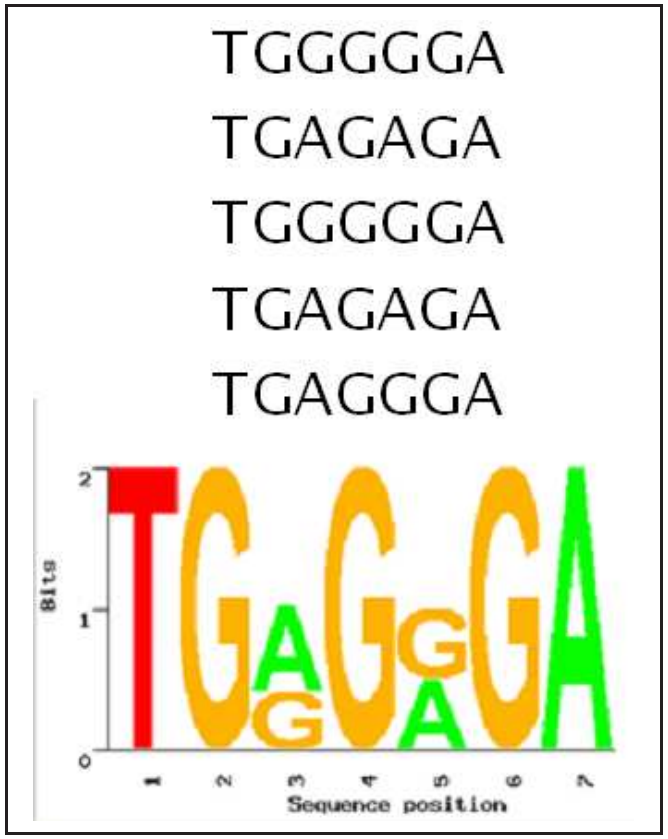
# Multiple Sequence Alignment using Profile HMM

based on Chapter 5 and Section 6.5 from

*Biological Sequence Analysis*

by R. Durbin et al., 1998




Acknowledgements:

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# PLAN

1. From profiles to Profile HMMs
2. Setting the parameters of a profile HMM;
   the optimal (MAP) model construction
3. Basic algorithms for profile HMMs
4. Profile HMM training from unaligned sequences:
   Getting the model and the multiple alignment simultaneously
5. Profile HMM variants for non-global alignments
6. Weighting the training sequences

# 1 From profiles to Profile HMMs

## Problem

**Given a multiple alignment (obtained either manually or using one of the methods presented in Ch. 6 and Ch. 7), and the profile associated to a set of marked (X = match) columns,**
**design a HMM that would perform sequence alignments to that profile.**

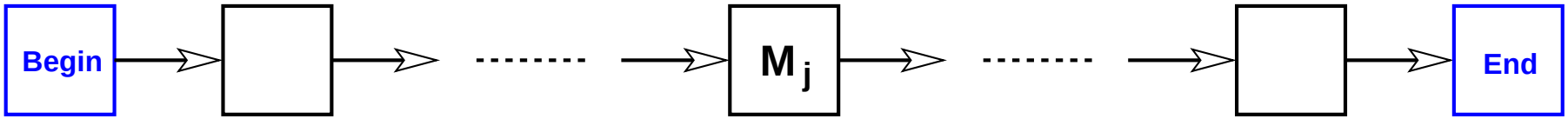
## Example

| | X | X | . | . | . | X |
|---|---|---|---|---|---|---|
| bat | A | G | - | - | - | C |
| rat | A | - | A | G | - | C |
| cat | A | G | - | A | A | - |
| gnat | - | - | A | A | A | C |
| goat | A | G | - | - | - | C |

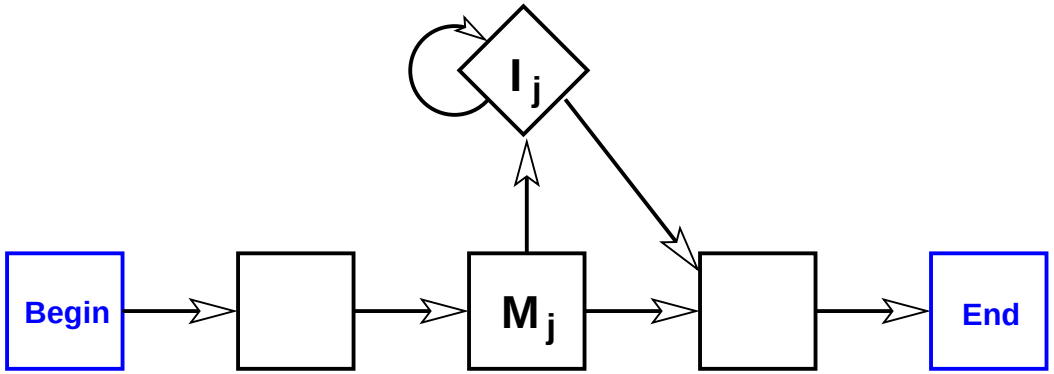
| | 1 | 2 | . . . | 3 |
|---|---|---|---|---|
| A | 4/5 | 0 | | 0 |
| C | 0 | 0 | | 4/5 |
| G | 0 | 3/5 | | 0 |
| T | 0 | 0 | | 0 |
| - | 1/5 | 2/5 | | 1/5 |

# Building up a solution

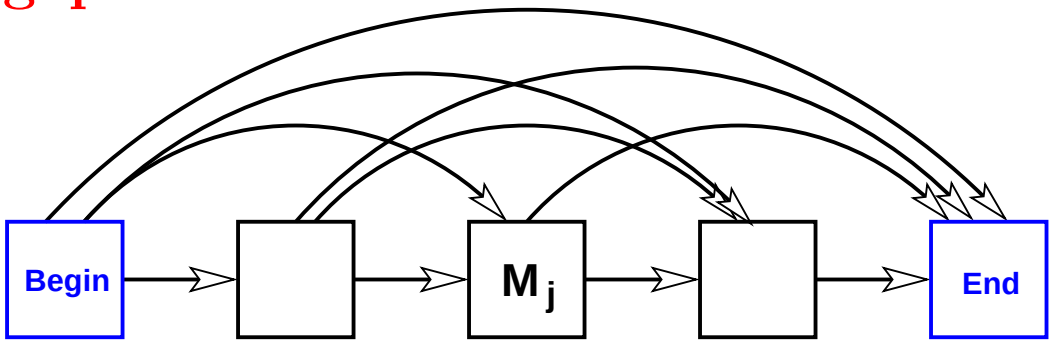
At first sight, not taking into account gaps:



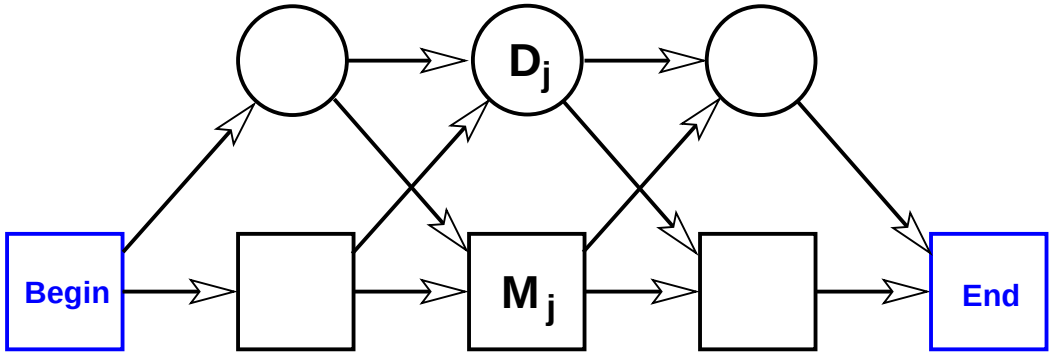

What about **insert residues?**



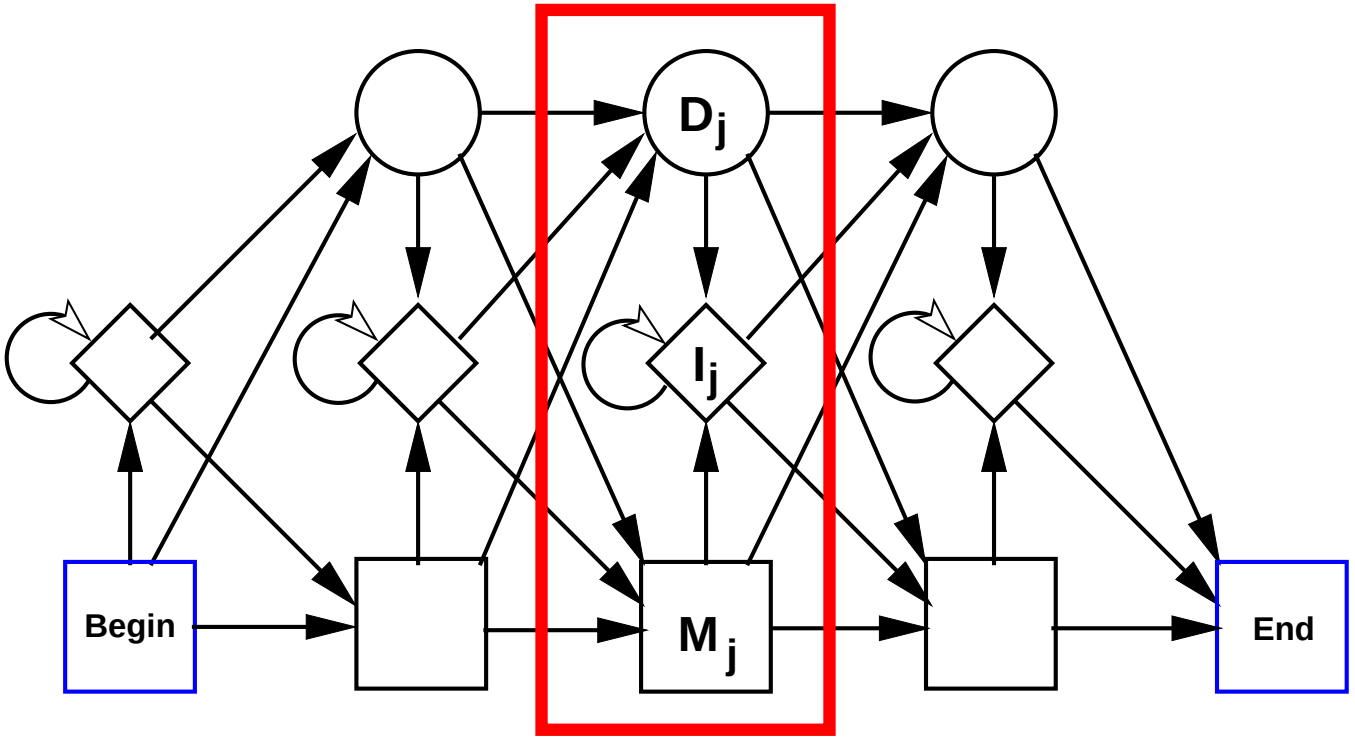
## What about gaps?




## A better treatment of gaps:



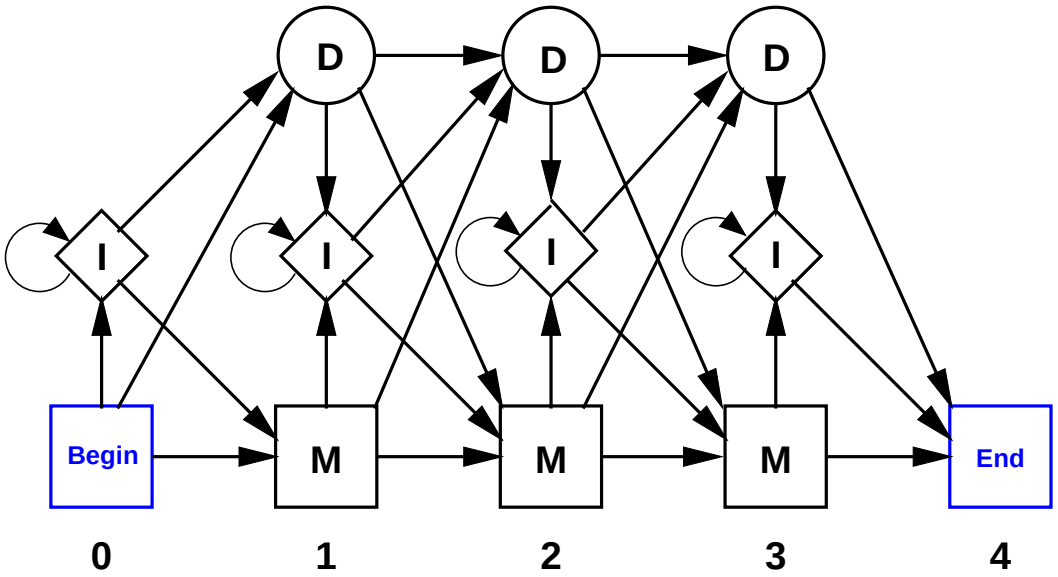
## Could we put it all together?



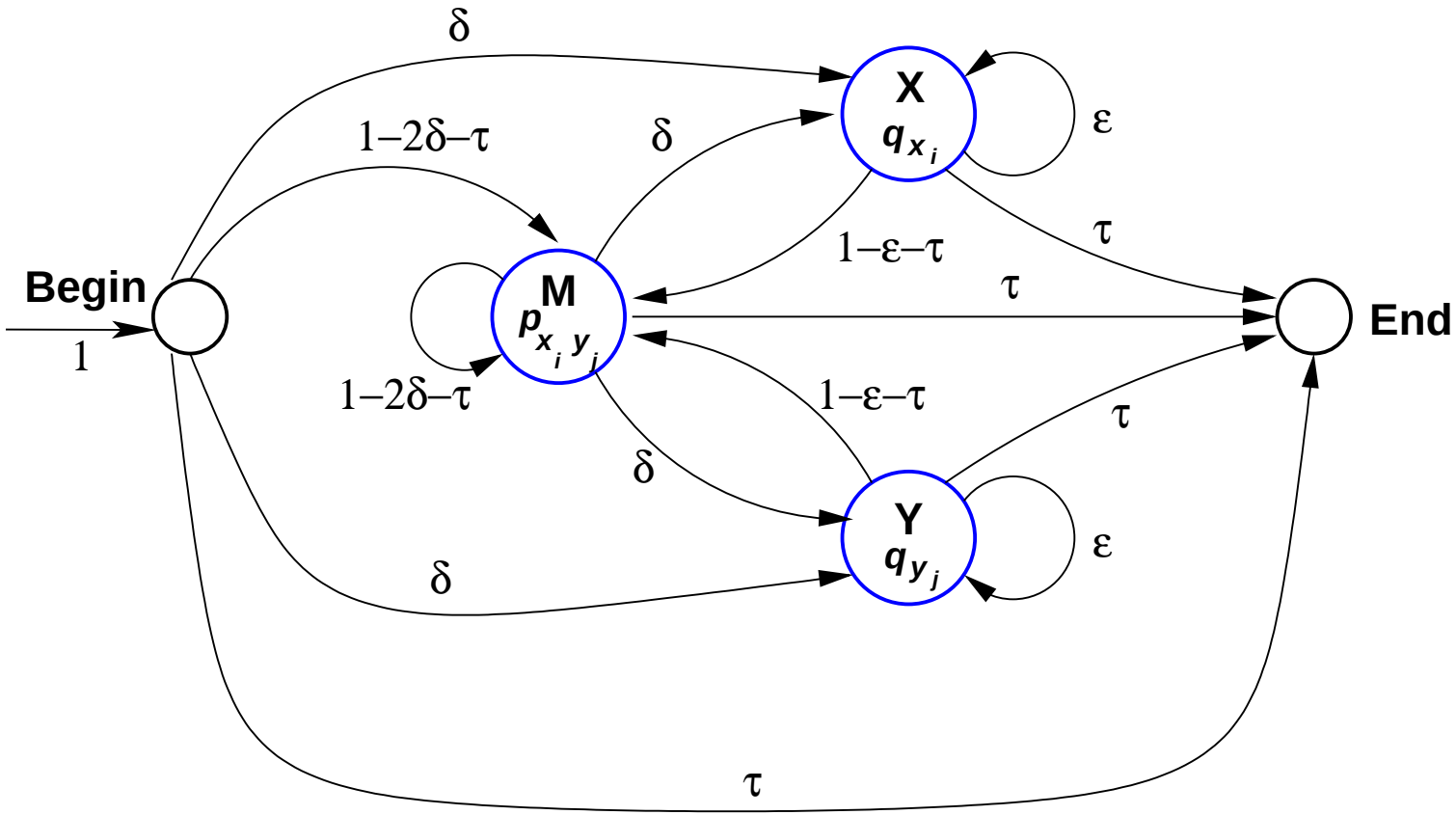

**Transition structure of a profile HMM**

# Does it work?

| | X | X | . | . | . | X |
|---|---|---|---|---|---|---|
| bat | A | G | - | - | - | C |
| rat | A | - | A | G | - | C |
| cat | A | G | - | A | A | - |
| gnat | - | - | A | A | A | C |
| goat | A | G | - | - | - | C |
| | 1 | 2 | . | . | . | 3 |



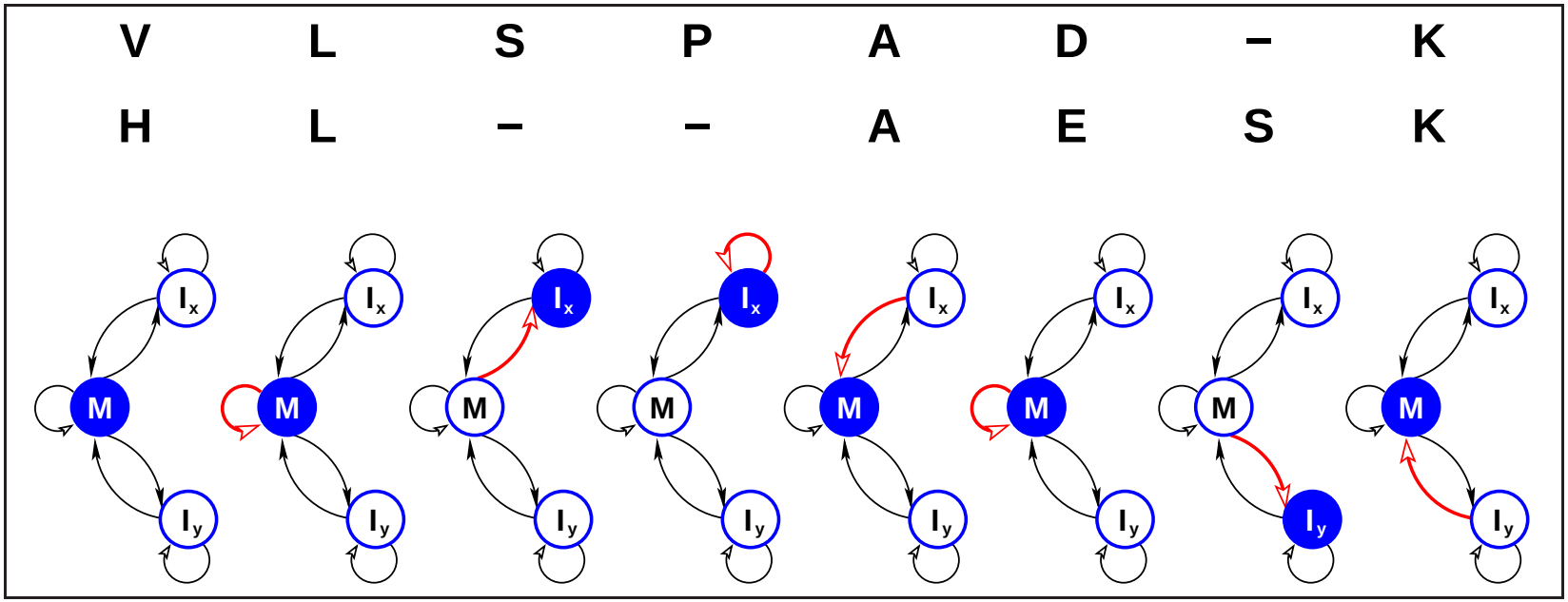
# Any resemblance to pair HMMs?




Doesn't seem so...

# However, remember...

An example of the state assignments for global alignment using the affine gap model:




When making the extension to multiple sequence alignment,
think of generating only one string (instead of a pair of strings);
use $I_x$ for inserting residues, and $I_y$ to produce a gap;
use one triplet of states (M, $I_x$, $I_y$) for each column in the alignment;
finally define (appropriate) edges in the resulting FSA.

## Consequence

**It shouldn't be difficult to re-write the basic HMM algorithms for profile HMMs!**

**one moment, though...**

# 2 Setting the parameters of a Profile HMM

## 2.1 Using Maximum Likelihood Estimates (MLE) for transition and emission probabilities

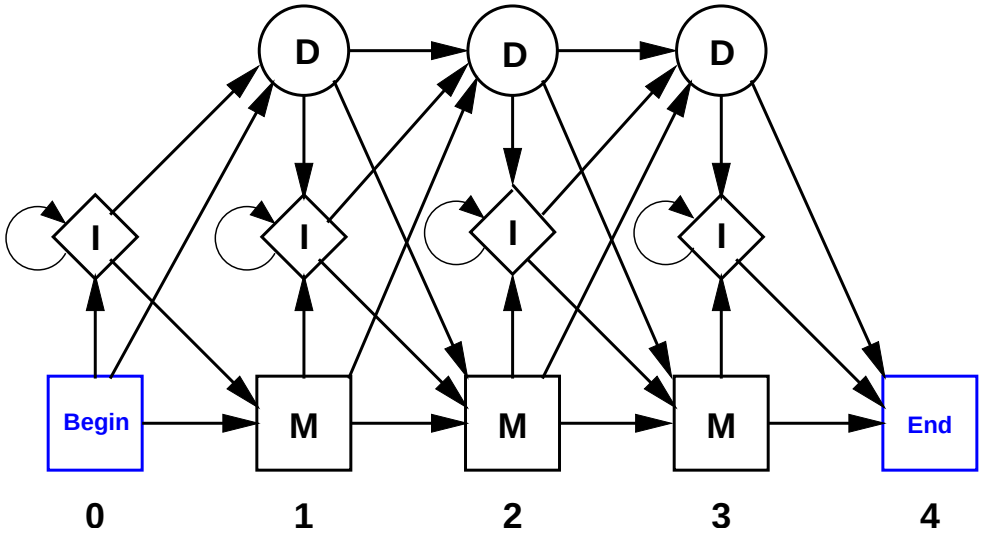
**For instance — assuming a given multiple alignment with match states marked (X) —, the emission probabilities are computed as**

$$e_{M_j}(a) = \frac{c_{ja}}{\sum_{a'} c_{ja'}}$$

**where $c_{ja}$ is the observed frequency of residue $a$ in the column $j$ of the multiple alignment.**

# Counts for our example

| | X | X | . | . | . | X |
|---|---|---|---|---|---|---|
| bat | A | G | - | - | - | C |
| rat | A | - | A | G | - | C |
| cat | A | G | - | A | A | - |
| gnat | - | - | A | A | A | C |
| goat | A | G | - | - | - | C |
| | 1 | 2 | . | . | . | 3 |




| | | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| match emissions | A | – | 4 | 0 | 0 |
| | C | – | 0 | 0 | 4 |
| | G | – | 0 | 3 | 0 |
| | T | – | 0 | 0 | 0 |
| insert emissions | A | 0 | 0 | 6 | 0 |
| | C | 0 | 0 | 0 | 0 |
| | G | 0 | 0 | 1 | 0 |
| | T | 0 | 0 | 0 | 0 |
| state transitions | M–M | 4 | 3 | 2 | 4 |
| | M–D | 1 | 1 | 0 | 0 |
| | M–I | 0 | 0 | 1 | 0 |
| | I–M | 0 | 0 | 2 | 0 |
| | I–D | 0 | 0 | 1 | 0 |
| | I–I | 0 | 0 | 4 | 0 |
| | D–M | – | 0 | 0 | 1 |
| | D–D | – | 1 | 0 | 0 |
| | D–I | – | 0 | 2 | 0 |

# What about zero counts, i.e. unseen emissions/transitions?

One solution: **use pseudocounts** (generalising Laplace's law...):

$$e_{M_j}(a) = \frac{c_{ja} + Aq_a}{\sum_{a'} c_{ja'} + A}$$

where $A$ is a weight put on pseudocounts as compared to real counts ($c_{ja}$), and $q_a$ is the frequency with which $a$ appears in a random model.

$A = 20$ works well for protein alignments.

**Note:** At the intuitive level, using pseudocount makes a lot of sense:

- $e_{M_j}(a)$ is approximately equal to $q_a$ if very little data is available, i.e. all real counts are very small compared to $A$;
- when a large amount of data is available, $e_{M_j}(a)$ is essentially equal to the maximum likelihood solution.

For **other solutions** (e.g. Dirichlet mixtures, substitution matrix mixtures, estimation based on an ancestor), you may see Durbin et al., 1998, Section 6.5.

# 2.2 Setting the $L$ parameter

- The process of model construction represents a way to decide which columns of the alignment should be assigned to match states, and which to insert states.
- There are $2^L$ combinations of marking for alignment of $L$ columns, and hence $2^L$ different profile HMMs to choose from.

  In a marked column, symbols are assigned to match states and gaps are assigned to delete states

  In an unmarked column, symbols are assigned to insert states and gaps are ignored.
- There are at least tree ways to determine the marking:
  - manual construction: the user marks alignment columns by hand;
  - heuristic construction: e.g. a column might be marked when the proportion of gap symbols in it is below a certain threshold;
  - Maximum A Posteriori (MAP) model construction: next slides.

# The MAP (maximum a posteriori) model construction

- **Objective:** we search for the model $\mu$ that maximises the likelihood of the given data, namely:

$$\underset{\mu}{\operatorname{argmax}}\, P(\mathcal{C} \mid \mu)$$

  where $\mathcal{C}$ is a set of aligned sequences.
  **Note:** The sequences in $\mathcal{C}$ are assumed to be statistically independent.

- **Idea:** The MAP model construction algorithm recursively calculates $S_j$, the log probability of the optimal model for the alignment up to and including column $j$, assuming that the column $j$ is marked.

- **More specifically:** $S_j$ is calculated from smaller subalignments ending at a marked column $i$, by incrementing $S_i$ with the summed log probability of the transitions and emissions for the columns between $i$ and $j$.

# MAP model construction algorithm: Notations

- $c_{xy}$ — the observed state transition counts
- $a_{xy}$ — the transition probabilities, estimated from the $c_{xy}$ in the usual fashion (MLE)

$$a_{xy} = \frac{c_{xy}}{\sum_y c_{xy}}$$

- $S_j$ — the log probability of the optimal model for the alignment up to and including column $j$, assuming that column $j$ is marked
- $\mathcal{T}_{ij}$ — the summed log probability of all the state transitions between marked columns $i$ and $j$

$$\mathcal{T}_{ij} = \sum_{x,y \in M,D,I} c_{xy} \log a_{xy}$$

- $\mathcal{M}_j$ — the log probability contribution for match state symbol emissions in the column $j$
- $\mathcal{L}_{i,j}$ — the log probability contribution for the insert state symbol emissions for the columns $i+1, \ldots, j-1$ (for $j - i > 1$).

# The MAP model construction algorithm

**Initialization:** $S_0 = 0$, $\mathcal{M}_{L+1} = 0$

**Recurrence:**

**for** $j = 1, \ldots, L+1$

$S_j = \max_{0 \leq i < j} S_i + \mathcal{T}_{ij} + \mathcal{M}_j + \mathcal{L}_{i+1,j-1} + \lambda$

$\sigma_j = \arg\max_{0 \leq i < j} S_i + \mathcal{T}_{ij} + \mathcal{M}_j + \mathcal{L}_{i+1,j-1} + \lambda$

**Traceback:**

**from** $j = \sigma_{L+1}$, **while** $j > 0$:

**mark column** $j$ **as a match column;** $j = \sigma_j$

**Complexity:**

$\mathcal{O}(L)$ **in memory and** $\mathcal{O}(L^2)$ **in time for an alignment of** $L$ **columns... with some care in implementation!**

**Note:** $\lambda$ **is a penalty used to favour models with fewer match states. In Bayesian terms,** $\lambda$ **is the log of the prior probability of marking each column. It implies a simple but adequate exponentially decreasing prior distribution over model lengths.**

# 3 Basic algorithms for Profile HMMs

## Notations

- $v_{M_j}(i)$ — **the probability of the best path matching the subsequence $x_{1\ldots i}$ to the (profile) submodel up to the column $j$, ending with $x_i$ being emitted by the state $M_j$;**

  $v_{I_j}(i)$ — **the probability of the best path ending in $x_i$ being emitted by $I_j$;**

  $v_{D_j}(i)$ — **the probability of the best path ending in $D_j$ ($x_i$ being the last character emitted before $D_j$).**

- $V_{M_j}(i)$, $V_{I_j}(i)$, $V_{D_j}(i)$ — **the log-odds scores corresponding respectively to $v_{M_j}(i)$, $v_{I_j}(i)$, $v_{D_j}(i)$.**

- $f_{M_j}(i)$ — **the combined probability of all alignments up to $x_i$ that end in state $M_j$, and similarly $f_{I_j}(i)$, $f_{D_j}(i)$.**

- $b_{M_j}(i)$, $b_{I_j}(i)$, $b_{D_j}(i)$ — **the corresponding backward probabilities.**

# The Viterbi algorithm for profile HMM

## Initialization:

**rename the Begin state as $M_0$, and set $v_{M_0}(0) = 1$;**
**rename the End state as $M_{L+1}$**

## Recursion:

$$v_{M_j}(i) = e_{M_j}(x_i) \max \begin{cases} v_{M_{j-1}}(i-1)\, a_{M_{j-1}M_j} \\ v_{I_{j-1}}(i-1)\, a_{I_{j-1}M_j} \\ v_{D_{j-1}}(i-1)\, a_{D_{j-1}M_j} \end{cases}$$

$$v_{I_j}(i) = e_{I_j}(x_i) \max \begin{cases} v_{M_j}(i-1)\, a_{M_j I_j}, \\ v_{I_j}(i-1)\, a_{I_j I_j} \\ v_{D_j}(i-1)\, a_{D_j I_j} \end{cases}$$

$$v_{D_j}(i) = \max \begin{cases} v_{M_{j-1}}(i)\, a_{M_{j-1}D_j} \\ v_{I_{j-1}}(i)\, a_{I_{j-1}D_j} \\ v_{D_{j-1}}(i)\, a_{D_{j-1}D_j} \end{cases}$$

## Termination:

**the final score is $v_{M_{L+1}}(n)$, calculated using the top recursion relation.**

# The Viterbi algorithm for profile HMMs: log-odds version

## Initialization:

$V_{M_0}(0) = 0$; **(the Begin state is $M_0$, and the End state is $M_{L+1}$)**

## Recursion:

$$V_{M_j}(i) = \log \frac{e_{M_j}(x_i)}{q_{x_i}} + \max \begin{cases} V_{M_{j-1}}(i-1) + \log a_{M_{j-1}M_j} \\ V_{I_{j-1}}(i-1) + \log a_{I_{j-1}M_j} \\ V_{D_{j-1}}(i-1) + \log a_{D_{j-1}M_j} \end{cases}$$

$$V_{I_j}(i) = \log \frac{e_{I_j}(x_i)}{q_{x_i}} + \max \begin{cases} V_{M_j}(i-1) + \log a_{M_jI_j}, \\ V_{I_j}(i-1) + \log a_{I_jI_j} \\ V_{D_j}(i-1) + \log a_{D_jI_j} \end{cases}$$

$$V_j^D(i) = \max \begin{cases} V_{M_{j-1}}(i) + \log a_{M_{j-1}D_j} \\ V_{I_{j-1}}(i) + \log a_{I_{j-1}D_j} \\ V_{D_{j-1}}(i) + \log a_{D_{j-1}D_j} \end{cases}$$

## Termination:

**the final score is $V_{M_{L+1}}(n)$, calculated using the top recursion relation.**

# The Forward algorithm for profile HMMs

**Initialization:** $f_{M_0}(0) = 1$

**Recursion:**

$$f_{M_j}(i) = e_{M_j}(x_i)[f_{M_{j-1}}(i-1)a_{M_{j-1}M_j} + f_{I_{j-1}}(i-1)a_{I_{j-1}M_j} + f_{D_{j-1}}(i-1)a_{D_{j-1}M_j}]$$

$$f_{I_j}(i) = e_{I_j}(x_i)[f_{M_j}(i-1)a_{M_jI_j} + f_{I_j}(i-1)a_{I_jI_j} + f_{D_j}(i-1)a_{D_jI_j}]$$

$$f_{D_j}(i) = f_{M_{j-1}}(i)a_{M_{j-1}D_j} + f_{I_{j-1}}(i)a_{I_{j-1}D_j} + f_{D_{j-1}}(i)a_{D_{j-1}D_j}$$

**Termination:**

$$f_{M_{L+1}}(n+1) = f_{M_L}(n)a_{M_LM_{L+1}} + f_{I_L}(n)a_{I_LM_{L+1}} + f_{D_L}(n)a_{D_LM_{L+1}}$$

# The Backward algorithm for profile HMMs

## Initialization:

$b_{M_{L+1}}(n+1) = 1;$
$b_{M_L}(n) = a_{M_L M_{L+1}};\ b_{I_L}(n) = a_{I_L M_{L+1}};\ b_{D_L}(n) = a_{D_L M_{L+1}}$

## Recursion:

$$b_{M_j}(i) = b_{M_{j+1}}(i+1)a_{M_j M_{j+1}}e_{M_{j+1}}(x_{i+1}) + b_{I_j}(i+1)a_{M_j I_j}e_{I_j}(x_{i+1}) + b_{D_{j+1}}(i)a_{M_j D_{j+1}}$$

$$b_{I_j}(i) = b_{M_{j+1}}(i+1)a_{I_j M_{j+1}}e_{M_{j+1}}(x_{i+1}) + b_{I_j}(i+1)a_{I_j I_j}e_{I_j}(x_{i+1}) + b_{D_{j+1}}(i)a_{I_j D_{j+1}}$$

$$b_{D_j}(i) = b_{M_{j+1}}(i+1)a_{D_j M_{j+1}}e_{M_{j+1}}(x_{i+1}) + b_{I_j}(i+1)a_{D_j I_j}e_{I_j}(x_{i+1}) + b_{D_{j+1}}(i)a_{D_j D_{j+1}}$$

# The Baum-Welch (Expectation-Maximization) algorithm for Profile HMMs: re-estimation equations

**Expected emission counts from sequence $x$:**

$$E_{M_j}(a) = \frac{1}{P(x)} \sum_{i|x_i=a} f_{M_j}(i) b_{M_j}(i)$$

$$E_{I_j}(a) = \frac{1}{P(x)} \sum_{i|x_i=a} f_{I_j}(i) b_{I_j}(i)$$

**Expected transition counts from sequence $x$:**

$$A_{X_j M_{j+1}} = \frac{1}{P(x)} \sum_i f_{X_j}(i) a_{X_j M_{j+1}} e_{M_{j+1}}(x_{i+1}) b_{M_{j+1}}(i+1)$$

$$A_{X_j I_j} = \frac{1}{P(x)} \sum_i f_{X_j}(i) a_{X_j I_j} e_{I_j}(x_{i+1}) b_{I_j}(i+1)$$

$$A_{X_j D_{j+1}} = \frac{1}{P(x)} \sum_i f_{X_j}(i) a_{X_j D_{j+1}} b_{D_{j+1}}(i)$$

**where $X_j$ is one of $M_j$, $I_j$, and $D_j$.**

# Avoiding local maxima

- The Baum-Welch algorithm is guaranteed to find a local maximum on the probability "surface" but there is no guarantee that this local optimum is anywhere near the global optimum.
- A more involved approach is to use some form of stochastic search algorithm that "bumps" Baum-Welch off from local maxima:
  - noise injection during Baum-Welch re-estimation,
  - simulated annealing Viterbi approximation of EM,
  - Gibbs sampling.

  For details, see Durbin et al. Section 6.5, pages 154–158.

# 4 Getting simultaneously the model and the multiple alignment

## Profile HMM training from unaligned sequences

**Initialization:**

Choose the length of the profile HMM (i.e., the number of match states), and initialize the transition and emission parameters.

A commonly used rule is to set the profile HMM's length to be the average length of the training sequences.

**Training:**

Estimate the model using the Baum-Welch algorithm or its Viterbi alternative.

Start Baum-Welch from multiple different points to see if it all converges to approximately the same optimum.

If necessary, use a heuristic method for avoiding local optima. (See the previous slide.)

**Multiple alignment:**

Align all sequences to the final model using the Viterbi algorithm and build a multiple alignment.

## Further comments on profile HMM's initial parameters:

- One possibility:
  guess a multiple alignment of some or all given sequences.
- A further possibility:
  derive the model's initial parameters from the Dirichlet prior over parameters (see Ch. 11).
- Alternatively:
  use frequencies derived from the prior to initialise the model's parameters, then use this model to generate a small number of random sequences, and finally use the resulting counts as 'data' to estimate an initial model.
- Note:
  The model should be encouraged to use 'sensible' transitions; for instance transitions into match states should be large compared to other transition probabilities.

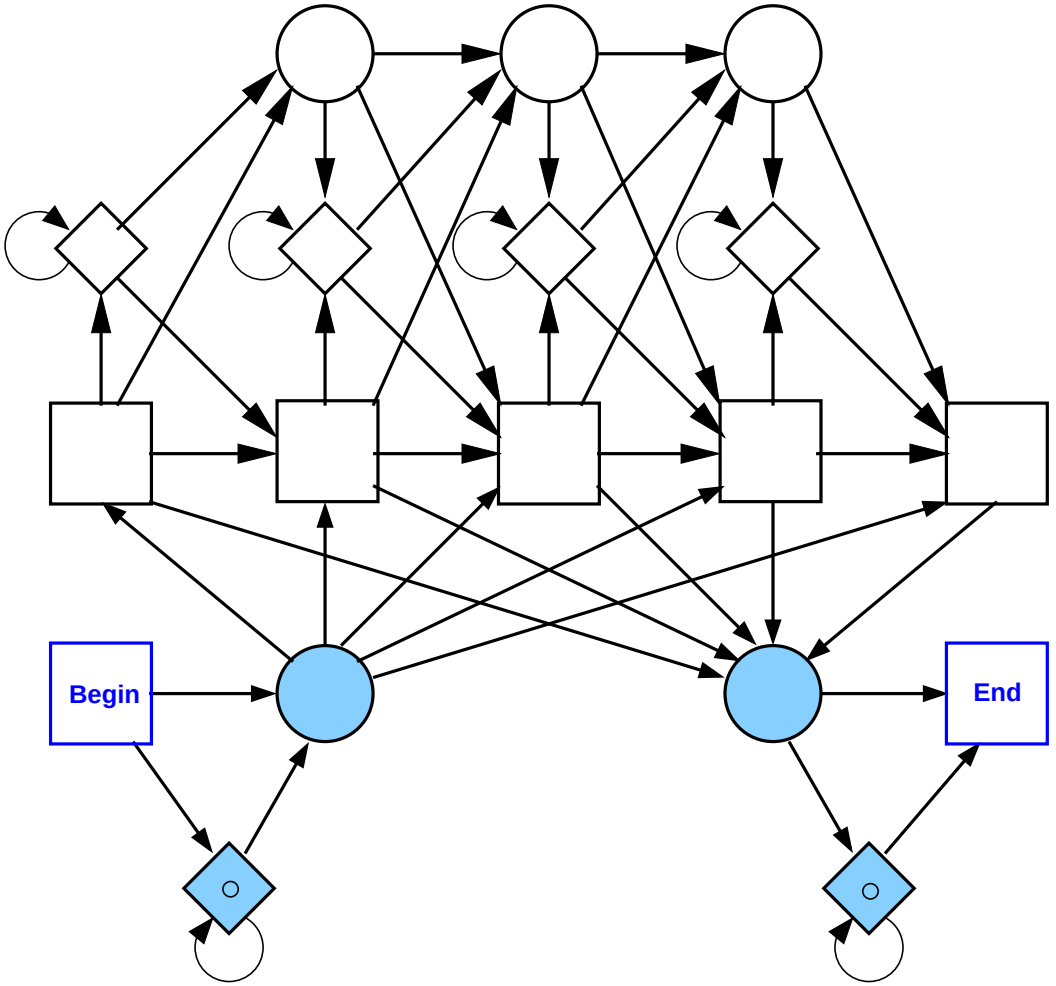
# Model surgery

After training a model we can analyse the alignment it produces:

- From counts estimated by the forward-backward procedure we can see how much a certain transition is used by the training sequences.
- The usage of a match state is the sum of counts for all letters emitted in the state.
- If a certain match state is used by less than half the number of given sequences, the corresponding module (triplet of match, insert, delete states) should be deleted.
- Similarly, if more than half (or some other predefined fraction) of the sequences use the transitions into a certain insert state, this should be expanded to some number of new modules (usually the average number of insertions).

# 5 Profile HMMs for non-global alignments

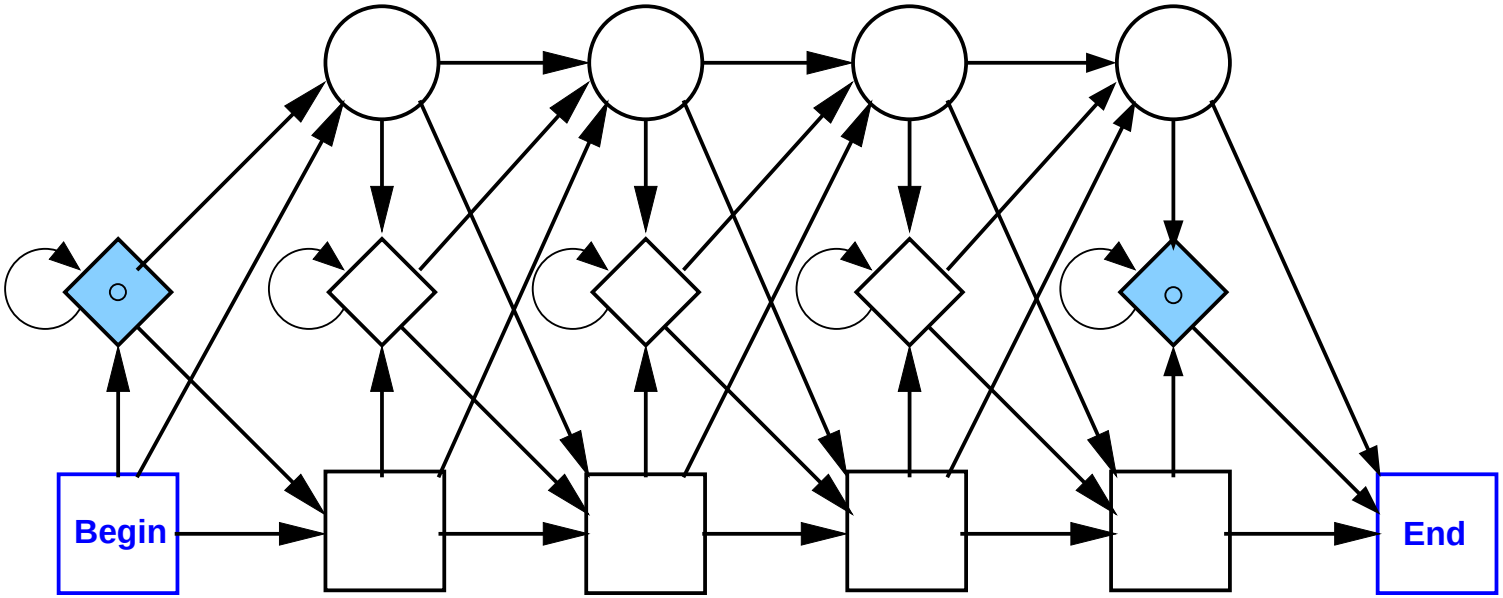
**Local**
**multiple alignment**



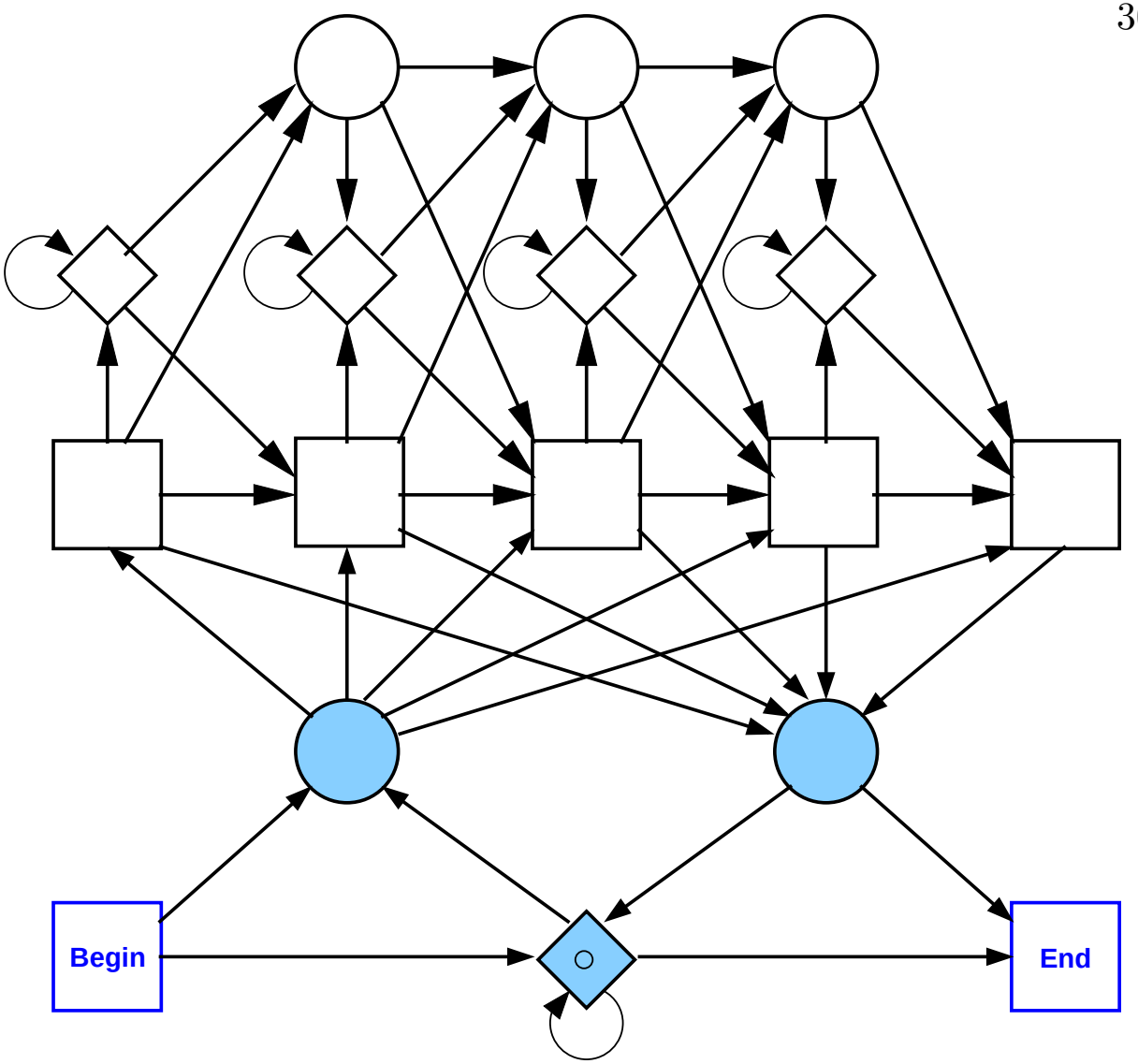
**The looping probabilities of the flanking (blue diamond) states should be close to 1; let's set them to $1 - \eta$.**

**Transition probabilities from the left flanking state to the match states:**
**one option: all $\eta/L$**
**another option: $\eta/2$ for the transition into the first match state, and $\eta/2(L-1)$ for the other positions.**

## For the rare case when the first residue might be missing:



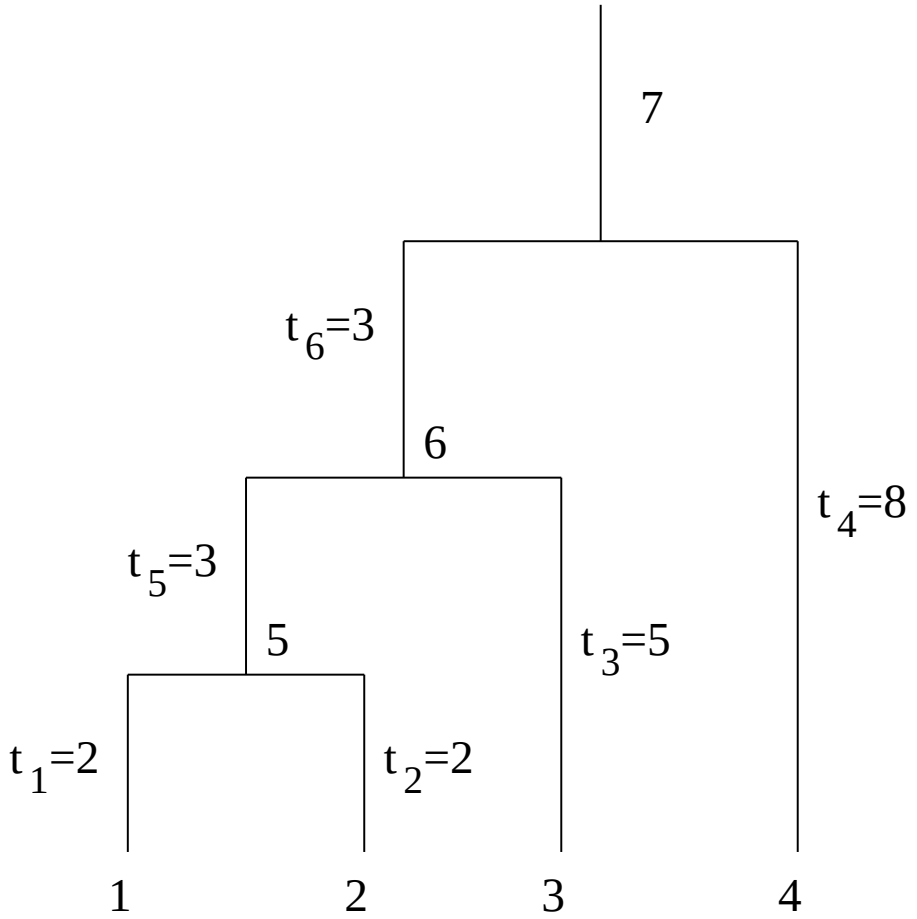
A profile HMM for repeat matches to subsections of the profile model



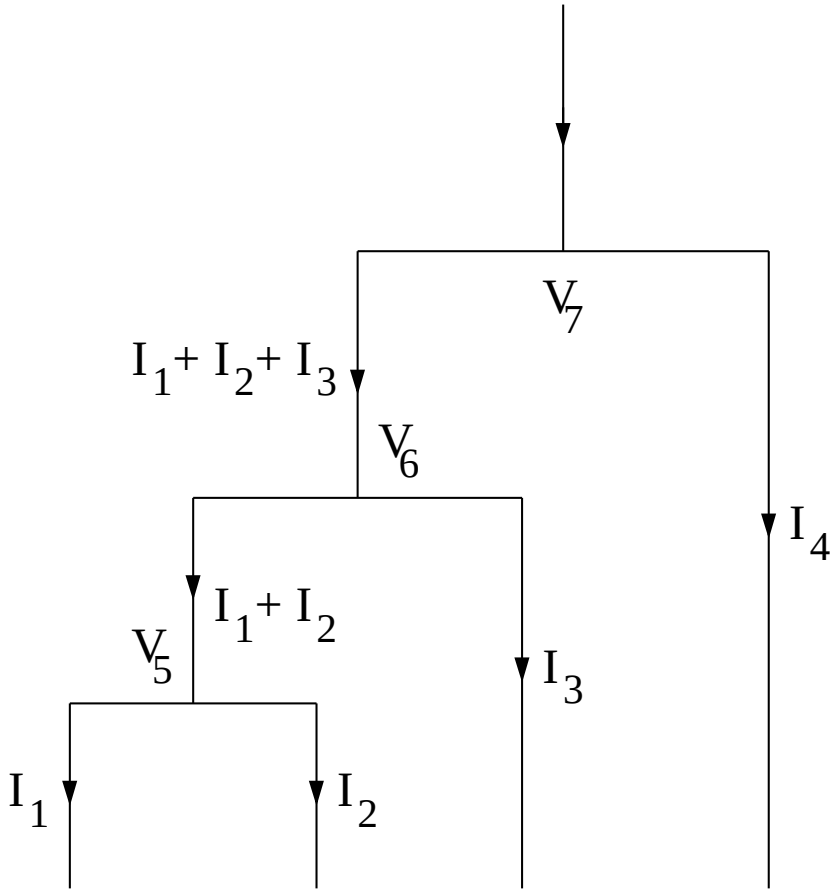
# 6 Weighting the training sequences

When the (training) sequences in the given multiple alignment are not statistically independent, one may use some simple weighting schemes derived from a phylogenetic tree.

Example of a phylogenetic tree:

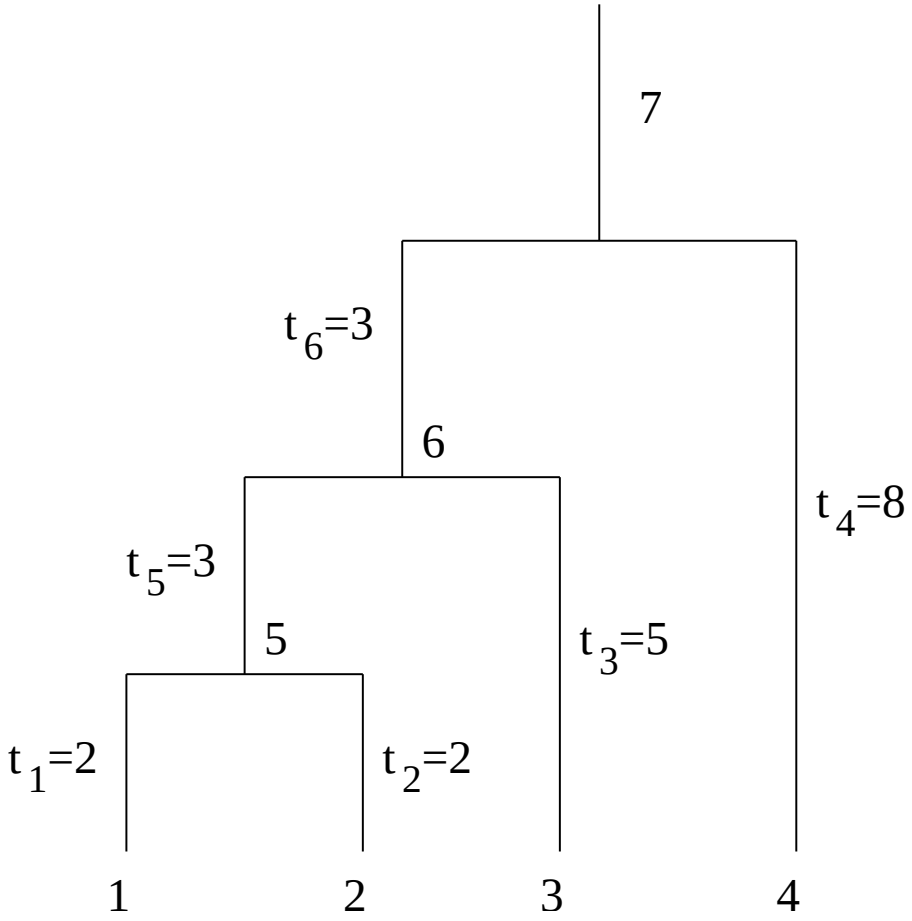


# 6.1 A weighting scheme using Kirchhof's laws [Thompson et al., 1994]




- **Let $I_n$ and $V_n$ be the current and respectively the voltage at node $n$. We can set the resistance equal to the edge length/time.**
- **Equations:**
  $V_5 = 2I_1 = 2I_2$
  $V_6 = 2I_1 + 3(I_1 + I_2) = 5I_3$
  $V_7 = 8I_4 = 5I_3 + 3(I_1 + I_2 + I_3)$
- **Result:**
  $I_1 : I_2 : I_3 : I_4 = 20 : 20 : 32 : 47$

# 6.2 Another simple algorithm [Gerstein et al., 1994]




- **Initially the weights are set to the edge lengths of the leafs:** $w_1 = w_2 = 2, w_3 = 5, w_4 = 8$.
- **Then**

$$\Delta\omega_i = t_n \frac{\omega_i}{\sum_{\text{leaves } k \text{ below } n} \omega_k}$$

**So, at node 5:**
$w_1 = w_2 = 2 + 3/2 = 3.5$
**At node 6:**
$w_1 = w_2 = 3.5 + 3 \times 3.5/12$,
$w_3 = 5 + 3 \times 5/12$

- **Result:**
$w_1 : w_2 : w_3 : w_4 = 35 : 35 : 50 : 64$

**For details on other, more involved weighting schemes,**

- **Root weights from Gaussian parameters**
- **Voronoi weights**
- **Maximum discrimination weights**
- **Maximum entropy weights**

**you may see Durbin et al., Section 5.8, pages 126–132**

# Examples

**(including the use of weights in the computation of parameters for the HMM profile):**

**pr. 5.6, 5.10 in [Borodovsky, Ekisheva, 2006]**