Statistical Machine Learning Methods for Bioinformatics

II. Hidden Markov Model for Biological Sequences

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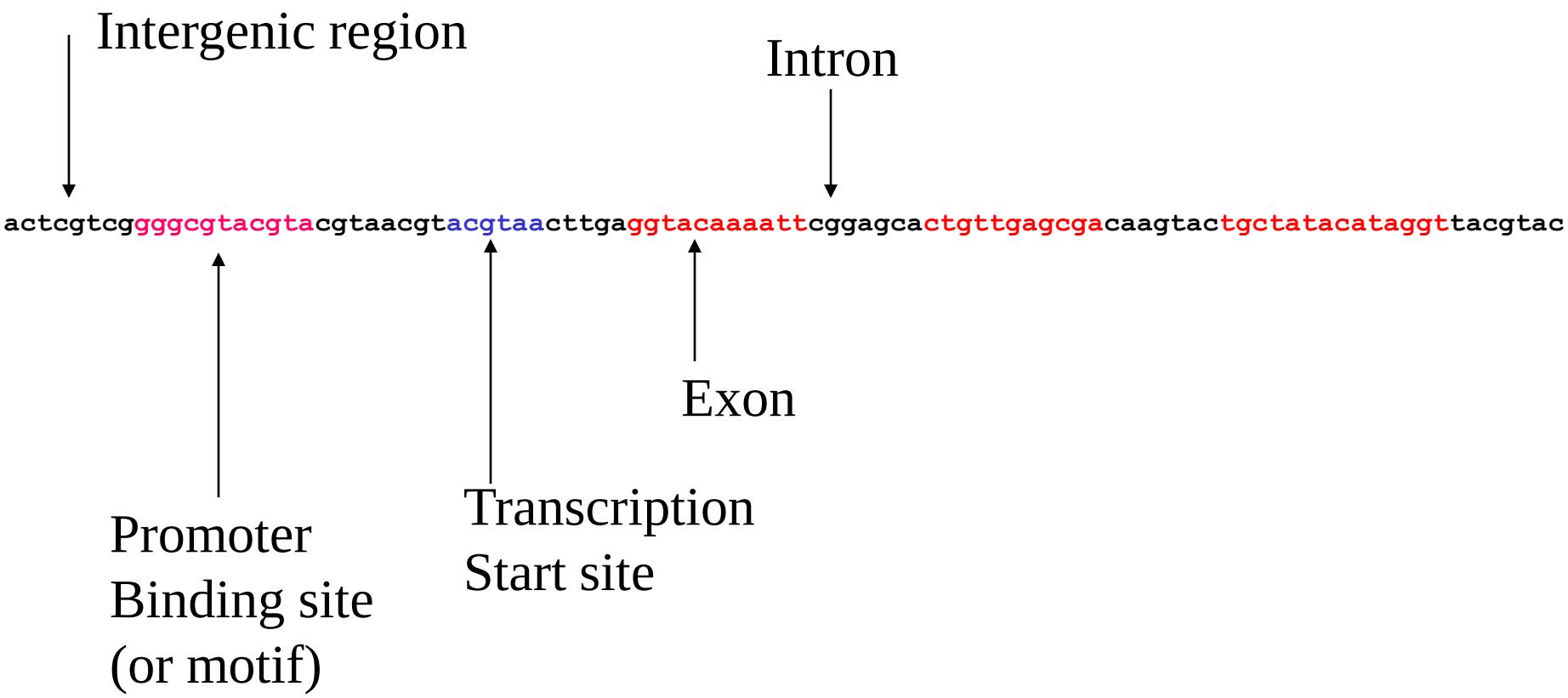
Application of HMM in Biological Sequence Analysis

- DNA binding sites (motif)
- Gene structure prediction
- **Protein sequence modeling (learning, profile)**
- **Protein sequence alignment (decoding)**
- **Protein database search (scoring, e.g. fold recognition)**
- Protein structure prediction

Motif and Gene Structure

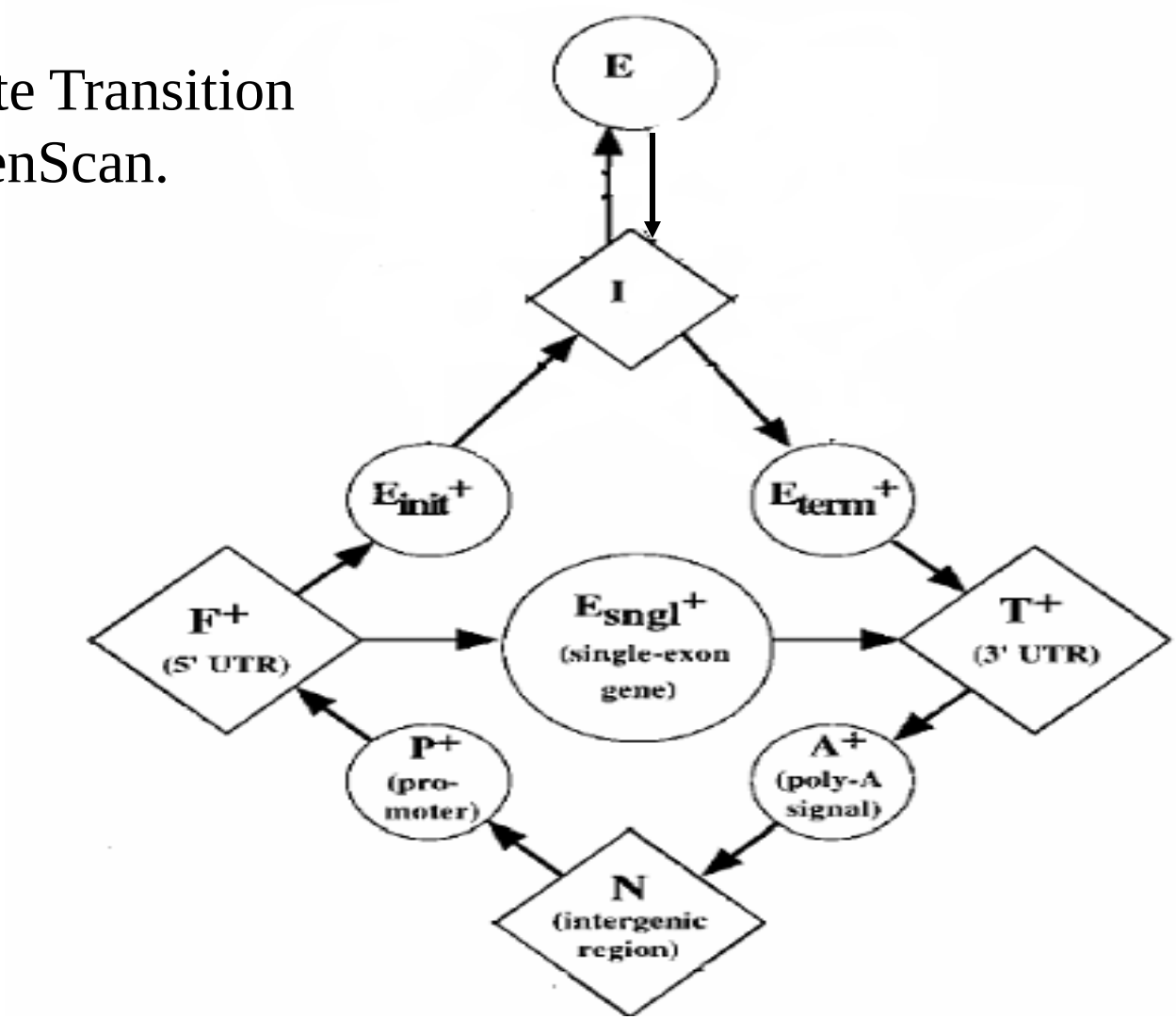


HMM has been used for modeling binding site and gene structure prediction.

GENSCAN

(genes.mit.edu/GENSCAN.html)

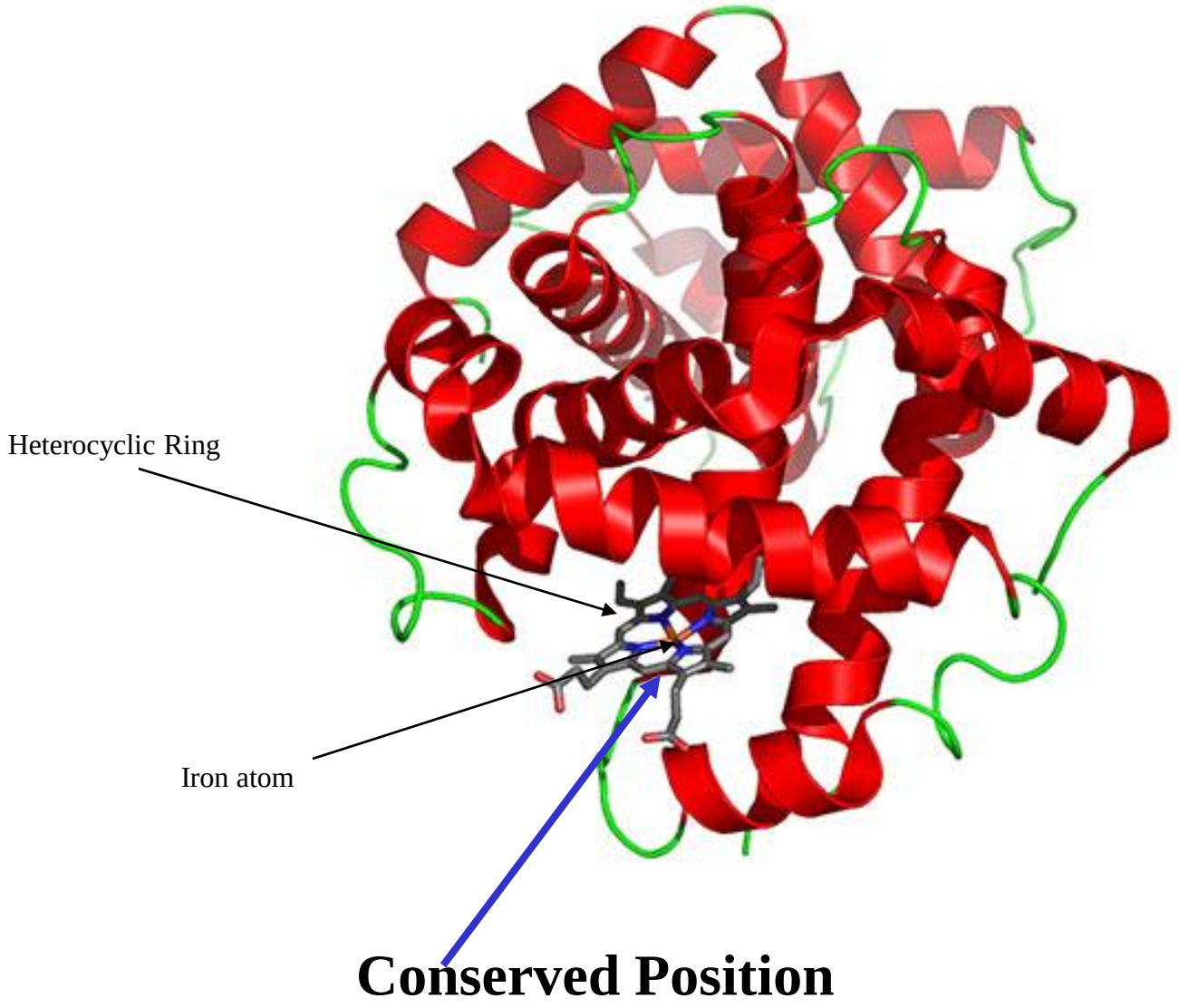
Simplified State Transition Diagram of GenScan.



Model Protein Family (Profile HMM)

- Create a statistical model (HMM) for a group of related protein sequences (e.g protein family)
- Identify core (conserved) elements of homologous sequences
- Positional evolutionary information (e.g. insertion and deletion)

Example: Hemoglobin Transports Oxygen



Why do We Build a Profile (Model)?

- Understand the conservation (core function and structure elements) and variation
- Sequence generation
- Multiple sequence alignments
- Profile-sequence alignment (more sensitive than sequence-sequence alignment)
- Fold recognition
- Profile-profile alignment

Protein Family

```
seq1 VRRNNMGMPLIESSSYHDALFTLGYAGDRISQMLGMRLLAQGRLSEMAGADALDV
seq2 NIYIDSNGIAHIYANNLHDLFLAEGYYEASQRLFEIELFGLAMGNLSSWVGAKALSS
seq3 SAETYRDAWGIPHLRADTPHELARAQGTARDRAWQLEVERHRAQGTSASFLGPEALSW
seq4 DRLGVVTIDAANQLDAMRALGYAQERYFEMDLMRRAPAGELSELFGAKAVDL
```

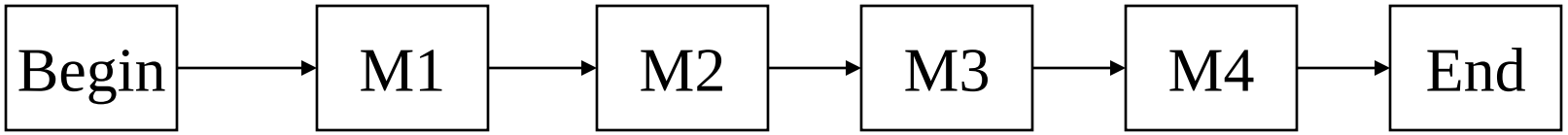
```
seq1 ---VRRNNMGMPLIESSSYHDALFTLGY--AGDRISQMLGMRLLAQGRLSEMAGADALDV
seq2 --NIYIDSNGIAHIYANNLHDLFLAEGYYEASQRLFEIELFG-LAMGNLSSWVGAKALSS
seq3 SAETYRDAWGIPHLRADTPHELARAQGT--ARDRAWQLEVERHRAQGTSASFLGPEALSW
seq4 -----DRLGVVTIDAANQLDAMRALGY--AQERYFEMDLMRRAPAGELSELFGAKAVDL
```

Imagine these sequences evolve from a single ancestral sequence and undergo evolutionary mutations. How to use a HMM to model?

Key to Build a HMM is to Set Up States

- Think about the positions of the ancestral sequence is undergoing mutation events to generate new sequences in difference species. A position can be modeled by a **dice**.
- **Match** (match or mutate): the position is kept with or without variations / mutations.
- **Delete**: the position is deleted
- **Insert**: amino acids are inserted between two positions.

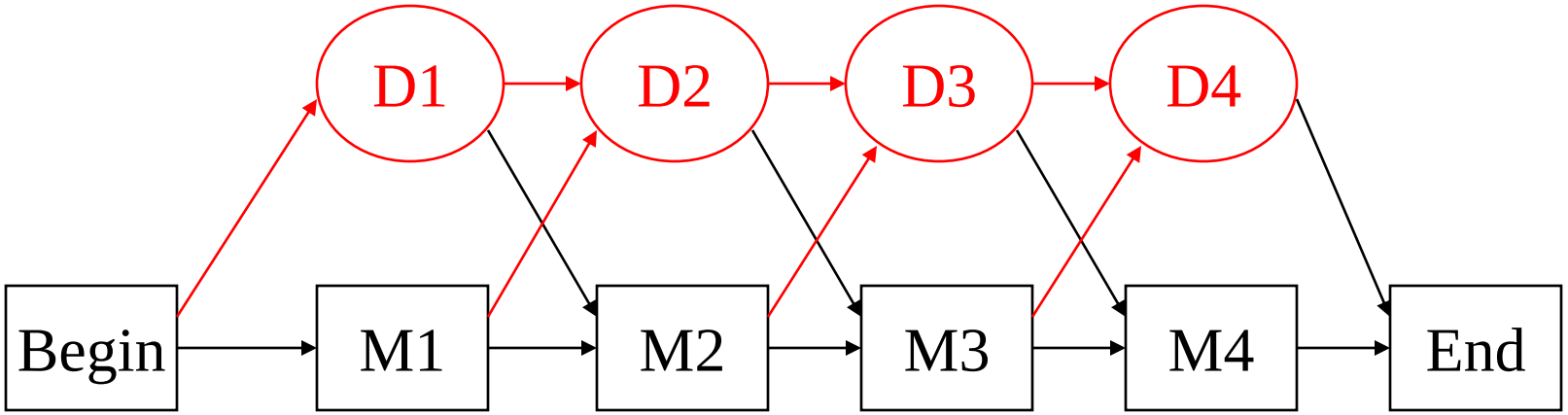
Hidden Markov Model



Each match state has an emission distribution of 20 amino acids; one match state for a position.

Krogh et al. 1994, Baldi et al. 1994

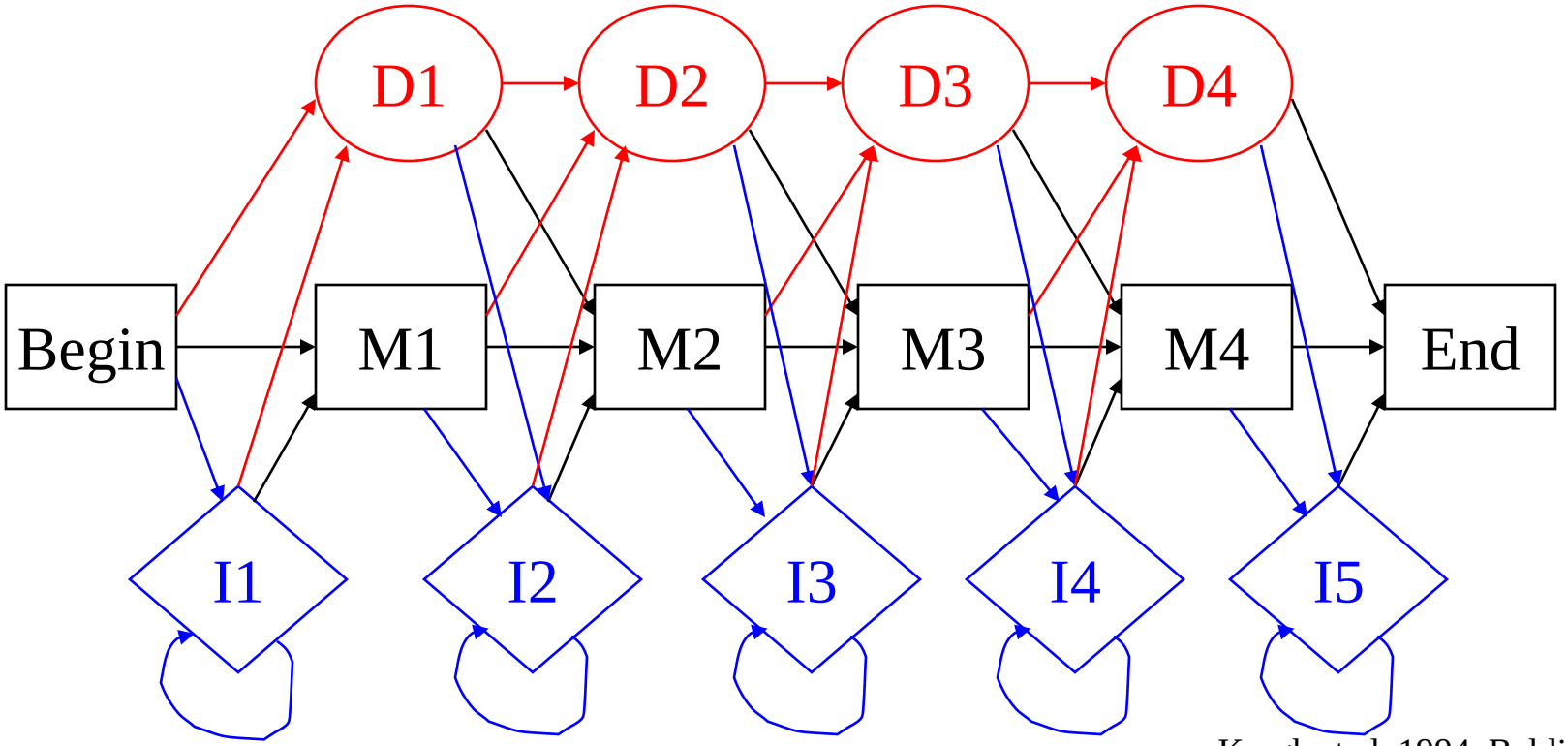
Hidden Markov Model



Each match state has an emission distribution of 20 amino acids.
Deletion state is a mute state (emitting a dummy)

Krogh et al. 1994, Baldi et al. 1994

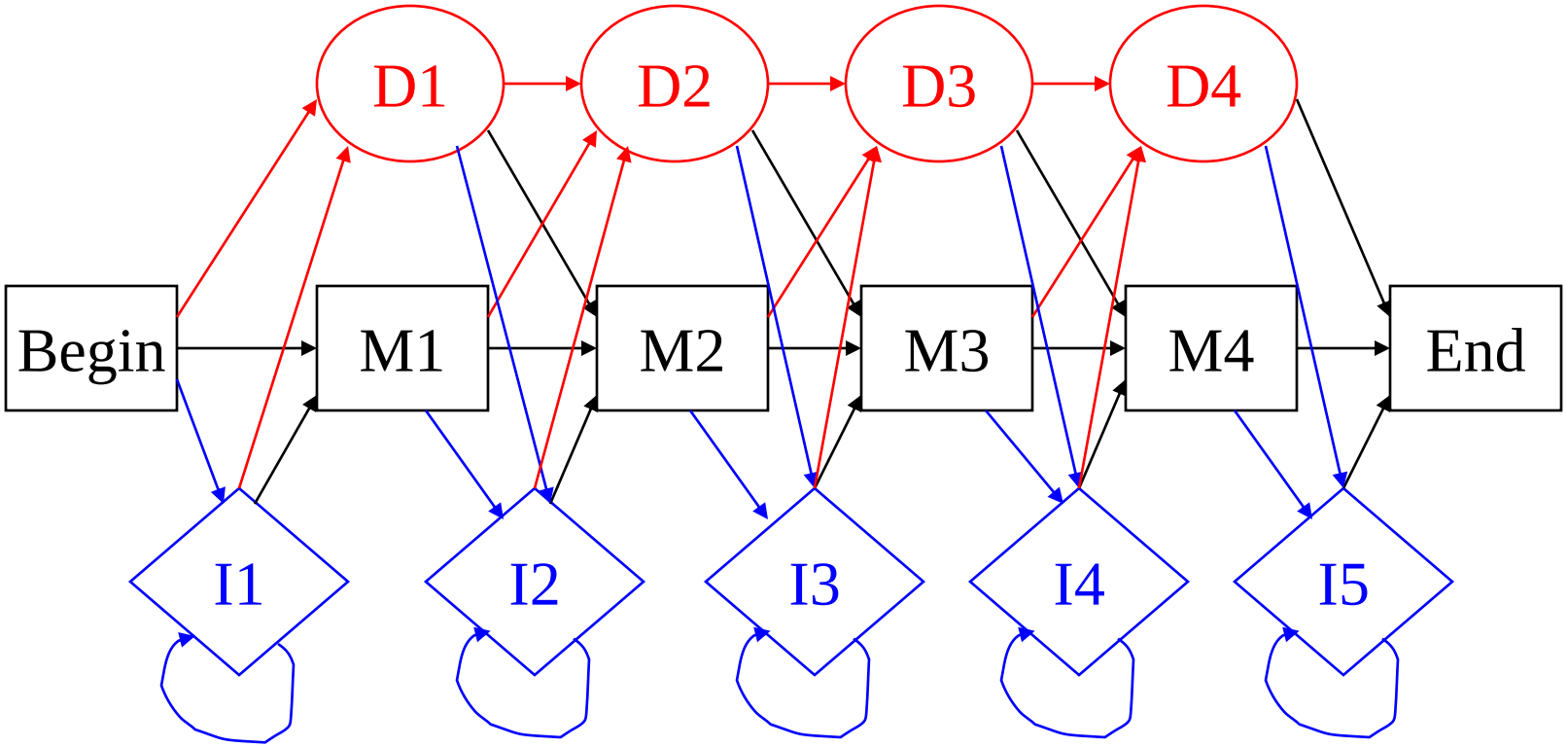
Hidden Markov Model



Krogh et al. 1994, Baldi et al. 1994

Each match state has an emission distribution of 20 amino acids.
Each insertion state has an emission distribution of 20 amino acids.
Variants of architecture exist. (see Eddy, bioinformatics, 1997)

Hidden Markov Model

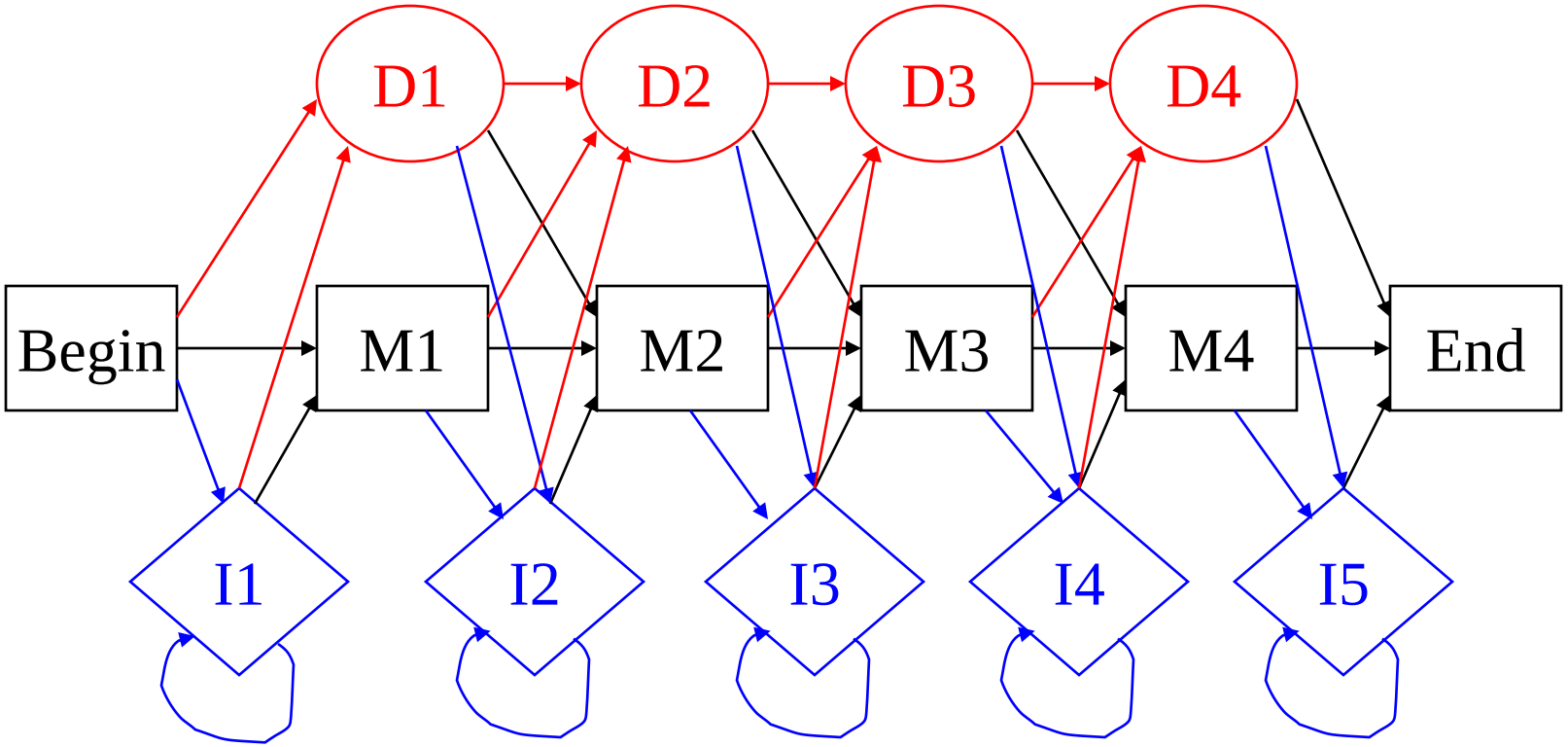


How many states? (M positions: length of model)

M (match) + M (deletion) + (M+1) (insertion) + 2 = 3M + 3

Krogh et al. 1994, Baldi et al. 1994

Hidden Markov Model



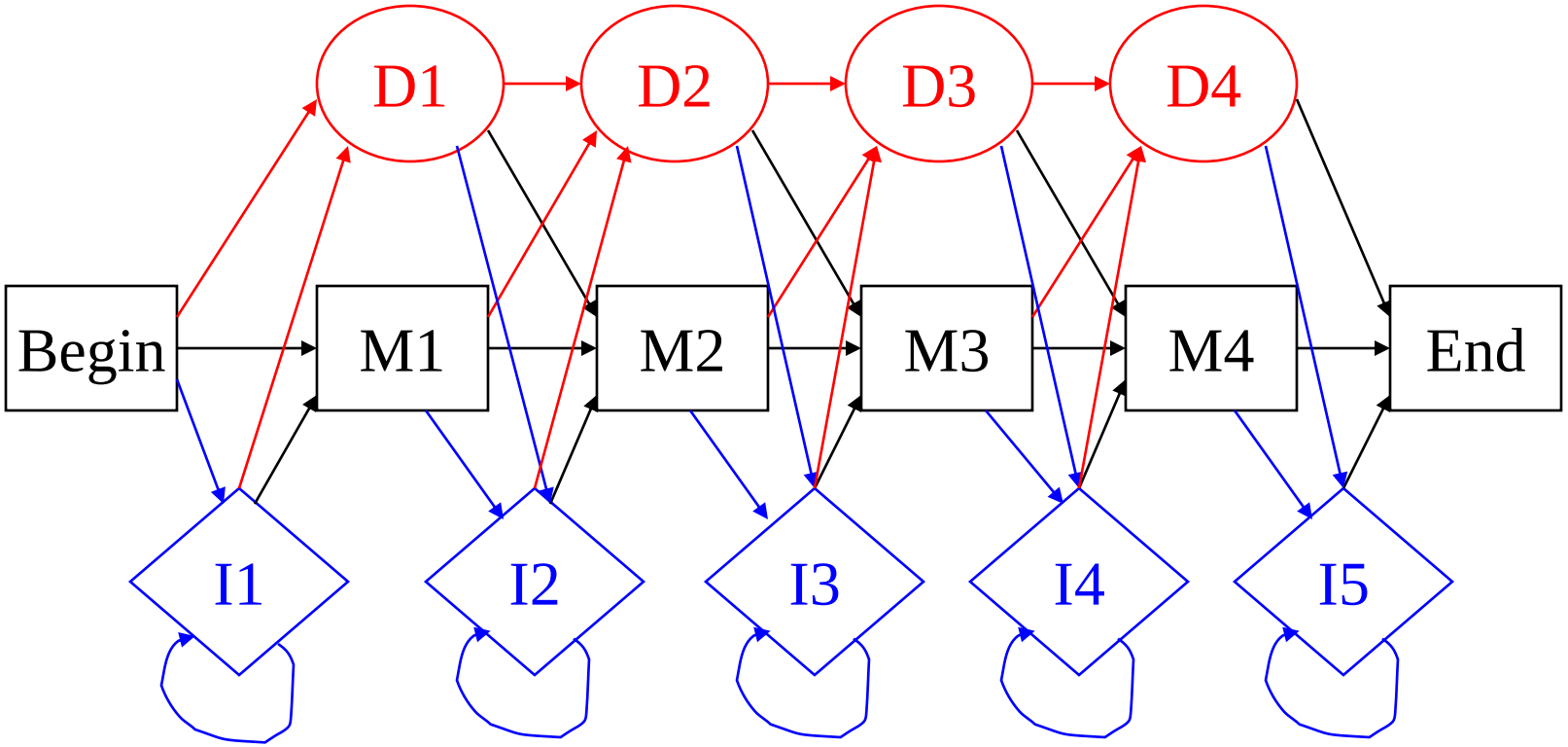
How many transitions? (M positions: length of model)

Deletion: 3M – 1, Match: 3M – 1, Insertion: 3(M+1) – 1, B/E: 3

Total = 9M +3.

Krogh et al. 1994, Baldi et al. 1994

Hidden Markov Model



How many emissions? (M positions: length of model)

M * 20 (match) + (M+1)*20 (insertion) = 40M + 20

Krogh et al. 1994, Baldi et al. 1994

Initialization of HMM

- How to decide model length (the number of match states)?
- How to initialize transition probabilities?
- How to initialize emission probabilities?

How to Decide Model Length?

- **Learn**: Use a range of model length (centered at the average sequence length). If transition probability from a match (M_i) state to a delete state (D_{i+1}) > 0.5, remove the $M_{i+1.}$ If transition probability from a match (M_i) state to an insertion state (I_{i+1}) > 0.5, add a match state.
- **Get from multiple alignment**: assign a match state to any column with <50% gaps.

How to Initialize Parameters?

- Uniform initialization of transition probabilities is ok in most cases.
- Uniform initialization of emission probability of insert state is ok in many cases.
- Uniform initialization of emission probability of match state is **bad**. (lead to bad local minima)
- Using amino acid distribution to initialize the emission probabilities is better. (need regularization / smoothing to avoid zero)

Initialize from Multiple Alignments

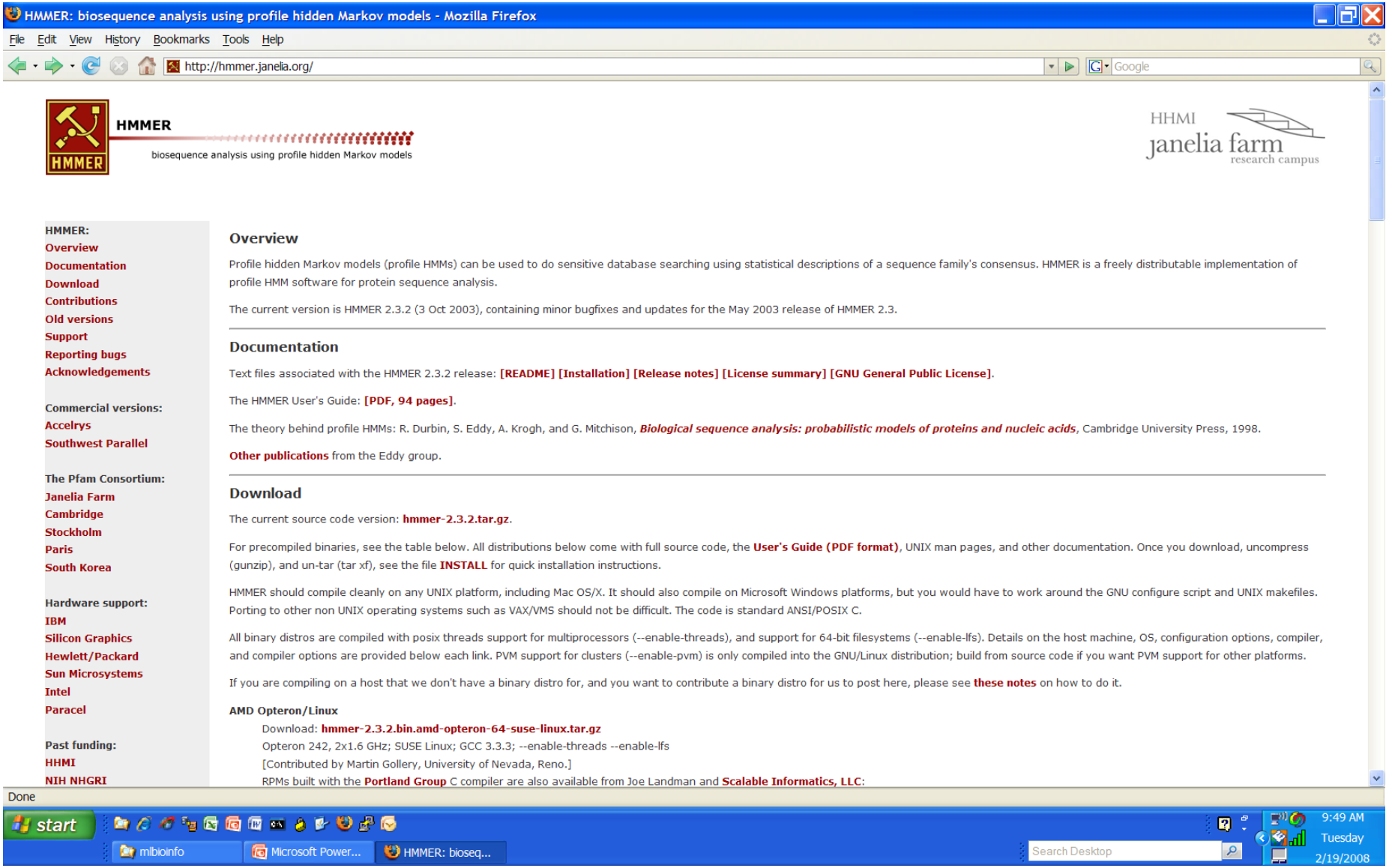
```
seq1 ---VRRNNMGMPLIESSSYHDALFTLGY--AGDRISQMLGMRLLAQGRLSEMAGADALDV
seq2 --NIYIDSNGIAHIYANNLHDLFLAEGYYEASQRLFEIELFG-LAMGNLSSWVGAKALSS
seq3 SAETYRDAWGIPHLRADTPHELARAQGT--ARDRAWQLEVERHRAQGTSASFLGPEALSW
seq4 -----DRLGVVTIDAANQLDAMRALGY--AQERYFEMDLMRRAPAGELSELFGAKAVDL
```

First, assign match / main states, delete states, insert states from MSA
Get the path of each sequence
Count the amino acid frequencies emitted from match or insert states, which are converted into probabilities for each state (need smoothing/ regularization / pseudo-count).
Count the number of state transitions and use them to initialize transition probabilities.

Estimate Parameters (Learning)

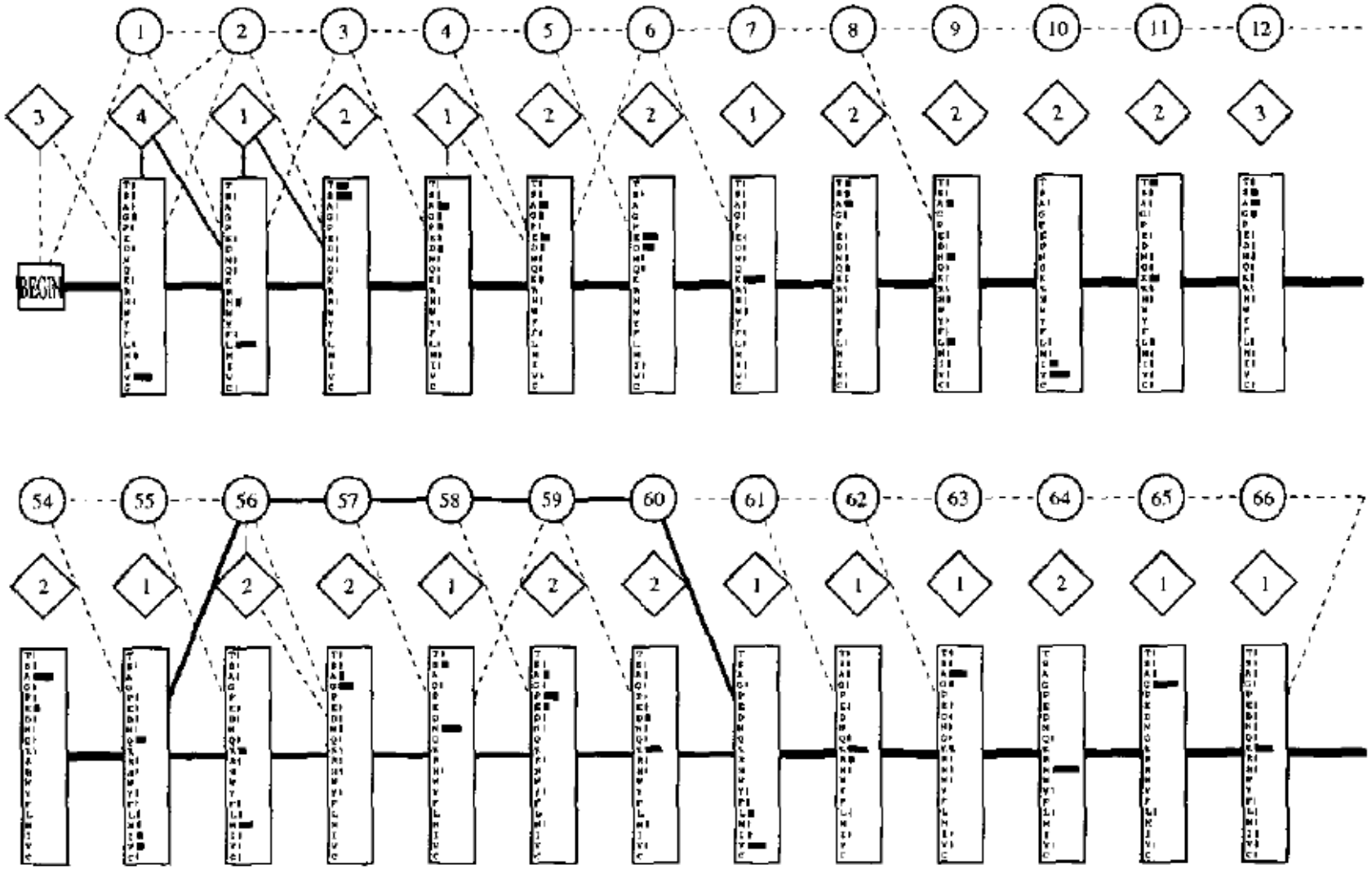
- We want to find a set of parameters to maximize the probability of the observed sequences in the family: maximum likelihood: P(sequences | model) = P(sequence 1| model) * … * P(sequence n | model).
- Baum-Welch's algorithm (or EM algorithm) (see my previous lectures about HMM theory)

Demo of HMMER



http://hmmer.janelia.org/

Visualization of Features and Structure in HMM



Myoglobin protein family. How to interpret it?

Krogh et al. 94

Demo of HMMEditor

J. Dai and J. Cheng. **HMMEditor: a visual editing tool for profile hidden Markov model**. *BMC Genomics*, in press, 2007.

Tool: http://casp.rnet.missouri.edu/hmmeditor/

Paper: http://www.biomedcentral.com/qc/1471-2164/9/S1/S8

Protein Family Profile HMM Databases

- Pfam database (Sonnhammer et al., 1997, 1998) (domain database) (http://pfam.wustl.edu/)
- PROSITE profiles database (Bairoch et al., 1997) (motif database)

What Can We Do With the HMM?

- **Recognition / database search**: does a new sequence belong to the family? (database search)
- **Idea**: The sequences belonging to the family (or generated from HMM) should receive higher probability than the sequence not belong to the family (unrelated sequences).

Two Ways to Search

- Build a HMM for a query family, and search HMM against of a database of sequences
- Build a HMM for each sequence in the database. Search a query sequence against the database of HMMs.

Compute P(Sequence | HMM)

- Forward algorithm to compute P(sequence | model)
- We work on: -log(P(sequence|M): distance from the sequence to the model. (negative log likelihood score)
- Unfortunately, -log(P(sequence|M) is length dependent. So what can we do?

Normalize the Score into Z-score

- Search the profile against a large database such as Swiss-Prot
- Plot –log(P(seuqence|model), NLL scores, against sequence length.

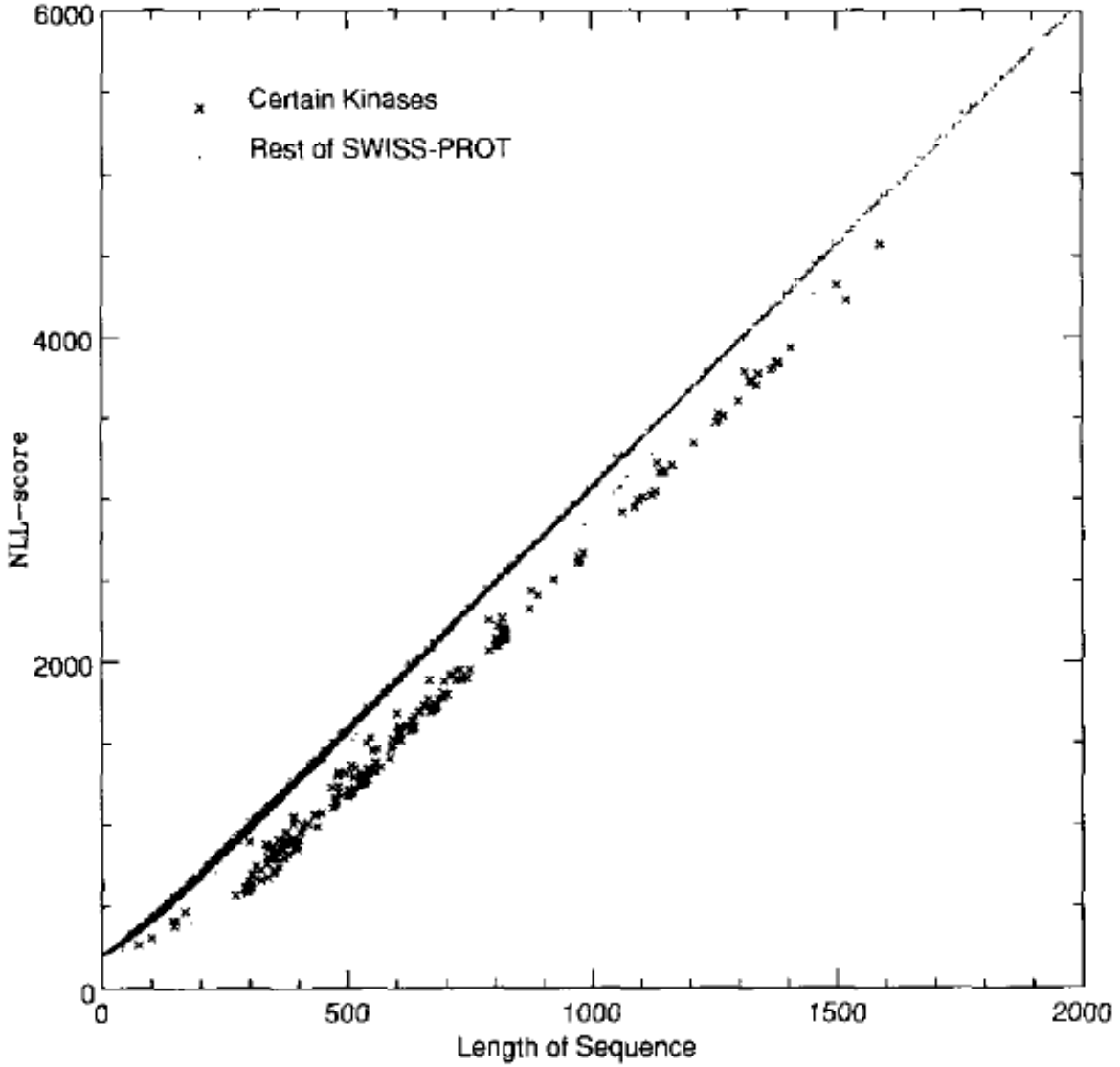


Figure 9. Scatter plot of NLL-score versus length for sequences in SWISS-PROT using the Kinase HMM.

NLL score is linear to sequence length.

NLL scores of the same family is lower than un-related sequences

We need normalization.

Krogh et al. 94

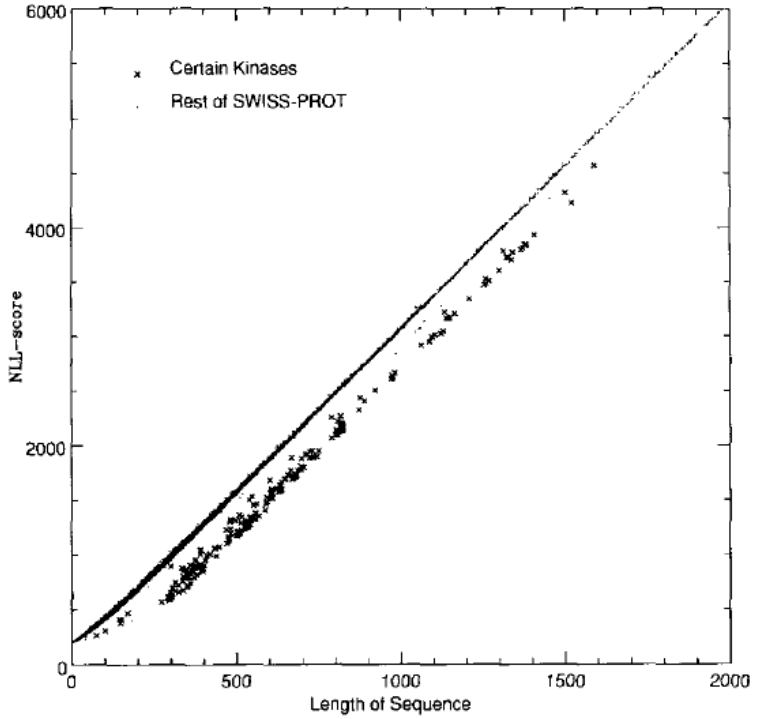


Figure 9. Scatter plot of NLL-score versus length for sequences in SWISS-PROT using the Kinase HMM.

NULL model of unrelated sequences:

Length	Mean (u)	Std (σ)
100	500	5
101	550	6

Compute Z-score: $|s - u| / \sigma$

$\sigma > 4$: the sequence is very different from unrelated sequence.
(for non-database search, a randomization can work.)

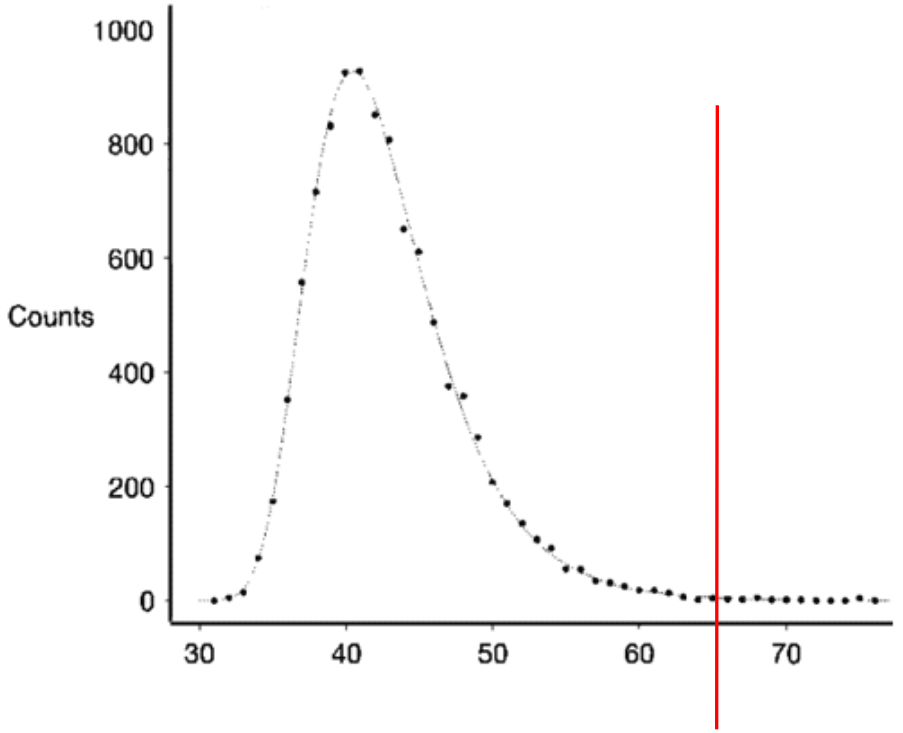
Extreme Value Distribution
(Karlin and Altschul)

http://www.people.virginia.edu/~wrp/cshl02/Altschul/Altschul-3.html

$$P(S \geq x) = 1 - \exp(-Kmn\, e^{-\lambda x})$$

P-value

E-value



K and lamda are statistical parameters. m,n model and sequence length.

Application: Soybean Project

- 2000 proteins in Arabidopsis
- These sequences are clustered into 64 families by biologists (known)

- 4000 new proteins in Soybean?
- How to assign them into those families?

How to solve the problem?

Iterative Data Search (SAM)

- Use BLAST to search a query sequence database to gather an initial MSA
- **Repeat**

  Build an HMM from MSA (training)

  Search the HMM against sequence database to get more related sequences

  Create a new MSA from all related sequences
- **Until** a predefined number of iterations or no new sequences are found.

(Idea is very similar as PSI-BLAST)

Insight I: Evaluating a Sequence Against a Profile HMM is Fold Recognition Process (or Similarity Recognition)

- Check if a sequence is in the same family (or superfamily) as the protein family (superfamily) used to build the profile HMM.
- If they are in the same family, they will share the similar protein structure (fold), possibly protein function.
- The known structure can be used to model the structure of the proteins without known structure.

Insight II: Evaluating a Sequence Against a HMM is Sequence-Profile Alignment

- Align a query sequence against a HMM of the target sequence to get the most likely path (Viterbi algorithm) (or vice versa)
- Match the path of the query sequence with the path of the target sequence, we get their alignment.
- Represented work: SAM or HMMer.

Pairwise Alignment via HMM

Seq 1: A T G R K E
Path : M_1 I_1 I_1 M_2 D_3 M_4 I_4

Seq 2: V C K E R P
Path : M_1 I_1 M_2 M_3 M_4 I_4

Path: M_1 M_2 M_3 M_4
Seq 1: A T G R - K E
Seq 2: V C - K E R P

HMM for Multiple Sequence Alignment

- Build a HMM for a group of sequences
- Align each sequence against HMM using Viterbi algorithm to find the most likely path. (dynamic programming)
- Match the main/match states of these paths together.
- Add gaps for delete states
- For insertion between two positions, use the longest insertion of a sequence as template. Add gaps to other sequence if necessary. (see Krogh's paper)

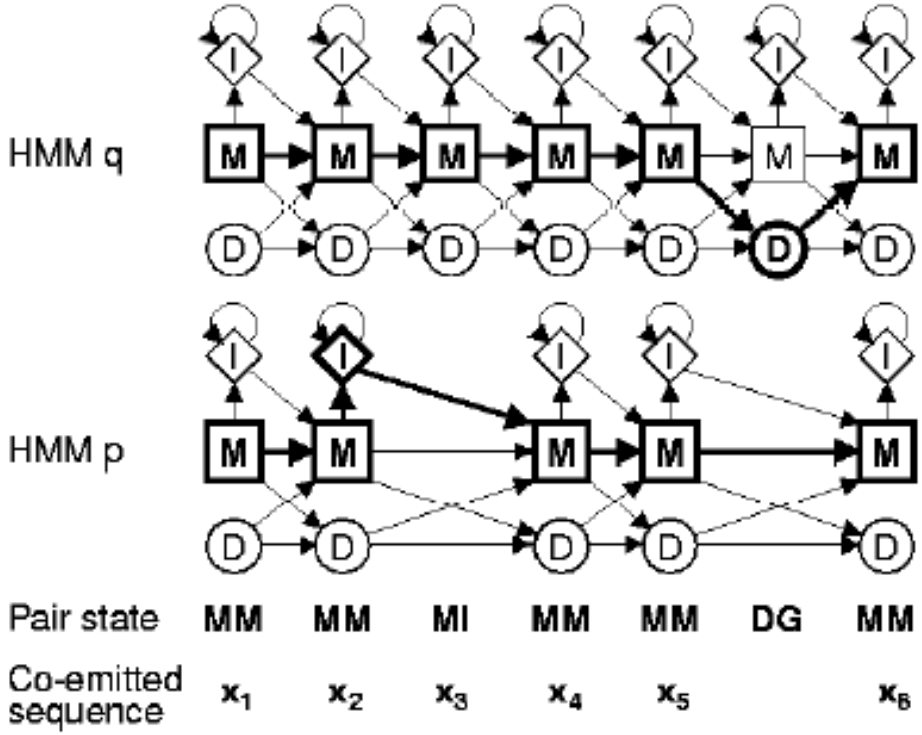
How About Evaluating the Similarity between HMMs?

- Can we evaluate the similarity of two HMMs?

- Can we align two profile HMMs? (profile-profile alignment). Compare HMM with HMM.

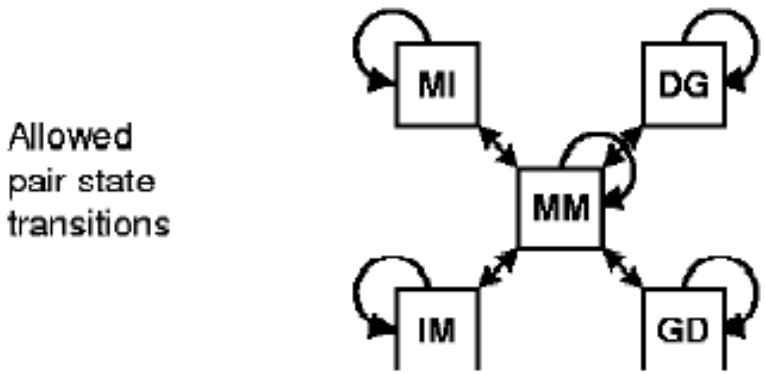
J. Soeding, Bioinformatics, 2005

HMM-HMM Comparison
Profile-Profile Alignment



Generalized Sequence (of States) 1

Generalized Sequence (of States) 2



Goal: find a sequence of paired states with maximum log sum of odds score

J. Soeding, Bioinformatics, 2005

Derivation of Objective Function

$$
\begin{aligned}
S_{\mathrm{LSO}} &= \log \sum_{x_1,\ldots,x_L} \frac{\prod_{l=1}^{L} q^{\mathrm{P}}_{k(l)}(x_l)\, p^{\mathrm{P}}_{k(l)}(x_l) \times \mathcal{P}_{tr}}{\prod_{l=1}^{L} f(x_l)} \\
&= \log \sum_{x_1=1}^{20} \cdots \sum_{x_L=1}^{20} \prod_{l=1}^{L} \frac{q^{\mathrm{P}}_{k(l)}(x_l)\, p^{\mathrm{P}}_{k(l)}(x_l)}{f(x_l)} \times \mathcal{P}_{\mathrm{tr}} \\
&= \log \prod_{l=1}^{L} \left(\sum_{a=1}^{20} \frac{q^{\mathrm{P}}_{k(l)}(a)\, p^{\mathrm{P}}_{k(l)}(a)}{f(a)} \right) + \log \mathcal{P}_{\mathrm{tr}} \\
&= \sum_{k:X_k Y_k = MM} S_{\mathrm{aa}}(q_{i(k)}, p_{j(k)}) + \log \mathcal{P}_{\mathrm{tr}}.
\end{aligned}
$$

In the last line we have introduced the column score,

$$
S_{\mathrm{aa}}(q_i, p_j) = \log \sum_{a=1}^{20} \frac{q_i(a)\, p_j(a)}{f(a)},
$$

Co-emission score of all possible sequences of a path through two HMMs

J. Soeding, Bioinformatics, 2005

Dynamic Programming for a path of paired states through two HMMs

$$S_{MM}(i,j) = S_{\mathrm{aa}}(q_i, p_j)$$

$$+ \max \begin{cases} S_{MM}(i-1,j-1) + \log[q_{i-1}(M,M)\, p_{j-1}(M,M)] \\ S_{MI}(i-1,j-1) + \log[q_{i-1}(M,M)\, p_{j-1}(I,M)] \\ S_{IM}(i-1,j-1) + \log[q_{i-1}(I,M)\, p_{j-1}(M,M)] \\ S_{DG}(i-1,j-1) + \log[q_{i-1}(D,M)\, p_{j-1}(M,M)] \\ S_{GD}(i-1,j-1) + \log[q_{i-1}(M,M)\, p_{j-1}(D,M)] \end{cases}$$

$$S_{MI}(i,j) = \max \begin{cases} S_{MM}(i-1,j) + \log[q_{i-1}(M,M)\, p_j(M,I)] \\ S_{MI}(i-1,j) + \log[q_{i-1}(M,M)\, p_j(I,I)] \end{cases}$$

$$S_{DG}(i,j) = \max \begin{cases} S_{MM}(i-1,j) + \log[q_{i-1}(M,D)] \\ S_{DG}(i-1,j) + \log[q_{i-1}(D,D)] \end{cases}$$

and similarly for $S_{IM}(i,j)$ and $S_{GD}(i,j)$.

J. Soeding, Bioinformatics, 2005

COACH Approach

- Given two families of sequences, build a multiple alignment (MSA) for each one of them.
- Build HMM from one MSA
- Align another MSA against the HMM. (match each column of amino acids against states in the HMM)

R. C. Edgar and K. Sjolander. Bioinformatics, 2004

How to Do Local Alignment

- COACH approach

  With respect to sequence: add an insertion state right after the start state and right before the end state.

  With respect to HMM: start state can jump to any match state and any match state can jump to end state.

- HHSearch approach

  Semi-global: set $S_{MM}(i,0) = S_{MM}(0,j) = 0$, and S_{MI}, S_{IM}, S_{DG}, S_{GD} are initialized to $-\infty$ to force the first pair of states is MM.

  Local: add a 0 to avoid a path with negative score into dynamic programming formula (See Soeding, bioinformatics, 2005)

Sequence Weighting

- **Henikoff-Henikoff**: sum of position-based weight. For each position, each type of amino acid is assigned weight 1. The weight is of each amino acid is 1 / frequency of the amino acid at the position. The weight of a sequence is the sum of positional weights. (gap is not counted. A position with more than 50% gaps may be removed from counting.) An easy, useful algorithm
- **Tree algorithm**: construct a phylogenetic tree. Start from root, weight 1 flows down. At any branch, the weight is cut to half.

Pseudo-Count

- PSI-BLAST pseudo-count
  (Altschul et al., 1997)

  For each position of PSSM, score is log (Q_i / P_i). Q_i is the estimated probability of residue i to be found in the column. P_i is the background probability.

  Small sample size require prior knowledge of residue i to better estimate Q_i. Best method is Dirichlet Mixtures. A very good and simple method is a data-dependent pseudocount method. This method uses the prior knowledge of amino acid relationships embodied in the substitution matrix S_{ij} to generate residue pseudocount frequencies g_i. (f_j is the frequency of residue j). α is set to Nc -1 (Nc is the number of columns). β is set to 10.

$$g_i = \sum_j \frac{f_j}{P_j} q_{ij}$$

$$s_{ij} = [\ln(q_{ij}/P_i P_j)]/\lambda_u$$

$$q_{ij} = P_i P_j e^{\lambda_u s_{ij}}$$

$$Q_i = \frac{\alpha f_i + \beta g_i}{\alpha + \beta}$$

Null Model

- Background null model (log-odds)
- Reverse null model (SAM)
- Sum of log-odds score of local alignment obeys extreme-value distribution (same as PSI-BLAST): good for estimating the significance of sequence-HMM match.
- Sum of log-odds score of global alignment is length dependent.

HMM Software and Code

- HMMER: http://hmmer.wustl.edu
- SAM: http://www.cse.ucsc.edu/research/combio/sam.html
- HHSearch: http://toolkit.tuebingen.mpg.de
- PRC-HMM: http://supfam.mrc-lmb.cam.ac.uk/PRC/
- COACH: http://www.drive5.com/lobster/
- HMM-HMM comparison: http://www.brics.dk/~cstorm/hmmcomp/
- MUSCLE: http://www.drive5.com/muscle/

Project 1: Multiple Sequence Alignment Using HMM

- Dataset: BaliBASE: http://www-bio3d-igbmc.u-strasbg.fr/balibase/
- Generate multiple alignment using Clustalw (http://www.ch.embnet.org/software/ClustalW.html) for a family of sequences
- Construct a HMM for a family of sequences (initialization, number of states)
- Estimate the parameters of HMM using the sequences
- Analyze the emission probability of states
- Analyze the transition probability between states (visualization is good)
- Compute the probability of each sequence
- Generate multiple sequence alignments and compare it with the initial multiple alignment
- Implement your own HMM or use open source code.
- If you use open source code, thoroughly decode the structure of the program and implementation techniques, and write them in your paper.

Reference: A. Krogh et al, JMB, 1994 and open source HMM.

Project 2: Profile-Profile Alignment Using HMM

- Goal: implement a profile-profile alignment tool using open source HMM code such as SAM, HMMer, HHSearch and COACH.
- You can use the source of HHSearch or COACH, but you need to thoroughly describe the structure of the code.
- Reference papers (methods):

1. R. C. Edgar and K. Sjolander. COACH: Profile-Profile Alignment of Protein Families Using Hidden Markov Models. Bioinformatics. 2004.

2. J. SÖding. Protein Homology Detection by HMM-HMM Comparison. Bioinformatics. 2005.

3. Lyngsø,R.B., Pedersen,C.N.S. and Nielsen,H. (1999) Metrics and similarity measures for hidden markov models. *Proc. Int. Conf. Intell. Syst. Mol. Biol.*, pp. 178–186.