CAP 5510: Introduction to Bioinformatics
CGS 5166: Bioinformatics Tools

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Problem 1:

- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov models: M+ and M —
  - Then compare

How to distinguish?

- Compute

$$S(x) = \log\left(\frac{P(x \mid M+)}{P(x \mid M-)}\right) = \sum_{i=1}^{L} \log\left(\frac{p_{x(i-1)xi}}{m_{x(i-1)xi}}\right) = \sum_{i=1}^{L} r_{x(i-1)xi}$$

r=p/m	A	C	G	T
A	-0.740	0.419	0.580	-0.803
C	-0.913	0.302	1.812	-0.685
G	-0.624	0.461	0.331	-0.730
T	-1.169	0.573	0.393	-0.679

Score(GCAC)
= .461-.913+.419
< 0.
GCAC not from CpG island.

Score(GCTC)
= .461-.685+.573
> 0.
GCTC from CpG island.

Problem 1:

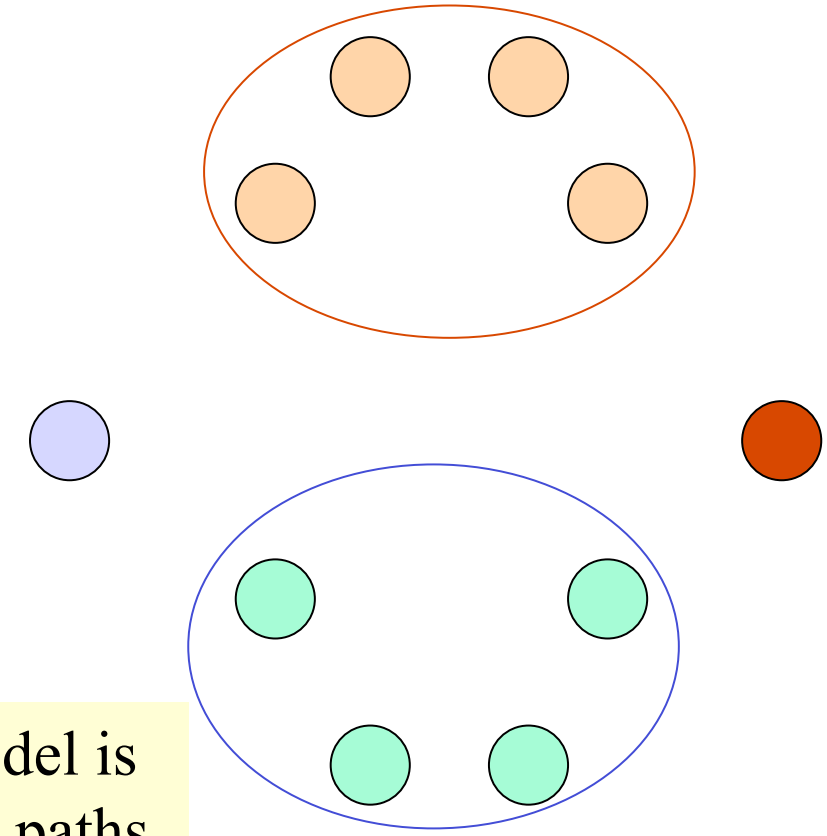
- Input: Small sequence S
- Output: Is S from a CpG island?
  - Build Markov Models: M+ & M-
  - Then compare

Problem 2:

- Input: Long sequence S
- Output: Identify the CpG islands in S.
  - Markov models are inadequate.
  - Need Hidden Markov Models.

Hidden Markov Model (HMM)

- States
- Transitions
- Transition Probabilities
- Emissions
- Emission Probabilities



- What is hidden about HMMs?

Answer: The path through the model is hidden since there are many valid paths.

How to Solve Problem 2?

- Solve the following problem:

Input: Hidden Markov Model M,

parameters Θ, emitted sequence S

Output: Most Probable Path Π

How: Viterbi's Algorithm (Dynamic Programming)

Define $\Pi[i,j]$ = MPP for first j characters of S ending in state i

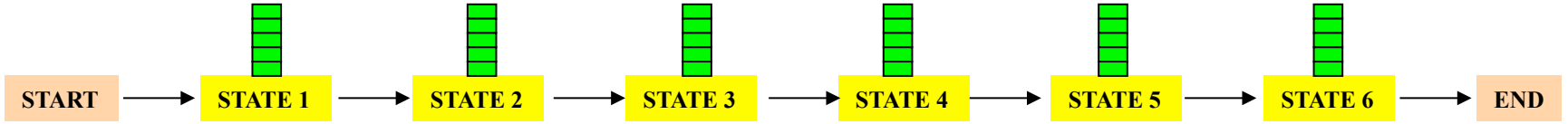
Define P[i,j] = Probability of $\Pi[i,j]$

- **Compute state i with largest P[i,j].**

Profile HMMs

PROFILE METHOD, [M. Gribskov et al., '90]

Location in Seq.	Sequence 1 2 3 4 5 6	Protein Name
14	G V S A S A	Ka RbtR
32	G V S E M T	Ec DeoR
33	G V S P G T	Ec RpoD
76	G A G I A T	Ec TrpR
178	G C S R E T	Ec CAP
205	C L S P S R	Ec AraC
210	C L S P S R	St AraC
13	G V N K E T	Br MerR

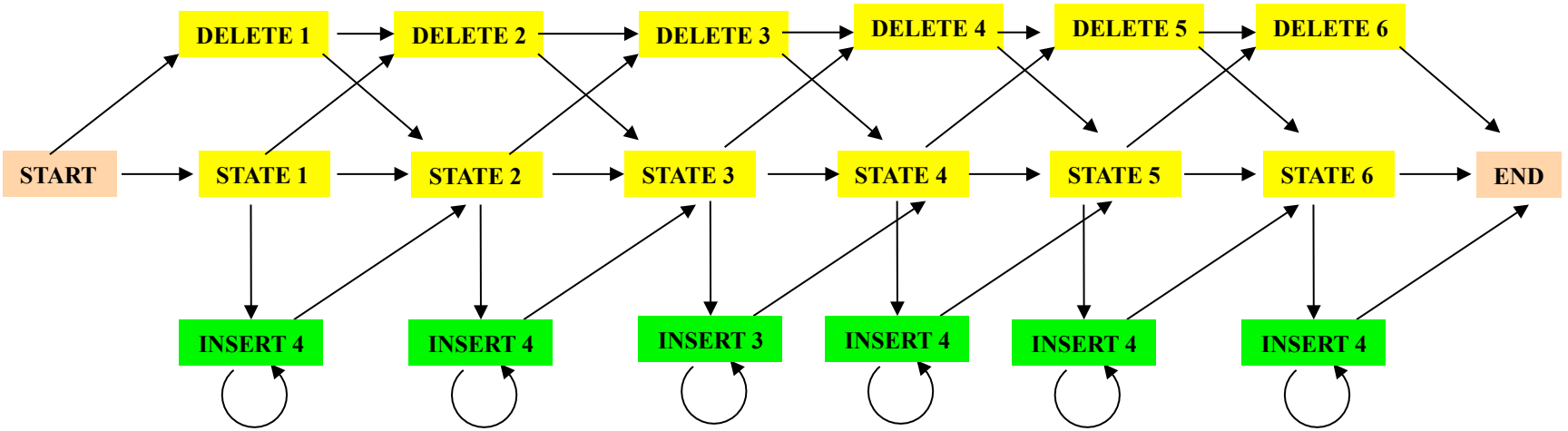


Profile HMMs from Multiple Alignments

```
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
```

Construct Profile HMM from above multiple alignment.

Profile HMMs with InDels



Missing transitions from DELETE j to INSERT j and from INSERT j to DELETE j+1.

HMM for Sequence Alignment

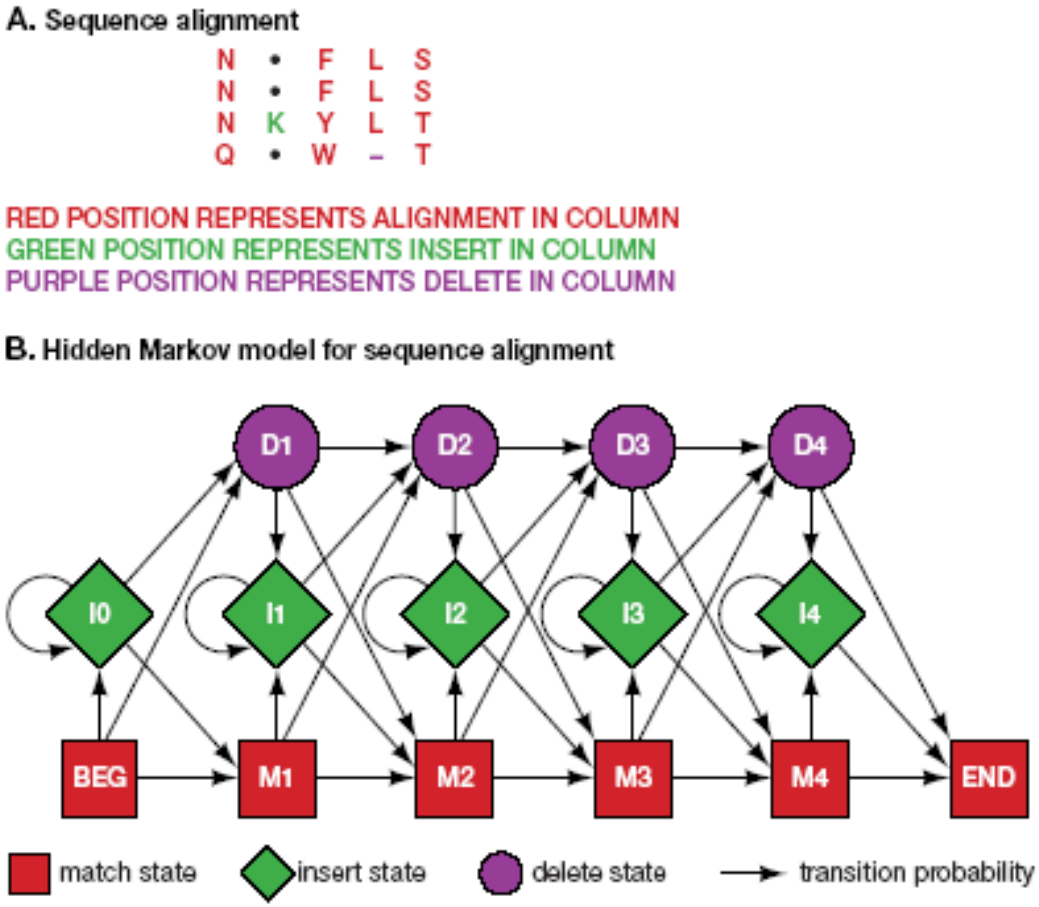


FIGURE 5.16. Relationship between the sequence alignment and the hidden Markov model of the alignment (Krogh et al. 1994). This particular form for the HMM was chosen to represent the sequence, structural, and functional variation expected in proteins. The model accommodates the identities, mismatches, insertions, and deletions expected in a group of related proteins. (*A*) A section of an msa. The illustration shows the columns generated in an msa. Each column may include matches and mismatches (*red* positions), insertions (*green* positions), and deletions (*purple* positions). (*B*) The HMM. Each column in the model represents the possibility of a match, insert, or delete in each column of the alignment in *A*. The HMM is a probabilistic representation of a section of the msa. Sequences can be generated from the HMM by starting at the beginning state labeled BEG and then by following any one of many pathways from one type of sequence variation to another (states) along the state transition arrows and terminating in the ending state labeled END. Any sequence can be generated by the model and each pathway has a probability associated with it. Each square match state stores an amino acid distribution such that the probability of finding an amino acid depends on

Profile HMM Software

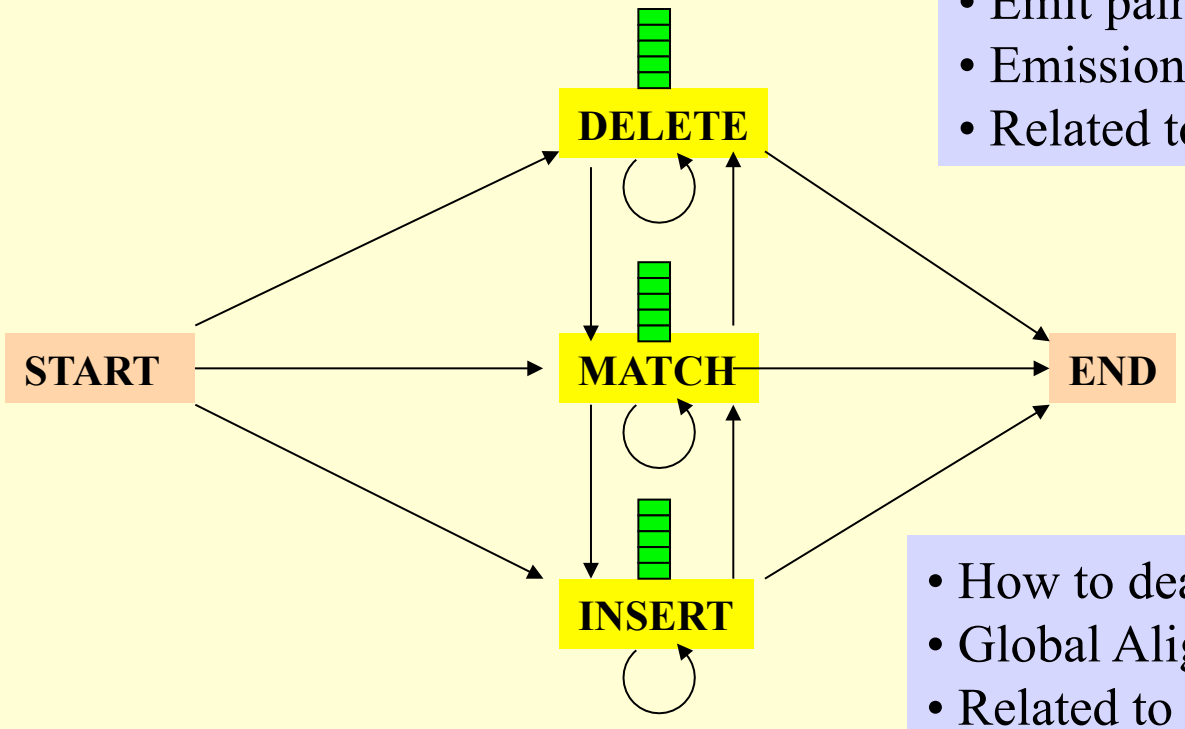
- HMMER http://hmmer.wustl.edu/
- SAM http://www.cse.ucsc.edu/research/compbio/sam.html
- PFTOOLS http://www.isrec.isb-sib.ch/ftp-server/pftools/
- HMMpro http://www.netid.com/html/hmmpro.html
- GENEWISE http://www.ebi.ac.uk/Wise2/
- PROBE ftp://ftp.ncbi.nih.gov/pub/neuwald/probe1.0/
- META-MEME http://metameme.sdsc.edu/
- BLOCKS http://www.blocks.fhcrc.org/
- PSI-BLAST http://www.ncbi.nlm.nih.gov/BLAST/newblast.html

- Read more about Profile HMMs at
  - http://www.csb.yale.edu/userguides/seq/hmmer/docs/node9.html

How to model Pairwise Sequence Alignment

LEAPVE

LAPVIE

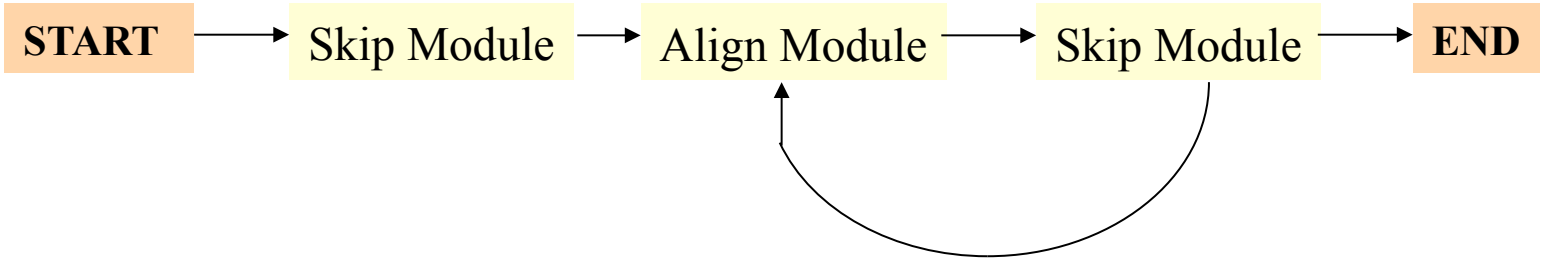


Pair HMMs
- Emit pairs of synbols
- Emission probs?
- Related to Sub. Matrices

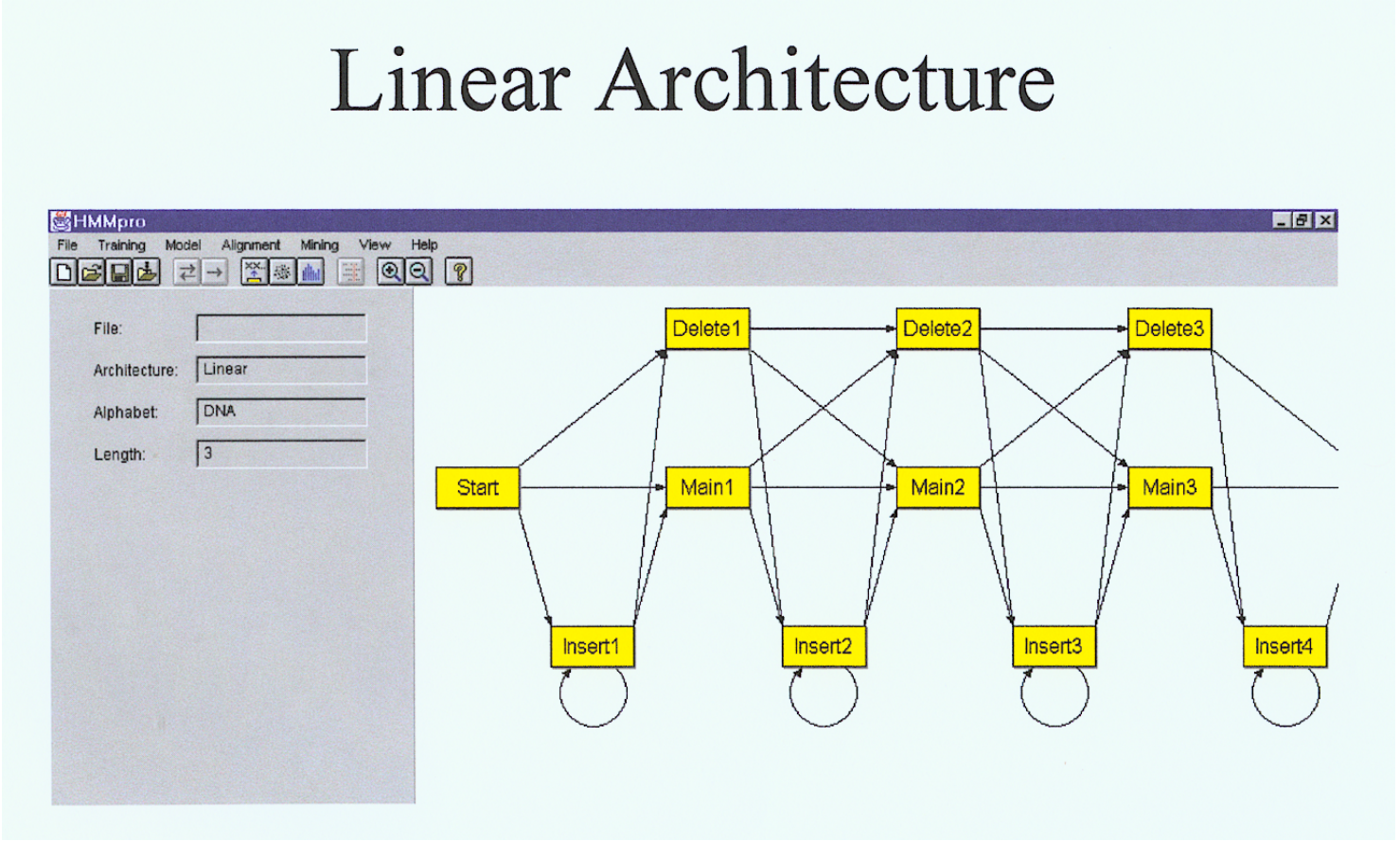
- How to deal with InDels?
- Global Alignment? Local?
- Related to Sub. Matrices

How to model Pairwise Local Alignments?

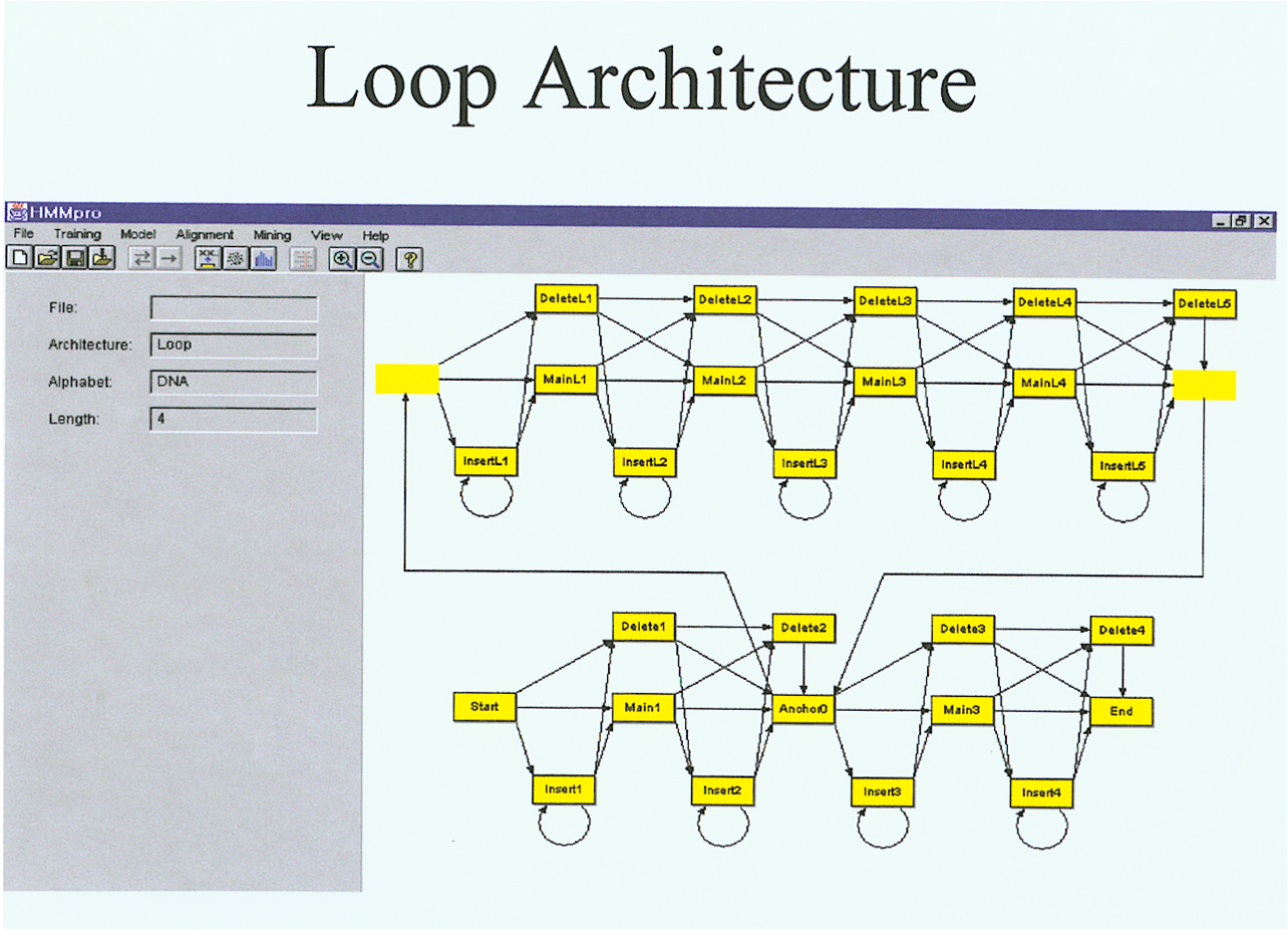
How to model Pairwise Local Alignments with gaps?



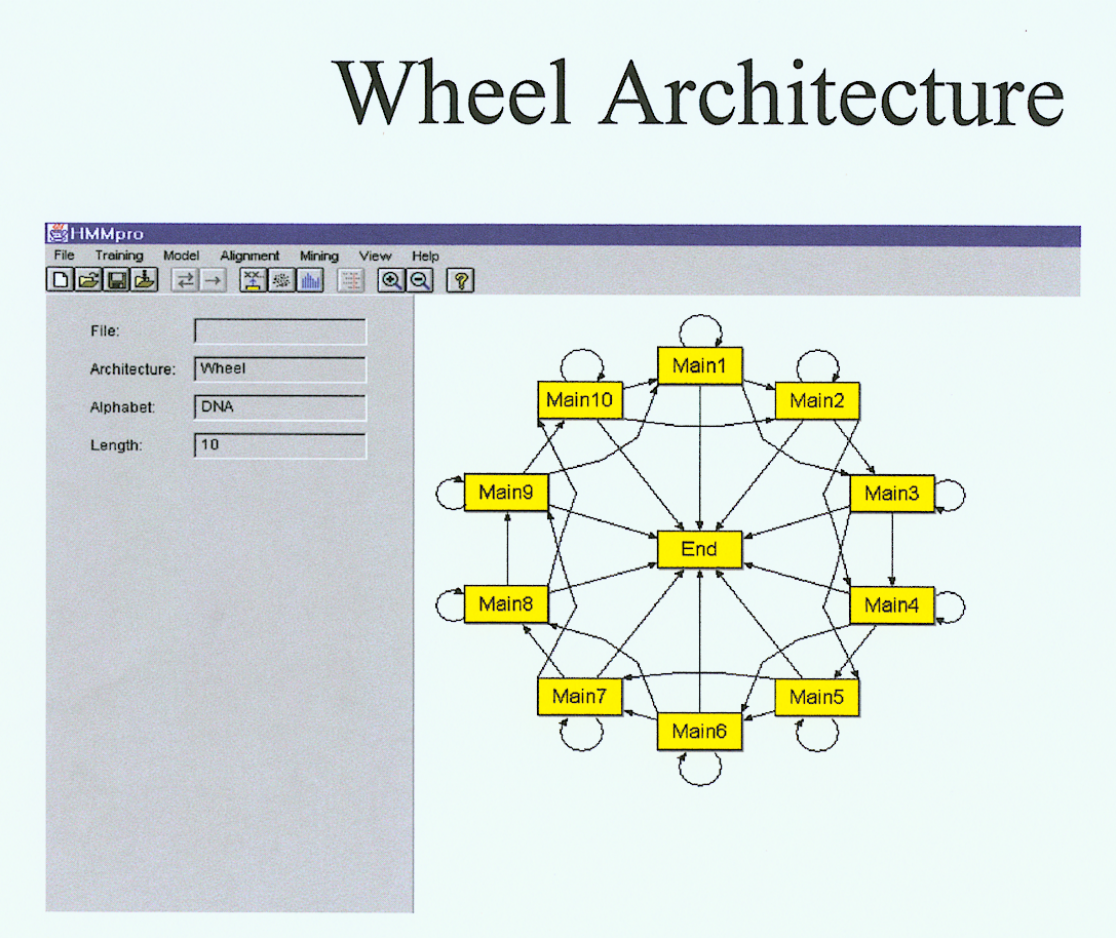
Standard HMM architectures



Standard HMM architectures



Standard HMM architectures



Problem 3: LIKELIHOOD QUESTION

- Input: Sequence S, model M, state i
- Output: Compute the probability of reaching state i with sequence S using model M
  - Backward Algorithm (DP)

Problem 4: LIKELIHOOD QUESTION

- Input: Sequence S, model M
- Output: Compute the probability that S was emitted by model M
  - Forward Algorithm (DP)

Problem 5: LEARNING QUESTION

- Input: model structure M, Training Sequence S
- Output: Compute the parameters Θ
- Criteria: ML criterion
    - maximize $P(S \mid M, \Theta)$ HOW???

Problem 6: DESIGN QUESTION

- Input: Training Sequence S
- Output: Choose model structure M, and compute the parameters Θ
    - No reasonable solution
    - Standard models to pick from

Iterative Solution to the LEARNING QUESTION (Problem 5)

- Pick initial values for parameters Θ_0
- Repeat

  Run training set S on model M

  Count # of times transition $i \Rightarrow j$ is made

  Count # of times letter x is emitted from state i

  Update parameters Θ
- Until (some stopping condition)

Entropy

- Entropy measures the variability observed in given data.

$$E = -\sum_{c} p_c \log p_c$$

- Entropy is useful in multiple alignments & profiles.

- Entropy is max when uncertainty is max.

G-Protein Couple Receptors

- Transmembrane proteins with 7 α-helices and 6 loops; many subfamilies
- Highly variable: 200-1200 aa in length, some have only 20% identity.
- [Baldi & Chauvin, '94] HMM for GPCRs
- HMM constructed with 430 match states (avg length of sequences) ; Training: with 142 sequences, 12 iterations

GPCR - Analysis

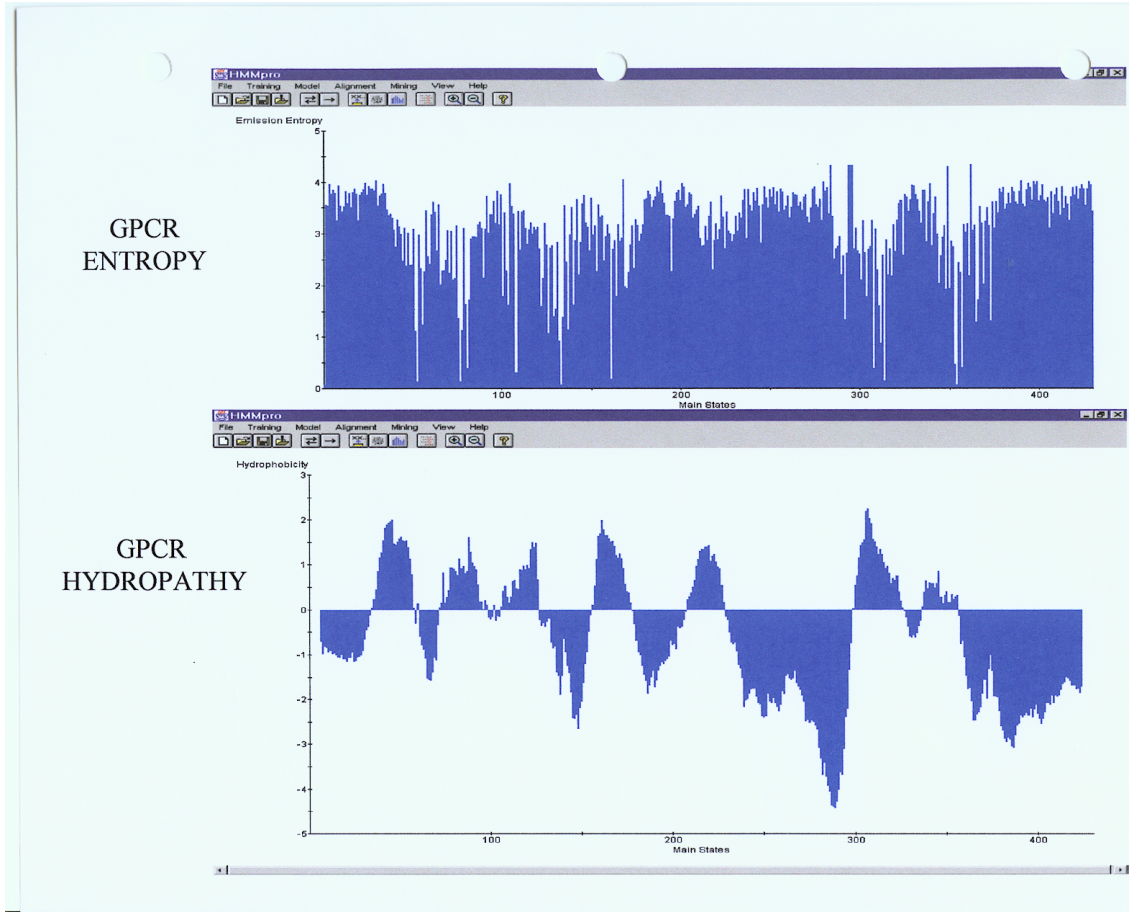
- Compute main state entropy values

$$H_i = -\sum_a e_{ia} \log e_{ia}$$

- For every sequence from test set (142) & random set (1600) & all SWISS-PROT proteins
  - Compute the negative log of probability of the most probable path π

$$Score(S) = -\log\left(P(\pi \mid S, M)\right)$$

GPCR Analysis



Entropy

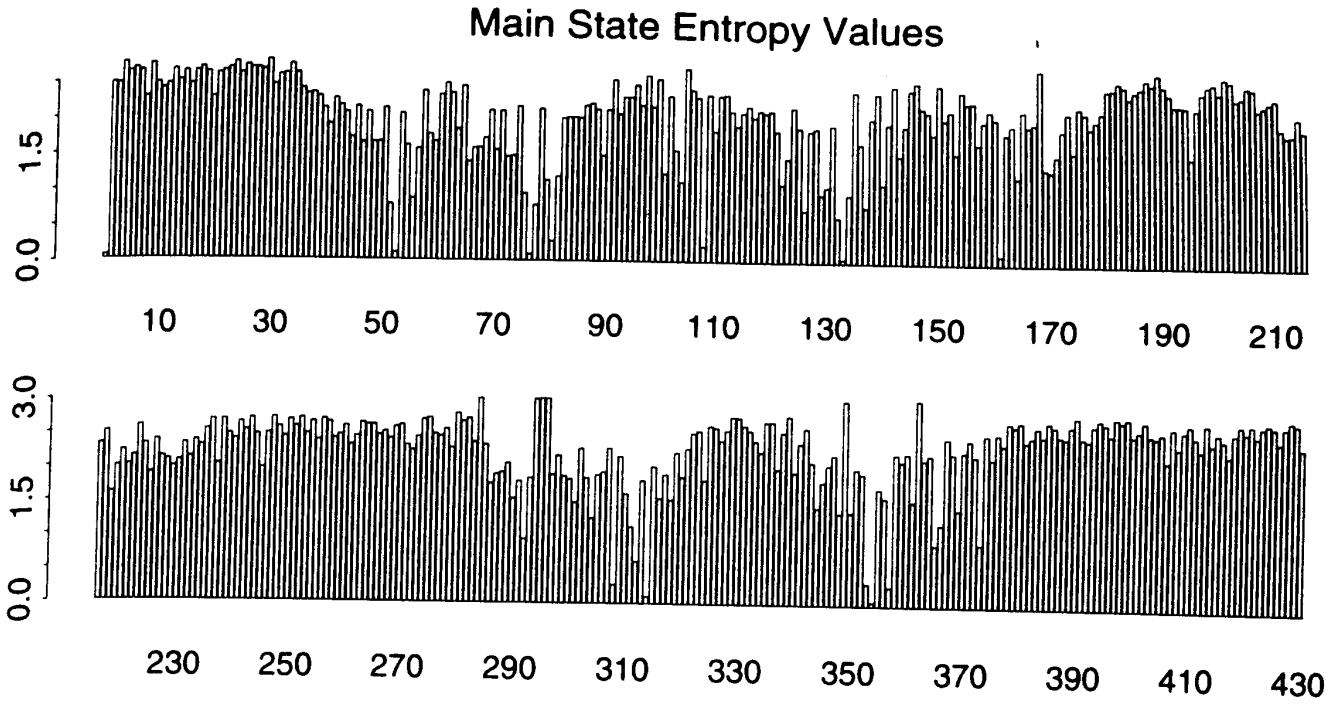


Figure 8.1: Entropy Profile of the Emission Probability Distributions Associated with the Main States of the HMM After 12 Cycles of Training.

GPCR Analysis (Cont'd)

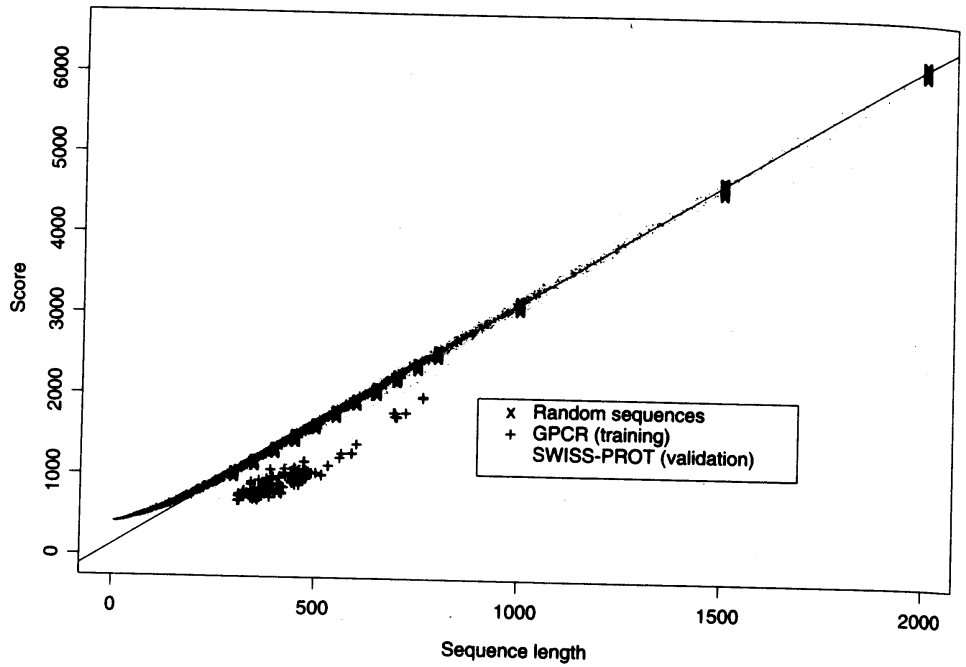


Figure 8.2: Scores (Negative Log-likelihoods of Optimal Viterbi Paths). Represented sequences consist of 142 GPCR training sequences, all sequences from the SWISS-PROT database of length less than or equal to 2000, and 220 randomly generated sequences with same average composition as the GPCRs of length 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800 (20 at each length). The regression line was obtained from the 220 random sequences. The horizontal distances in the histogram correspond to normalized scores (6).

Applications of HMM for GPCR

- Bacteriorhodopsin
  - Transmembrane protein with 7 domains
  - But it is not a GPCR
  - Compute score and discover that it is close to the regression line. Hence not a GPCR.
- Thyrotropin receptor precursors
  - All have long initial loop on INSERT STATE 20.
  - Also clustering possible based on distance to regression line.

HMMs – Advantages

- Sound statistical foundations
- Efficient learning algorithms
- Consistent treatment for insert/delete penalties for alignments in the form of locally learnable probabilities
- Capable of handling inputs of variable length
- Can be built in a modular & hierarchical fashion; can be combined into libraries.
- Wide variety of applications: Multiple Alignment, Data mining & classification, Structural Analysis, Pattern discovery, Gene prediction.

HMMs – Disadvantages

- Large # of parameters.
- Cannot express dependencies & correlations between hidden states.

References

- Krogh, Brown, Mian, Sjolander, Haussler, J. Mol. Biol. 235:1501-1531, 1994
- Gribskov, Luthy, Eisenberg, Meth. Enzymol. 183:146-159, 1995
- Gribskov, McLachlan, Eisenberg, Proc Natl. Acad. Sci. 84:4355-4358, 1996.