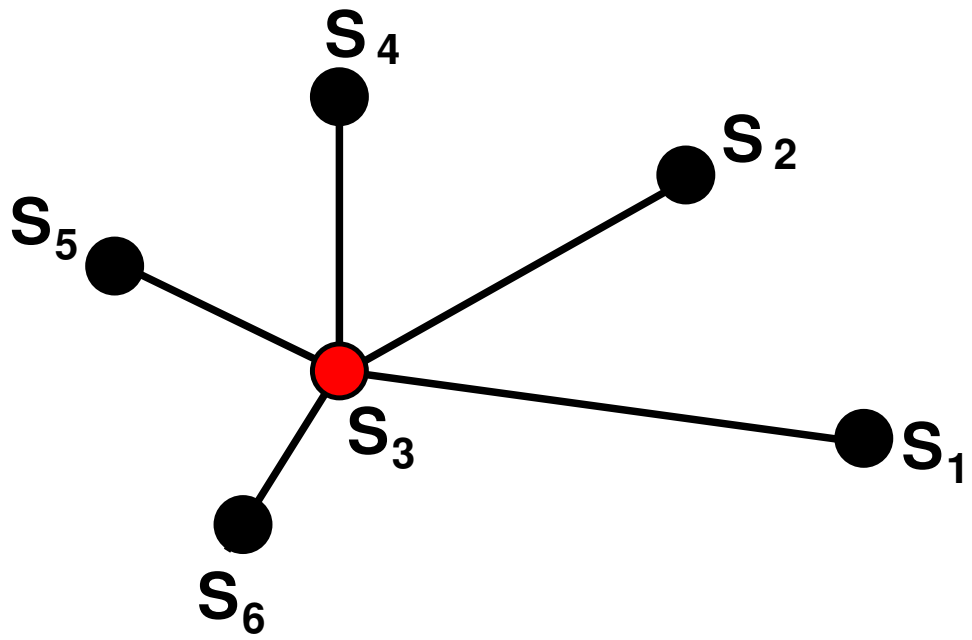
- Even for SP scores, MSA has exponential time complexity.
- Denote by $D(S,T)$ the **minimum cost** of aligning S with T.
- Let $\sigma(x,y)$ be our **cost function**, i.e. the cost of aligning the character x with the character y, for $x,y \in \Sigma \cup \{-\}$.
- Here we minimize costs σ instead of maximizing scores s.
  Example transformation: $\sigma(x,y) = \exp(-\lambda s(x,y))$.
- We assume that $\sigma(-,-) = 0$, $\sigma(x,y) = \sigma(y,x)$,
  and that the triangle inequality holds: $\sigma(x,y) \leq \sigma(x,z) + \sigma(z,y)$

Problem: The SP alignment problem.
INPUT: A set of sequences $\mathcal{S} = \{S_1, \ldots, S_k\}$.
QUESTION: Compute a global multiple alignment $\mathcal{M}$ with minimum SP-costs, given the above assumptions on $\sigma(\cdot,\cdot)$.

The Center Star Method for Alignment

Approximation algorithm for calculating the optimal multiple alignment under the SP metric with **approximation ratio of two.**

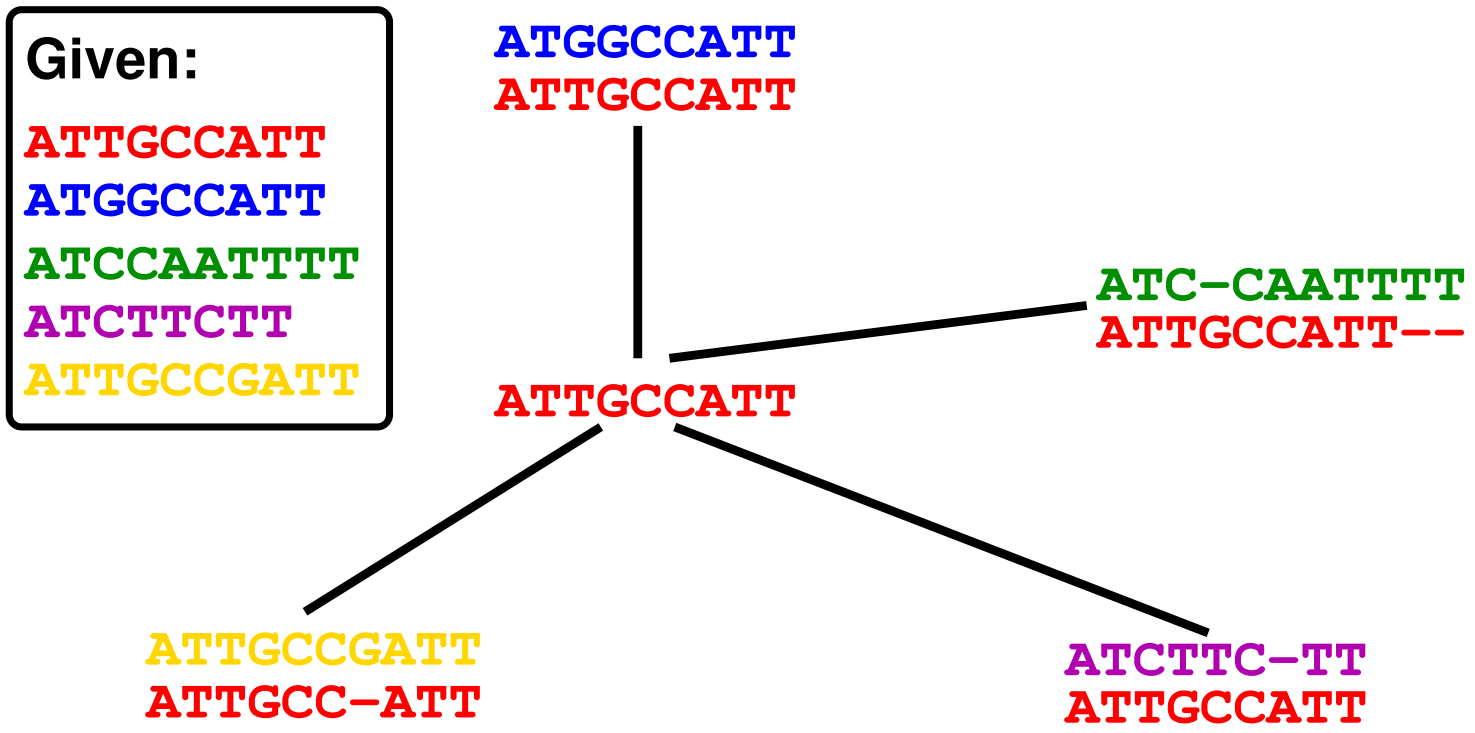
- **Center string:** String that minimizes $\sum_{S_j \in \mathcal{S}} D(S_c, S_j)$.
- **Center star:** A star tree of k nodes, center node labeled S_c, each of the $k-1$ remaining nodes labeled by $\mathcal{S} \setminus \{S_c\}$.



Type-2 approximation: explicit (star-)tree model, but position independent scoring.

The Center Star Algorithm

1. Find $S_t \in \mathcal{S}$ minimizing $\sum_{i \neq t} D(S_i, S_t)$ and let $\mathcal{M} = \{S_t\}$



2. Add sequences in $\mathcal{S} \setminus \{S_t\}$ to $\mathcal{M}$ one by one so that the **pairwise alignment** of every newly added sequence with S_t is optimal.
   Add spaces, when needed, to all pre-aligned sequences.

The Center Star Algorithm

Given:

ATTGCCATT
ATGGCCATT
ATCCAATTTT
ATCTTCTT
ATTGCCGATT

Pair:

ATGGCCATT
ATTGCCATT

ATC-CAATTTT
ATTGCCATT--

ATCTTC-TT
ATTGCCATT

ATTGCCGATT
ATTGCC-ATT

Alignment:

ATTGCCATT
ATGGCCATT

ATTGCCATT--
ATGGCCATT--
ATC-CAATTTT

ATTGCCATT--
ATGGCCATT--
ATC-CAATTTT
ATCTTC-TT--

ATTGCC-ATT--
ATGGCC-ATT--
ATC-CA-ATTTT
ATCTTC--TT--
ATTGCCGATT--

The Center Star Algorithm: Analysis

- $\mathcal{M}$: Multiple alignment produced by the center-star algorithm.
- $d(i,j)$: Cost of the resulting pairwise alignment of S_i and S_j, **induced by $\mathcal{M}$.**

  Note that $\underbrace{D(S_i, S_j)}_{\text{cost of best pairwise alignment}} \leq \underbrace{d(i,j)}_{\text{cost of induced alignment}}$
- SP-costs of center-star alignment: $\sigma(\mathcal{M}) = \sum_{i=1}^{k} \sum_{j=1, j\neq i}^{k} d(i,j)$
- $\mathcal{M}^*$: Optimal SP-alignment of all strings in $\mathcal{S}$ with costs $\sigma(\mathcal{M}^*)$.

Theorem 1.

$$\frac{\sigma(\mathcal{M})}{\sigma(\mathcal{M}^*)} =\leq \frac{2(k-1)}{k} \leq 2.$$

Theorem 2. *The running time of the center star algorithm for k strings with length $\leq n$ is $O(k^2 \cdot n^2)$.*

Proofs: see exercises.

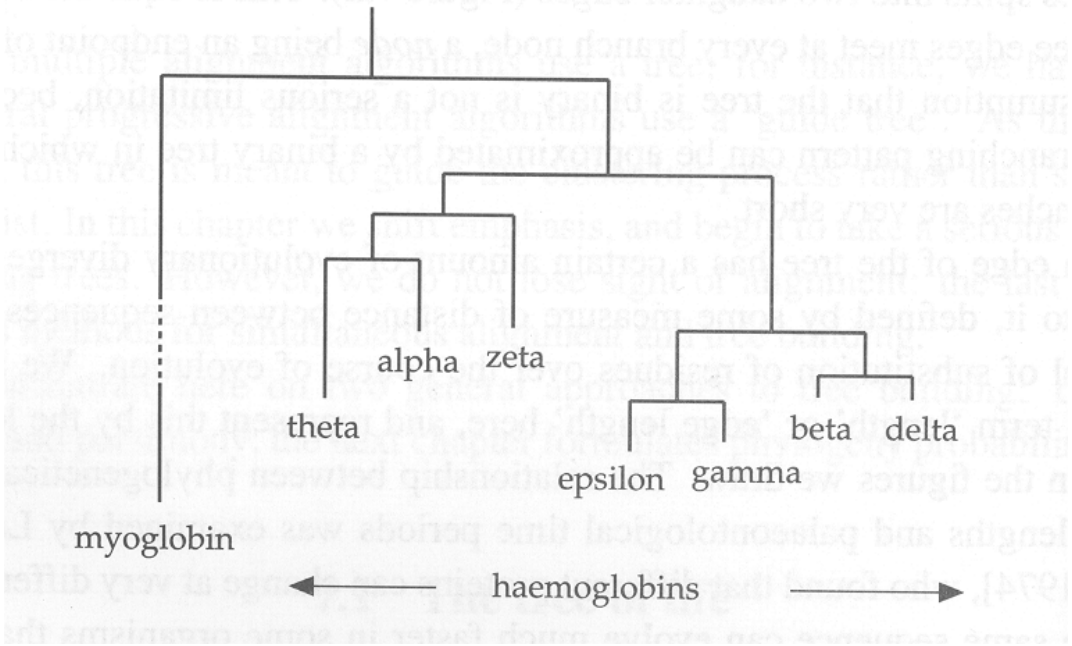
Progressive alignment heuristics

Idea: Use a binary "guide tree" instead of a star tree

(Guide tree defines a model of evolution)

Leaves: sequences, inner nodes: alignments

(sequence-sequence, sequence-profile, or profile-profile).



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Progressive alignment: ClustalW

ClustalW is a software package for multiple alignment (implementing an algorithm of Thompson, Higgins, Gibson 1994).

1. Calculate all pairwise alignment scores, convert to **pairwise distances.**

2. Use **Neighbor-Joining** algorithm to build a **tree** from the distances.

3. Align **sequence - sequence**, **sequence - profile, profile - profile.**

This algorithm makes use of many ad-hoc rules such as weighting, different matrix scores and special gap scores.