# HMM for sequence alignment: profile HMM

## Pair HMM

HMM for pairwise sequence alignment, which incorporates affine gap scores.

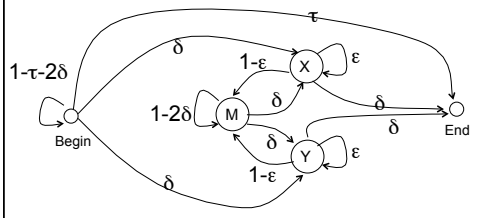
"Hidden" States

- Match (M)
- Insertion in *x* (X)
- insertion in *y* (Y)

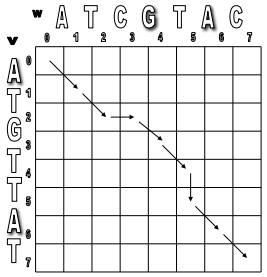
Observation Symbols

- Match (M): {(*a*,*b*)| *a*,*b* in ∑ }.
- Insertion in *x* (X): {(*a*,-)| *a* in ∑ }.
- Insertion in *y* (Y): {(-,*a*)| *a* in ∑ }.

## Pair HMMs



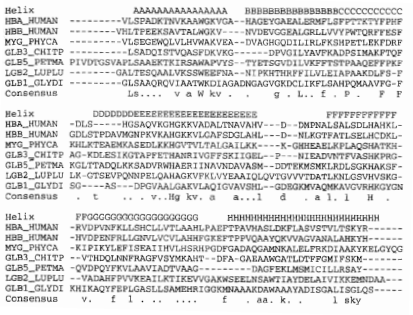

## Alignment: a path → a hidden state sequence




A T – G T T A T
A T C G T – A C

M M Y M M X M M

## Multiple sequence alignment (Globin family)




## Profile model (PSSM)

- A natural probabilistic model for a conserved region would be to specify independent probabilities $e_i(a)$ of observing nucleotide (amino acid) *a* in position *i*
- The probability of a new sequence x according to this model is

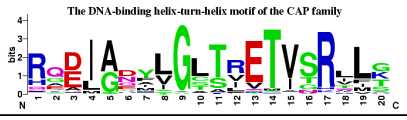
$$P(x \mid M) = \prod_{i=1}^{L} e_i(x_i)$$

## Profile / PSSM

•DNA / proteins Segments of the same length L;

•Often represented as Positional frequency matrix;

```
LTMTRGDIGNYLGLTVETISRLLGRFQKSGML
LTMTRGDIGNYLGLTIETISRLLGRFQKSGMI
LTMTRGDIGNYLGLTVETISRLLGRLQKMGIL
LAMSRNEIGNYLGLAVETVSRVFSRFQQNELI
LAMSRNEIGNYLGLAVETVSRVFTRFQQNGLI
LPMSRNEIGNYLGLAVETVSRVFTRFQQNGLL
VRMSREEIGNYLGLTLETVSRLFSRFGREGLI
LRMSREEIGSYLGLKLETVSRTLSKFHQEGLI
LPMCRRDIGDYLGLTLETVSRALSQLHTQGIL
LPMSRRDIADYLGLTVETVSRAVSQLHTDGVL
LPMSRQDIADYLGLTIETVSRTFTKLERHGAI
```

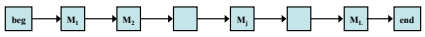



## Searching profiles: inference

- Give a sequence S of length L, compute the likelihood ratio of being generated from this profile vs. from background model:
  - R(S|P)= $\prod_{i=1}^{L} \frac{e_i(x_i)}{q_{x_i}}$
  - Searching motifs in a sequence: sliding window approach

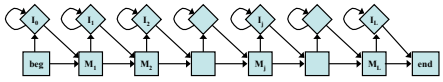
## Match states for profile HMMs

- Match states
  - Emission probabilities $e_{M_i}(a)$



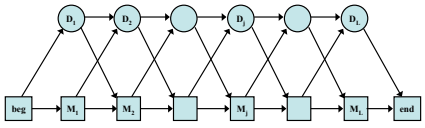

## Components of profile HMMs

- Insert states $e_{I_i}(a)$
  - Emission prob.
    - Can be the background distribution $q_a$.
  - Transition prob.
    - $M_i$ to $I_i$, $I_i$ to itself, $I_i$ to $M_{i+1}$
  - Log-score for a gap of length $k$ (not including the log-score from emission) $\log a_{M_i I_j} + \log a_{I_j M_{j+1}} + (k-1)\log a_{I_j I_j}$

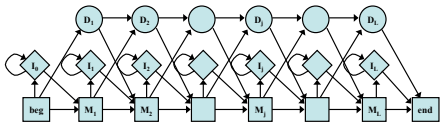



## Components of profile HMMs

- Delete states
  - No emission prob.
  - Cost of a deletion
    - $M_i$ to $D_{i+1}$, $D_i$ to $D_{i+1}$, $D_i$ to $M_{i+1}$
    - Each $D_i$ to $D_{i+1}$ might be different for different i




## Full structure of profile HMMs




This is the structure implemented in Hmmer, slightly different from the structure described in the textbook: there is no transition allowed from $D_j$ to $I_j$ or from $I_j$ to $D_{j+1}$. As a result, the recursive equation for Viterbi algorithm is different from the one described in the book too.

# Deriving HMMs from multiple alignments

- Key idea behind profile HMMs
  - Model representing the consensus for the alignment of sequence from the same family
  - Not the sequence of any particular member

```
HBA_HUMAN    ...VGA--HAGEY...
HBB_HUMAN    ...V----NVDEV...
MYG_PHYCA    ...VEA--DVAGH...
GLB3_CHITP   ...VKG------D...
GLB5_PETMA   ...VYS--TYETS...
LGB2_LUPLU   ...FNA--NIPKH...
GLB1_GLYDI   ...IAGADNGAGV...
                ***  *****
```

# Deriving HMMs from multiple alignments

- Basic profile HMM parameterization
  - Aim: making the higher probability for sequences from the family
- Parameters
  - the transition and emission probabilities: trivial if many of independent alignment sequences are given.

$$a_{kl} = \frac{A_{kl}}{\sum_{l'} A_{kl'}} \qquad e_k(a) = \frac{E_k(a)}{\sum_{a'} E_k(a')}$$

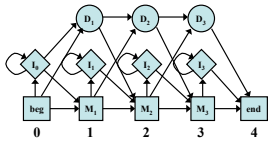
  - length of the model: heuristics or systematic way (e.g., using the MAP algorithm)

# Deriving HMMs from multiple alignments

**(a) Multiple alignment:**

```
            x x . . . x
bat         A G - - - C
rat         A G A G - C
cat         A G - A A G
gnat        - G A A A C
goat        A G - - - C
Matching    1 2 . . . 3
```

**(b) Profile-HMM architecture:**

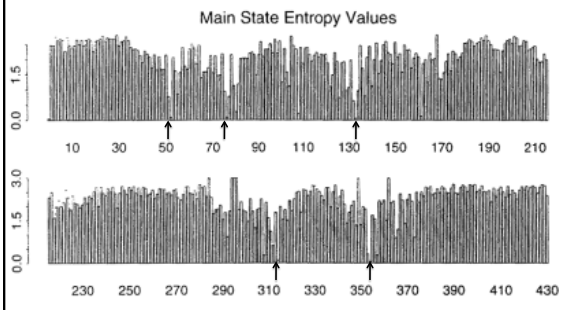



**(c) Observed emission/transition counts**

| | | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Emission from M | A | - | 4 | 0 | 0 |
| | C | - | 0 | 0 | 4 |
| | G | - | 0 | 5 | 1 |
| | T | - | 0 | 0 | 0 |
| Emission from I | A | 0 | 0 | 6 | 0 |
| | C | 0 | 0 | 0 | 0 |
| | G | 0 | 0 | 1 | 0 |
| | T | 0 | 0 | 0 | 0 |
| Transition probabilities | M-M | 4 | 4 | 2 | 4 |
| | M-D | 1 | 0 | 0 | 0 |
| | M-I | 0 | 0 | 3 | 0 |
| | I-M | 0 | 0 | 2 | 0 |
| | I-I | 0 | 0 | 4 | 0 |
| | D-M | - | 1 | 0 | 0 |
| | D-D | - | 0 | 0 | 0 |

A simple rule that works well is that columns that are more than half gap characters should be modeled by inserts.

# Sequence conservation: entropy of the emission probability distributions




High entropy indicates non-conserved positions (note: here negative entropy a plotted.

# Matching a sequence to a profile HMM (global alignment)

- Viterbi algorithm: pHMM θ (with L matching states) and a query sequence $x_1 x_2 .. x_N$

$$V_j^M(i) = e_{M_j}(x_i) \cdot \max\begin{cases} V_{j-1}^M(i-1) \cdot a_{M_{j-1}M_j}, \\ V_{j-1}^I(i-1) \cdot a_{I_{j-1}M_j}, \\ V_{j-1}^D(i-1) \cdot a_{D_{j-1}M_j}; \end{cases}$$

$$V_j^I(i) = e_{I_j}(x_i) \cdot \max\begin{cases} V_j^M(i-1) \cdot a_{M_jI_j}, \\ V_j^I(i-1) \cdot a_{I_jI_j}; \end{cases}$$

$$V_j^D(i) = \max\begin{cases} V_{j-1}^M(i) \cdot a_{M_{j-1}D_j}, \\ V_{j-1}^D(i) \cdot a_{D_{j-1}D_j}; \end{cases}$$

for i=1,…,L, j=1,…,N

Initialization:

$V_0^M(0) = 1; \quad V_{j>0}^M(0) = 0; \quad V_0^M(i>0) = 0;$

$V_j^I(0) = 0;$

$V_0^D(i) = 0;.$

Termination:

$$V = \max\left[V_L^M(N), V_L^I(N), V_L^D(N)\right]$$

Note: this is slightly different from the textbook; no transition from $D_i$ to $I_j$ or from $I_j$ to $D_{j+1}$.

# Viterbi algorithm: trace back

$$ptr_j^M(i) = \begin{cases} \nwarrow & if \quad V_j^M(i) = e_{M_j}(x_i) \cdot V_{j-1}^M(i-1) \cdot a_{M_{j-1}M_j} \\ \uparrow & if \quad V_j^M(i) = e_{M_j}(x_i) \cdot V_{j-1}^I(i-1) \cdot a_{I_{j-1}M_j} \\ \leftarrow & if \quad V_j^M(i) = e_{M_j}(x_i) \cdot V_{j-1}^D(i-1) \cdot a_{D_{j-1}M_j} \end{cases}$$

$$ptr_j^I(i) = \begin{cases} \nwarrow & if \quad V_j^I(i) = e_{I_j}(x_i) \cdot V_j^M(i-1) \cdot a_{M_jI_j}, \\ \uparrow & if \quad V_j^I(i) = e_{I_j}(x_i) \cdot V_j^I(i-1) \cdot a_{I_jI_j}; \end{cases}$$

$$ptr_j^D(i) = \begin{cases} \nwarrow & if \quad V_j^D(i) = e_{D_j}(x_i) \cdot V_{j-1}^M(i) \cdot a_{M_{j-1}D_j}, \\ \rightarrow & if \quad V_j^D(i) = e_{D_j}(x_i) \cdot V_{j-1}^D(i) \cdot a_{D_{j-1}D_j}; \end{cases}$$

$$ptr_{ter} = \begin{cases} M & if \quad V = V_L^M(N) \\ I & if \quad V = V_L^I(N) \\ D & if \quad V = V_L^D(N) \end{cases}$$

PrintStateSeq(ptr$^M$, ptr$^I$, ptr$^D$, ptr$_{ter}$, N, L)

```
PrintStateSeq(ptrM, ptrI, ptrD, ptrter, i, j)
  if i=0 or j=0
      return;
  if ptrter = "M"
    if ptrM(L) = "↖"
        printStateSeq(ptrM, ptrI, ptrD, "M", i-1, j-1)
    else if ptrM(L) = "↑"
        printStateSeq(ptrM, ptrI, ptrD, "I", i-1, j-1)
    else if ptrM(L) = "←"
        printStateSeq(ptrM, ptrI, ptrD, "D", i-1, j-1)
    print Mj
  else if ptrter = "I"
    if ptrM(L) = "↖"
        printStateSeq(ptrM, ptrI, ptrD, "M", i-1, j)
    else if ptrM(L) = "↑"
        printStateSeq(ptrM, ptrI, ptrD, "I", i-1, j)
    print Ij
  else
    if ptrM(L) = "↖"
        printStateSeq(ptrM, ptrI, ptrD, "M", i, j-1)
    else if ptrM(L) = "←"
        printStateSeq(ptrM, ptrI, ptrD, "D", i, j-1)
    print Dj
```

# Matching a sequence to a profile HMM (global alignment)

- Forward: pHMM θ (with L matching states) and a query sequence $x_1x_2..x_N$

$$F_j^{\mathrm{M}}(i) = e_{\mathrm{M}_j}(x_i)\cdot\left[F_{j-1}^{\mathrm{M}}(i-1)\cdot a_{\mathrm{M}_{j-1}\mathrm{M}_j} + F_{j-1}^{\mathrm{I}}(i-1)\cdot a_{\mathrm{I}_{j-1}\mathrm{M}_j} + F_{j-1}^{\mathrm{D}}(i-1)\cdot a_{\mathrm{D}_{j-1}\mathrm{M}_j}\right]$$

$$F_j^{\mathrm{I}}(i) = e_{\mathrm{I}_j}(x_i)\cdot\left[F_j^{\mathrm{M}}(i-1)\cdot a_{\mathrm{M}_j\mathrm{I}_j} + F_j^{\mathrm{I}}(i-1)\cdot a_{\mathrm{I}_j\mathrm{I}_j}\right]$$

$$F_j^{\mathrm{D}}(i) = F_{j-1}^{\mathrm{M}}(i)\cdot a_{\mathrm{M}_{j-1}\mathrm{D}_j} + F_{j-1}^{\mathrm{D}}(i)\cdot a_{\mathrm{D}_{j-1}\mathrm{D}_j}$$

for i=1,…,L, j=1,…,N

Initialization:

$F_0^M(0) = 1;\quad F_{j>0}^M(0) = 0;\quad F_0^M(i>0) = 0;$

$F_j^I(0) = 0;$

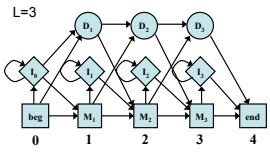
$F_0^D(i) = 0;$

Termination:

$$F = F_L^M(N) + F_L^I(N) + F_L^D(N)$$

Note: this is slightly different from the textbook; no transition from $D_j$ to $I_j$ or from $I_j$ to $D_{j+1}$.

# Example




| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Emission from M: A | – | 1 | .5 | 0 |
| C | – | 0 | 0 | .8 |
| G | – | 0 | .5 | .2 |
| T | – | 0 | 0 | 0 |
| Emission from I: A | .2 | .2 | .9 | .2 |
| C | .3 | .3 | 0 | .3 |
| G | .3 | .3 | .1 | .3 |
| T | .2 | .2 | 0 | .2 |
| Transition probabilities: M-M | .8 | 1 | .4 | 1 |
| M-D | .2 | 0 | 0 | 0 |
| M-I | 0 | 0 | .6 | 0 |
| I-M | .5 | .5 | .3 | .5 |
| I-I | .5 | .5 | .7 | .5 |
| D-M | – | 1 | .5 | 1 |
| D-D | – | 0 | .5 | 0 |

Query: AGG (N=3)

# Example: Viterbi algorithm

| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Emission from M: A | – | 1 | .5 | 0 |
| C | – | 0 | 0 | .8 |
| G | – | 0 | .5 | .2 |
| T | – | 0 | 0 | 0 |
| Emission from I: A | .2 | .2 | .9 | .2 |
| C | .3 | .3 | 0 | .3 |
| G | .3 | .3 | .1 | .3 |
| T | .2 | .2 | 0 | .2 |
| Transition probabilities: M-M | .8 | 1 | .4 | 1 |
| M-D | .2 | 0 | 0 | 0 |
| M-I | 0 | 0 | .6 | 0 |
| I-M | .5 | .5 | .3 | .5 |
| I-I | .5 | .5 | .7 | .5 |
| D-M | – | 1 | .5 | 1 |
| D-D | – | 0 | .5 | 0 |

Initialization

| j | i | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 0 | $V^M$ | 1 | 0 | 0 | 0 |
| | $V^I$ | 0 | | | |
| | $V^D$ | 0 | 0 | 0 | 0 |
| 1 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | | | | |
| 2 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | | | | |
| 3 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | | | | |

Query: X=AGG (N=3)

# Example: Viterbi algorithm

| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Emission from M: A | – | 1 | .5 | 0 |
| C | – | 0 | 0 | .8 |
| G | – | 0 | .5 | .2 |
| T | – | 0 | 0 | 0 |
| Emission from I: A | .2 | .2 | .9 | .2 |
| C | .3 | .3 | 0 | .3 |
| G | .3 | .3 | .1 | .3 |
| T | .2 | .2 | 0 | .2 |
| Transition probabilities: M-M | .8 | 1 | .4 | 1 |
| M-D | .2 | 0 | 0 | 0 |
| M-I | 0 | 0 | .6 | 0 |
| I-M | .5 | .5 | .3 | .5 |
| I-I | .5 | .5 | .7 | .5 |
| D-M | – | 1 | .5 | 1 |
| D-D | – | 0 | .5 | 0 |

| j | i | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 0 | $V^M$ | 1 | 0 | 0 | 0 |
| | $V^I$ | 0 | | | |
| | $V^D$ | 0 | 0 | 0 | 0 |
| 1 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | .2 | | | |
| 2 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | | | | |
| 3 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | | | | |

Query: X=AGG (N=3)

$$V_1^D(0) = \max\left(V_0^M(0)a_{M_0D_1}, V_0^D(0)a_{D_0D_1}\right)$$
$$= \max(0.2, 0) = 0.2$$

# Example: Viterbi algorithm

| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Emission from M: A | – | 1 | .5 | 0 |
| C | – | 0 | 0 | .8 |
| G | – | 0 | .5 | .2 |
| T | – | 0 | 0 | 0 |
| Emission from I: A | .2 | .2 | .9 | .2 |
| C | .3 | .3 | 0 | .3 |
| G | .3 | .3 | .1 | .3 |
| T | .2 | .2 | 0 | .2 |
| Transition probabilities: M-M | .8 | 1 | .4 | 1 |
| M-D | .2 | 0 | 0 | 0 |
| M-I | 0 | 0 | .6 | 0 |
| I-M | .5 | .5 | .3 | .5 |
| I-I | .5 | .5 | .7 | .5 |
| D-M | – | 1 | .5 | 1 |
| D-D | – | 0 | .5 | 0 |

| j | i | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 0 | $V^M$ | 1 | 0 | 0 | 0 |
| | $V^I$ | 0 | 0 | | |
| | $V^D$ | 0 | 0 | 0 | 0 |
| 1 | $V^M$ | 0 | .8 | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | .2 | | | |
| 2 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | 0 | | | |
| 3 | $V^M$ | 0 | | | |
| | $V^I$ | 0 | | | |
| | $V^D$ | 0 | | | |

Query: X=AGG (N=3)

$$V_1^M(1) = e_{M_1}(x_1)\cdot\max\left(V_0^M(0)a_{M_0M_1}, V_0^I(0)a_{I_0M_1}, V_0^D(0)a_{D_0M_1}\right)$$
$$= 1\times\max(1\times 0.8, 0\times 0.5, 0\times 1) = 0.8$$

# Example: Viterbi algorithm

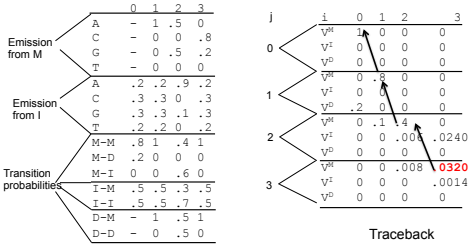
| | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| Emission from M: A | – | 1 | .5 | 0 |
| C | – | 0 | 0 | .8 |
| G | – | 0 | .5 | .2 |
| T | – | 0 | 0 | 0 |
| Emission from I: A | .2 | .2 | .9 | .2 |
| C | .3 | .3 | 0 | .3 |
| G | .3 | .3 | .1 | .3 |
| T | .2 | .2 | 0 | .2 |
| Transition probabilities: M-M | .8 | 1 | .4 | 1 |
| M-D | .2 | 0 | 0 | 0 |
| M-I | 0 | 0 | .6 | 0 |
| I-M | .5 | .5 | .3 | .5 |
| I-I | .5 | .5 | .7 | .5 |
| D-M | – | 1 | .5 | 1 |
| D-D | – | 0 | .5 | 0 |

| j | i | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 0 | $V^M$ | 1 | 0 | 0 | 0 |
| | $V^I$ | 0 | 0 | 0 | 0 |
| | $V^D$ | 0 | 0 | 0 | 0 |
| 1 | $V^M$ | 0 | .8 | 0 | 0 |
| | $V^I$ | 0 | 0 | 0 | 0 |
| | $V^D$ | .2 | 0 | 0 | 0 |
| 2 | $V^M$ | 0 | .1 | .4 | 0 |
| | $V^I$ | 0 | 0 | .006 | .0240 |
| | $V^D$ | 0 | 0 | 0 | 0 |
| 3 | $V^M$ | 0 | 0 | .008 | .0320 |
| | $V^I$ | 0 | 0 | 0 | .0014 |
| | $V^D$ | 0 | 0 | 0 | 0 |

Query: X=AGG (N=3)

$$V_3^M(3) = ?$$

# Example: Viterbi algorithm




Query: X=AGG (N=3)

# Searching with profile HMMs

- Main usage of profile HMMs
  - Detecting potential sequences in a family
  - Core algorithm: matching a sequence to a profile HMMs
    - Viterbi algorithm or forward algorithm
  - Comparing the resulting probability with random model (R): log-odd score

$$P(x \mid R) = \prod_i q_{x_i}$$

where $q_x$ is the frequency of observing $x_i$.

# Matching a sequence to a profile HMM (global alignment)

**Viterbi algorithm**

$$V_j^{\mathrm{M}}(i) = \log\frac{e_{\mathrm{M}_j}(x_i)}{q_{x_i}} + \max\begin{cases}\log V_{j-1}^{\mathrm{M}}(i-1) + \log a_{\mathrm{M}_{j-1}\mathrm{M}_j},\\ \log V_{j-1}^{\mathrm{I}}(i-1) + \log a_{\mathrm{I}_{j-1}\mathrm{M}_j},\\ \log V_{j-1}^{\mathrm{D}}(i-1) + \log a_{\mathrm{D}_{j-1}\mathrm{M}_j};\end{cases}$$

$$V_j^{\mathrm{I}}(i) = \log\frac{e_{\mathrm{I}_j}(x_i)}{q_{x_i}} + \max\begin{cases}\log V_j^{\mathrm{M}}(i-1) + \log a_{\mathrm{M}_j\mathrm{I}_j},\\ \log V_j^{\mathrm{I}}(i-1) + \log a_{\mathrm{I}_j\mathrm{I}_j};\end{cases}$$

$$V_j^{\mathrm{D}}(i) = \max\begin{cases}\log V_{j-1}^{\mathrm{M}}(i) + \log a_{\mathrm{M}_{j-1}\mathrm{D}_j},\\ \log V_{j-1}^{\mathrm{D}}(i) + \log a_{\mathrm{D}_{j-1}\mathrm{D}_j};\end{cases}$$

for i=1,…,L, j=1,…,N

Initialization:

$V_0^{\mathrm{M}}(0) = 0;\quad V_{j>0}^{\mathrm{M}}(0) = -\infty;\quad V_0^{\mathrm{M}}(i>0) = -\infty;$
$V_j^{\mathrm{I}}(0) = -\infty;$
$V_0^{\mathrm{D}}(i) = -\infty;.$

Termination:

$$V = \max\left[V_L^{\mathrm{M}}(N), V_L^{\mathrm{I}}(N), V_L^{\mathrm{D}}(N)\right]$$

# Matching a sequence to a profile HMM (global alignment)

**Forward algorithm**

$$F_j^{\mathrm{M}}(i) = \log\frac{e_{\mathrm{M}_j}(x_i)}{q_{x_i}} + \log[a_{\mathrm{M}_{j-1}\mathrm{M}_j}\exp(F_{j-1}^{\mathrm{M}}(i-1)) + a_{\mathrm{I}_{j-1}\mathrm{M}_j}\exp(F_{j-1}^{\mathrm{I}}(i-1)) + a_{\mathrm{D}_{j-1}\mathrm{M}_j}\exp(F_{j-1}^{\mathrm{D}}(i-1))];$$

$$F_j^{\mathrm{I}}(i) = \log\frac{e_{\mathrm{I}_j}(x_i)}{q_{x_i}} + \log[a_{\mathrm{M}_j\mathrm{I}_j}\exp(F_j^{\mathrm{M}}(i-1)) + a_{\mathrm{I}_j\mathrm{I}_j}\exp(F_j^{\mathrm{I}}(i-1))];$$

$$F_j^{\mathrm{D}}(i) = \log[a_{\mathrm{M}_{j-1}\mathrm{D}_j}\exp(F_{j-1}^{\mathrm{M}}(i)) + a_{\mathrm{I}_{j-1}\mathrm{D}_j}\exp(F_{j-1}^{\mathrm{I}}(i))];$$

Initialization:

$V_0^{\mathrm{M}}(0) = 0;\quad V_{j>0}^{\mathrm{M}}(0) = -\infty;\quad V_0^{\mathrm{M}}(i>0) = -\infty;$
$V_0^{\mathrm{I}}(0) = -\infty;$
$V_0^{\mathrm{D}}(i) = -\infty.$

Termination:

$$F = \log\left[\exp\left(F_L^{\mathrm{M}}(N)\right) + \exp\left(F_L^{\mathrm{I}}(N)\right) + \exp\left(F_L^{\mathrm{D}}(N)\right)\right]$$

# Significance of HMM alignment

- The log-odd score of local Viterbi alignment (V) alignment between a random sequence and a profile HMM follows a Gumbel (type I EVD) distribution

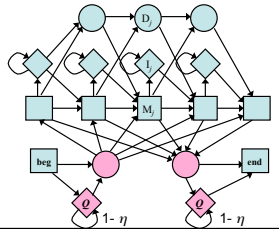
$$P(V \geq t) = 1 - \exp\left[-e^{-\lambda(t-\mu)}\right]$$

- With ~200 Viterbi, the location parameter μ can be accurately estimated;
- λ~log(z), z is the base of the log-odd score, e.g., z=2 when the sequence length approaches infinite
- The length effect can be corrected by $\lambda \sim \log 2 + \frac{1.44}{hN}$
  - Where, N is the length, and h is the average relative entropy per match state in the pHMM;
  - For typical Pfam models, N~140, h~1.8, λ~log2+0.0057, a small correction.

Eddy, PLoS Comp. Biol., 4:1, 2008

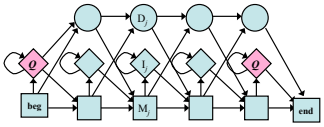
# Variants for non-global alignments

- Local alignment (Smith-Waterman type)
  - Emission prob. in flanking states use background values $q_x$.
  - Looping prob. close to 1, e.g. (1- $\eta$) for some small $\eta$.



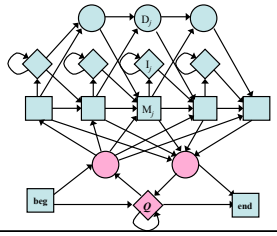
## Variants for non-global alignments

- Overlap (also called *glocal* or *fit*) alignment
  - The loop probability of the *first* and *last* insert states is much higher than the other insert states
  - When expecting to find either present as a whole or absent (e.g., of a protein domain within a protein)
  - Transition to first delete state allows missing first residue




## Variants for non-global alignments

- Repeat alignments
  - Transition from right flanking state back to random model
  - Can find multiple matching segments in query string

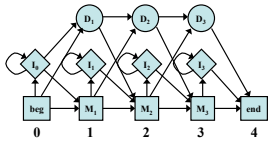



## Optimal model construction: different ways of marking columns

**(a) Multiple alignment:**

```
            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
Matching    1 2 . . . 3
```

**(b) Profile-HMM architecture:**




**(c) Observed emission/transition counts**

| | | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Emission from M | A | - | 4 | 0 | 0 |
| | C | - | 0 | 0 | 4 |
| | G | - | 0 | 3 | 0 |
| | T | - | 0 | 0 | 0 |
| Emission from I | A | 0 | 0 | 6 | 0 |
| | C | 0 | 0 | 0 | 0 |
| | G | 0 | 0 | 1 | 0 |
| | T | 0 | 0 | 0 | 0 |
| Transition probabilities | 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 |

## Optimal model construction

- MAP (match-insert assignment)
  - Recursive calculation of a number $S_j$
    - $S_j$: log prob. of the optimal model for alignment up to and including column $j$, assuming $j$ is marked.
    - $S_j$ is calculated from $S_i$ and summed log prob. between $i$ and $j$.
    - $T_{ij}$: summed log prob. of all the state transitions between marked $i$ and $j$.

$$T_{ij} = \sum_{x,y \in \{\mathrm{M,D,I}\}} c_{xy} \log a_{xy}$$

  - $c_{xy}$ are obtained from partial state paths implied by marking $i$ and $j$.

## Optimal model construction

- Algorithm: MAP model construction
  - Initialization:
    - $S_0 = 0, M_{L+1} = 0.$
  - Recurrence: for $j = 1,\ldots, L+1$:

$$S_j = \max_{0 \le i < j} \left( S_i + T_{ij} + M_j + I_{i+1,j-1} + \lambda \right)$$

$$\sigma_j = \arg\max_{0 \le i < j} \left( S_i + T_{ij} + M_j + I_{i+1,j-1} + \lambda \right)$$

  - Traceback: from $j = \sigma_{L+1}$, while $\sigma_j > 0$:
    - Mark column j as a match column
    - j ← $\sigma_j$.

## Weighting training sequences

- Input sequences are random?
- "Assumption: all examples are independent samples" might be incorrect
- Solutions
  - Weight sequences based on similarity: highly similar pair of training sequences receive lower weights

## Multiple sequence alignment (MSA) by training profile HMM

- Sequence profiles can be represented as probabilistic models like profile HMMs.
  - ML methods for building (training) profile HMM are based on multiple sequence alignment
  - Profile HMMs can also be trained from initially unaligned sequences using the Baum-Welch-like EM algorithm
    - Simultaneously aligning multiple sequences and building the profile HMM from the multiple alignment

## Multiple alignment with a known profile HMM

- A step backward: to derive a multiple alignment from a known profile HMM model
  - e.g., to align many sequences from the same family based on the HMM model built from the (seed) multiple alignment of a small representative set of sequences in the family.
- It just requires calculating a Viterbi alignment for each individual sequence
  - Match a sequence to a profile HMM: Viterbi algorithm
  - Residues aligned to the same match state in the profile HMM should be aligned in the same columns;
  - Given a preliminary alignment, HMM can align additional sequences.

## Multiple alignment with a known profile HMM

- Comparing with other MSA program
  - Profile HMM does not align inserts whereas other MSA algorithms align the whole sequences.

| Position | 1 | 2 | 3 | 4 | 5 | 6 | insert | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | P | H | F | – | D | LS | H | G | S | A | Q |
| | F | E | S | F | G | D | LSTPDAV | M | G | N | P | K |
| | F | D | R | F | K | H | LKTEAEM | K | A | S | E | D |
| | F | T | Q | F | A | G | KDLESI | K | G | T | A | P |
| | F | P | K | F | K | G | LTTADQL | K | K | S | A | D |
| | F | S | – | F | L | K | GTSEVP | Q | N | N | P | E |
| | F | G | – | F | S | G | AS | – | – | D | P | G |

## Training profile HMM from unaligned sequences

- Simultaneously aligning multiple sequences and building the profile HMM from the multiple alignment
  - Initialization: choose the length of the profile HMM and initialize parameters of the model
  - MSA: align all sequences to the final model using the Viterbi algorithm and build a multiple alignment as described in the previous section.
  - Training: estimate the model using the Baum-Welch algorithm
  - Iterating until the model (and the MSA) converges

## Profile HMM training from unaligned sequences

- Initial Model
  - The only decision that must be made in choosing an initial structure for Baum-Welch estimation is the length of the model M.
  - A commonly used rule is to set M be the average length of the training sequence.
  - We need some randomness in initial parameters to avoid local maxima.

## Multiple alignment by profile HMM training

- Avoiding Local maxima
  - Baum-Welch algorithm is guaranteed to find a LOCAL maxima.
    - Models are usually quite long and there are many opportunities to get stuck in a wrong solution.
  - Solution
    - Start many times from different initial models.
    - Use some form of stochastic search algorithm, e.g. simulated annealing.

## Multiple alignment by profile HMM training--Model surgery

- We can modify the model after (or during) training a model by manually checking the alignment produced from the model.
  - Some of the match states are redundant
  - Some insert states absorb too many sequences
- Model surgery
  - If a match state is used by less than ½ of training sequences, delete its module (match-insert-delete states)
  - If more than ½ of training sequences use a certain insert state, expand it into *n* new modules, where *n* is the average length of insertions
  - ad hoc, but works well

## Hmmer 3

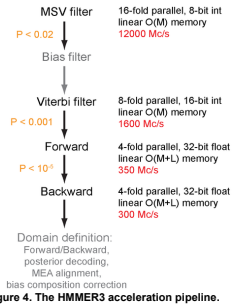



**Figure 4. The HMMER3 acceleration pipeline.**

Eddy SR (2011) Accelerated Profile HMM Searches. PLoS Comput Biol 7(10): e1002195. doi:10.1371/journal.pcbi.1002195
http://www.ploscompbiol.org/article/info:doi/10.1371/journal.pcbi.1002195



## Accelerated Profile HMM Searches

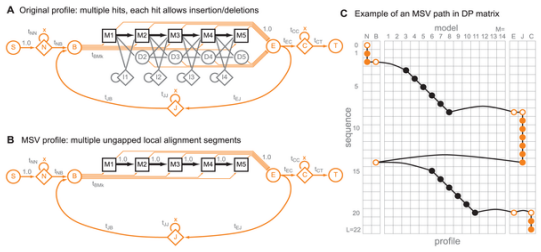



**Figure 1. The MSV profile.**

Eddy SR (2011) Accelerated Profile HMM Searches. PLoS Comput Biol 7(10): e1002195. doi:10.1371/journal.pcbi.1002195
http://www.ploscompbiol.org/article/info:doi/10.1371/journal.pcbi.1002195