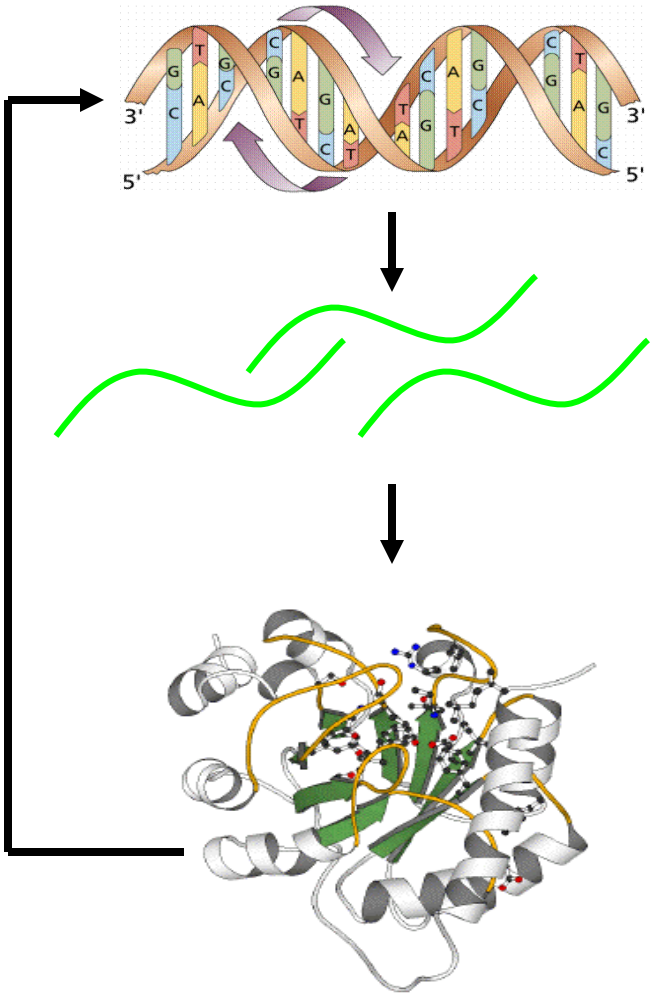
10-601
Machine Learning

Computational biology: Sequence alignment and profile HMMs

Central dogma



DNA

transcription

mRNA

translation

Protein

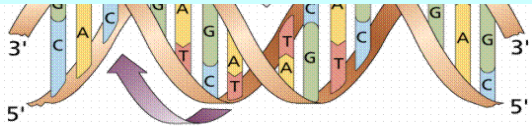
CCTGAGCCAACTATTGATGAA

CCUGAGCCAACUAUUGAUGAA

PEPTIDE

Central dogma

Can be measured using sequencing techniques



DNA

CCTGAGCCAACTATTGATGAA

transcription

Can be measured using microarrays

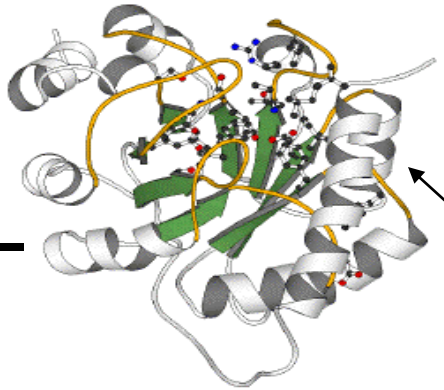
mRNA

CCUGAGCCAACUAUUGAUGAA

translation

Protein

PEPTIDE

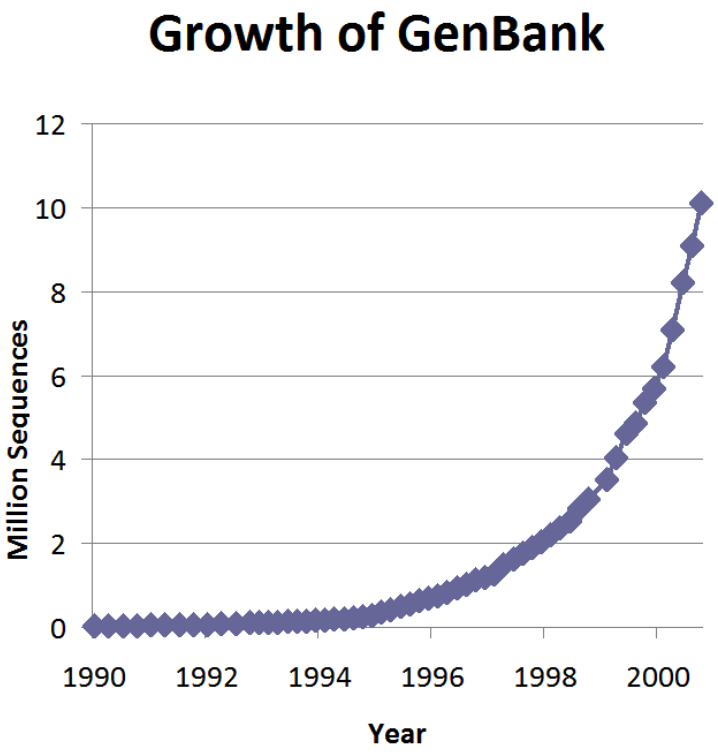


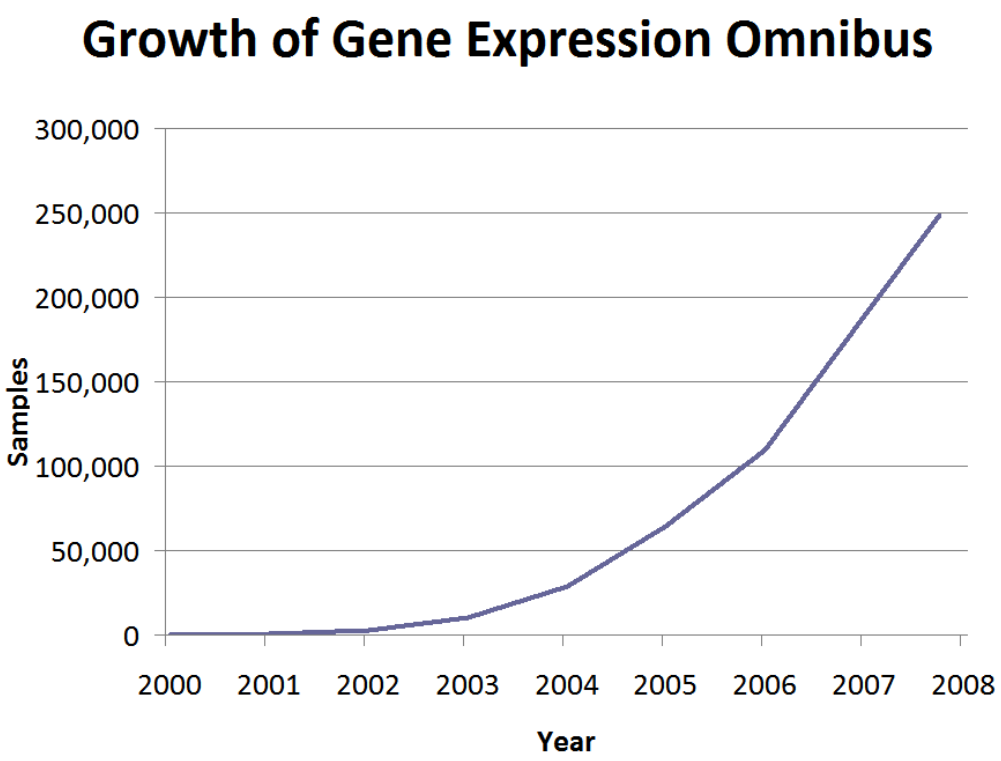
Can be measured using mass spectrometry

Comparison of Different Organisms

	Genome size	Num. of genes
E. coli	$.05*10^8$	4,200
Yeast	$.15*10^8$	6,000
Worm	$1*10^8$	18,400
Fly	$1.8*10^8$	13,600
Human	$30*10^8$	25,000
Plant	$1.3*10^8$	25,000

Growth in biological data



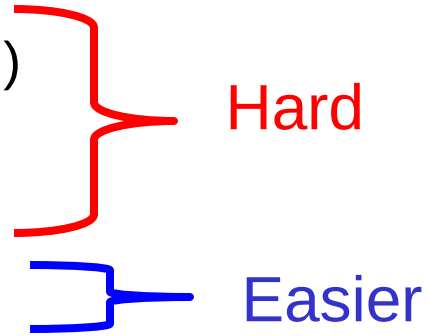


Lu et al *Bioinformatics* 2009

Assigning function to proteins

- One of the main goals of molecular (and computational) biology.
- There are 25000 human genes and the vast majority of their functions is still unknown
- Several ways to determine function

  - Direct experiments (knockout, overexpression)
  - Interacting partners
  - 3D structures
  - Sequence homology



Function from sequence homology

- We have a query gene: ACTGGTGTACCGAT
- Given a database containing genes with known function, our goal is to find similar genes from this database (possibly in another organism)
- When we find such gene we predict the function of the query gene to be similar to the resulting database gene
- Problems

  - How do we determine similarity?

Sequence analysis techniques

- A major area of research within computational biology.
- Initially, based on deterministic or heuristic alignment methods
- More recently, based on probabilistic inference methods

Sequence analysis

- Traditional

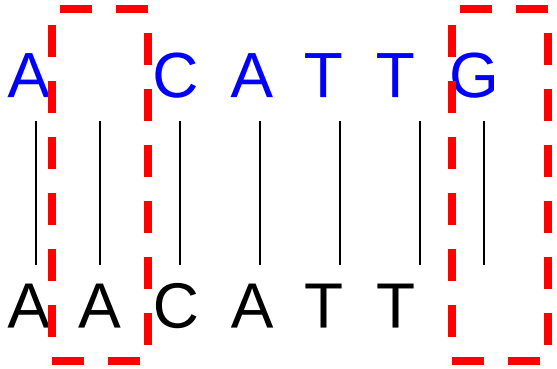
  - Dynamic programming

- Probabilsitic

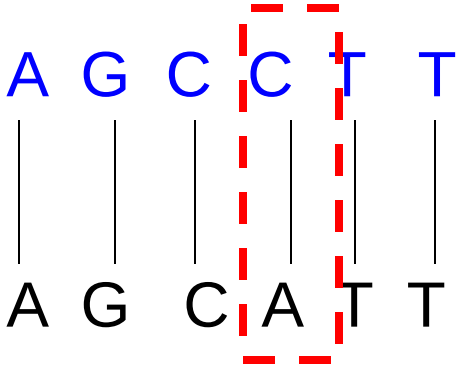
  - Profile HMMs

Pairwise sequence alignment

ACATTG
AACATT

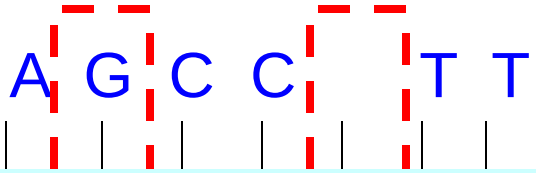


AGCCTT
AGCATT



Pairwise sequence alignment

AGCCTT
ACCATT



- We cannot expect the alignments to be perfect.
- Major reasons include insertion, deletion and substitutions.
- We need to allow gaps in the resulting alignment.

Scoring Alignments

- Alignments can be scored by comparing the resulting alignment to a background (random) model.

Independent

$$P(x, y \mid I) = \prod_i q_{x_i} \prod_j q_{x_j}$$

Related

$$P(x, y \mid M) = \prod_i p_{x_i y_i}$$

Score for alignment:

$$S = \sum_i s(x_i, y_i)$$

where:

$$s(a, b) = \log\left(\frac{p_{a,b}}{q_a q_b}\right)$$

Can be computed for each pair of letters

Scoring Alignments

- Alignments can be scored by comparing the resulting alignment to a background (random) model.

In other words, we are trying to find an alignment that maximizes the likelihood ratio of the aligned pair compared to the background model

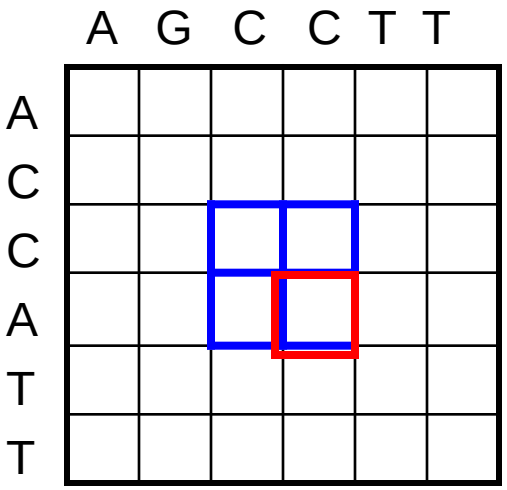
Score for alignment: $S = \sum_i s(x_i, y_i)$

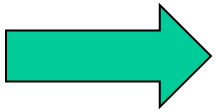
where: $s(a,b) = \log\left(\frac{p_{a,b}}{q_a q_b}\right)$

Computing optimal alignment: The Needham-Wuncsh algorithm

$$F(i,j) = \max \begin{cases} F(i-1,j-1)+s(x_i,x_j) \\ F(i-1,j)+d \\ F(i,j-1)+d \end{cases}$$

d is a penalty for a gap





F(i-1,j-1)	*F(i-1,j)*
F(i,j-1)	*F(i,j)*

Example

Assume a simple model where *S(a,b) = 1* if *a=b* and -5 otherwise.

Also, assume that d = -1

		A	G	C	C	T	T
	0	-1	-2	-3	-4	-5	-6
A	-1						
C	-2						
C	-3						
A	-4						
T	-5						
T	-6						

Example

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		A	G	C	C	T	T
	0	-1	-2	-3	-4	-5	-6
A	-1	1					
C	-2						
C	-3						
A	-4						
T	-5						
T	-6						

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		A	G	C	C	T	T
	0	-1	-2	-3	-4	-5	-6
A	-1	1	0				
C	-2	0					
C	-3						
A	-4						
T	-5						
T	-6						

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C	-2	0	-1				
C	-3	-1					
A	-4	-2					
T	-5	-3					
T	-6	-4					

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C	-2	0	-1	1	0	-1	-2
C	-3	-1	-2	0	2	1	0
A	-4	-2	-3	-1	1	0	-1
T	-5	-3	-4	-2	0	2	1
T	-6	-4	-5	-3	-1	1	3

Example

Assume a simple model where *S(a,b) = 1* if *a=b* and -5 otherwise.

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		A	G	C	C	T	T
	0	-1	-2	-3	-4	-5	-6
A	-1	1	0	-1	-2	-3	-4
C	-2	0	-1	1	0	-1	-2
C	-3	-1	-2	0	2	1	0
A	-4	-2	-3	-1	1	0	-1
T	-5	-3	-4	-2	0	2	1
T	-6	-4	-5	-3	-1	1	**3**

Example

Assume a simple model where *S(a,b) = 1* if *a=b* and -5 otherwise.

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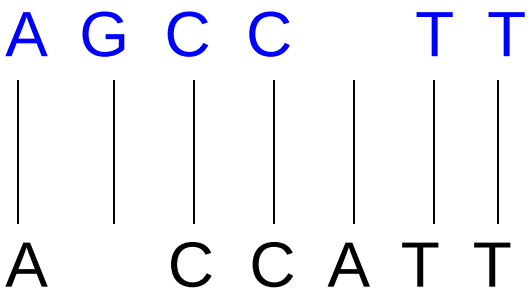


		A	G	C	C	T	T
	0	-1	-2	-3	-4	-5	-6
A	-1	1	0	-1	-2	-3	-4
C	-2	0	-1	1	0	-1	-2
C	-3	-1	-2	0	2	1	0
A	-4	-2	-3	-1	1	0	-1
T	-5	-3	-4	-2	0	2	1
T	-6	-4	-5	-3	-1	1	3

Running time

- The running time of an alignment algorithms if $O(n^2)$
- This doesn't sound too bad, or is it?

- The time requirement for doing global sequence alignment is too high in many cases.

- Consider a database with tens of thousands of sequences. Looking through all these sequences for the best alignment is too time consuming.

- In many cases, a much faster heuristic approach can achieve equally good results.

Sequence analysis

- Traditional
  - Dynamic programming √
- Probabilsitic
  - Profile HMMs

Protein families

- Proteins can be classified into families (and further into sub families etc.)
- A specific family includes proteins with similar high level functions
- For example:

  - Transcription factors
  - Receptors
  - Etc.

Family assignment is an important first step towards function prediction

Methods for Characterizing a Protein Family

- Objective: Given a number of related sequences, encapsulate what they have in common in such a way that we can recognize other members of the family.
- Some standard methods for characterization:
  - Multiple Alignments
  - Regular Expressions
  - Consensus Sequences
  - Hidden Markov Models

Multiple Alignment Process

- Process of aligning three or more sequences with each other
- We can determine such alignment by generalizing the algorithm to align two sequences
- Running time exponential in the number of sequences

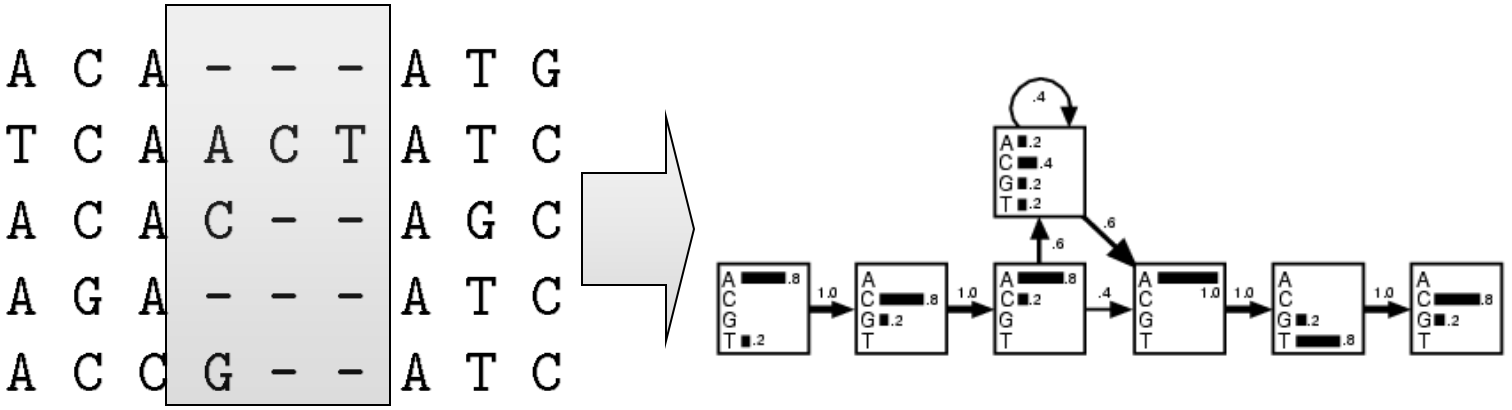
```
A C A - - - A T G
T C A A C T A T C
A C A C - - A G C
A G A - - - A T C
A C C G - - A T C
```

Training a HMM from an existing alignment

- Start with a predetermined number of states accounting for matches, insertions and deletions.
- For each position in the model, assign a column in the multiple alignment that is relatively conserved.
- Emission probabilities are set according to amino acid counts in columns.
- Transition probabilities are set according to how many sequences make use of a given delete or insert state.

MLE estimates

Remember the simple example



- Chose six positions in model.
- Highlighted area was selected to be modeled by an insert due to variability.
- Can also do neat tricks for picking length of model, such as model pruning.

So… what do we do with a model?

- Given a query protein:

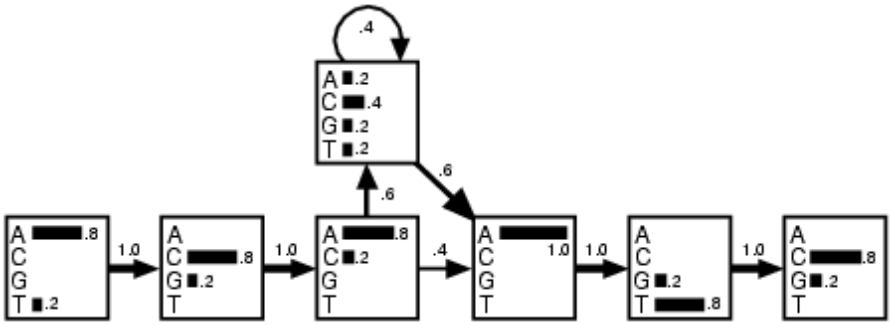
  - Design statistical tests to determine how likely it is to get this score from a random (gene) sequence

  - Use several protein family models for classifying new proteins, assign protein to most highly scoring family.

Choosing the best model: Aligning sequences to a models

- Compute the likelihood of the best set of states for this sequence
- We know how to do this: The Viterbi algortthm
- Time: O(N*M)

Scoring our simple HMM




```
A C A - - - A T G
T C A A C T A T C
A C A C - - A G C
A G A - - - A T C
A C C G - - A T C
```

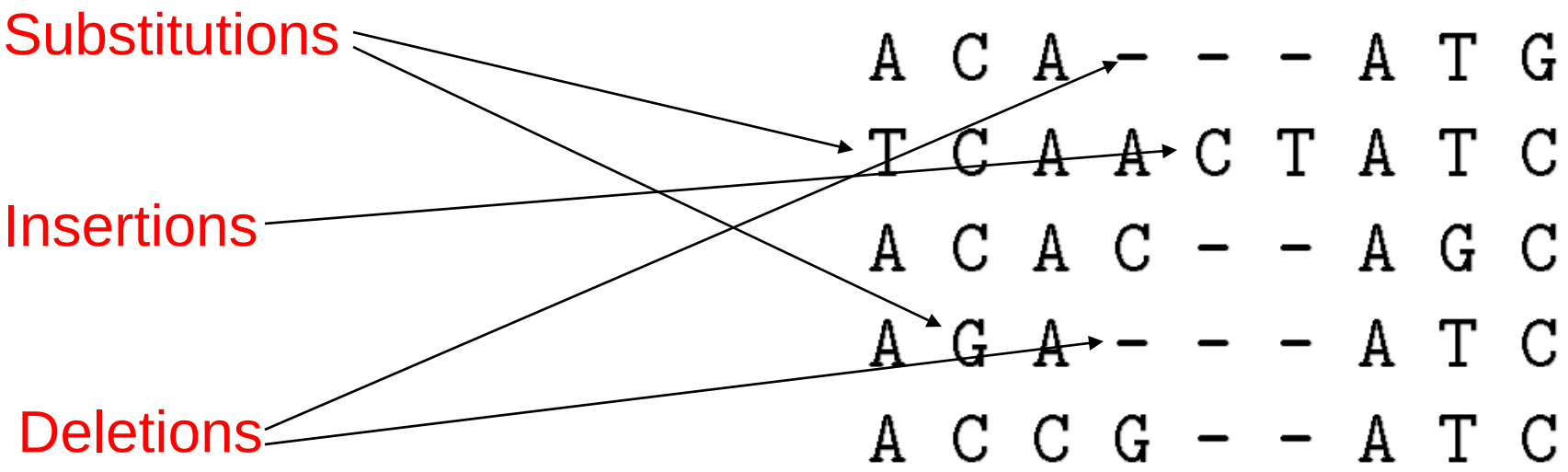
- #1 - “T G C T A G G” *vrs:* #2 - “A C A C A T C”
  - HMM:

#1 = Score of -0.97 #2 Score of 6.7 (Log odds)

Training from unaligned sequences

- Baum-Welch algorithm
  - Start with a model whose length matches the average length of the sequences and with random emission and transition probabilities.
  - Align all the sequences to the model.
  - Use the alignment to alter the emission and transition probabilities
  - Repeat. Continue until the model stops changing
- By-product: It produces a multiple alignment

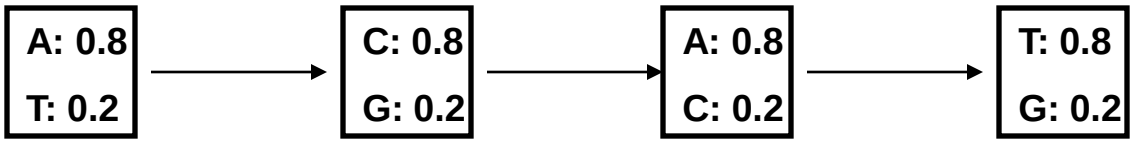
Multiple Alignment: Reasons for differences



Designing HMMs: Consensus (match) states

We first include states to output the consensus sequence

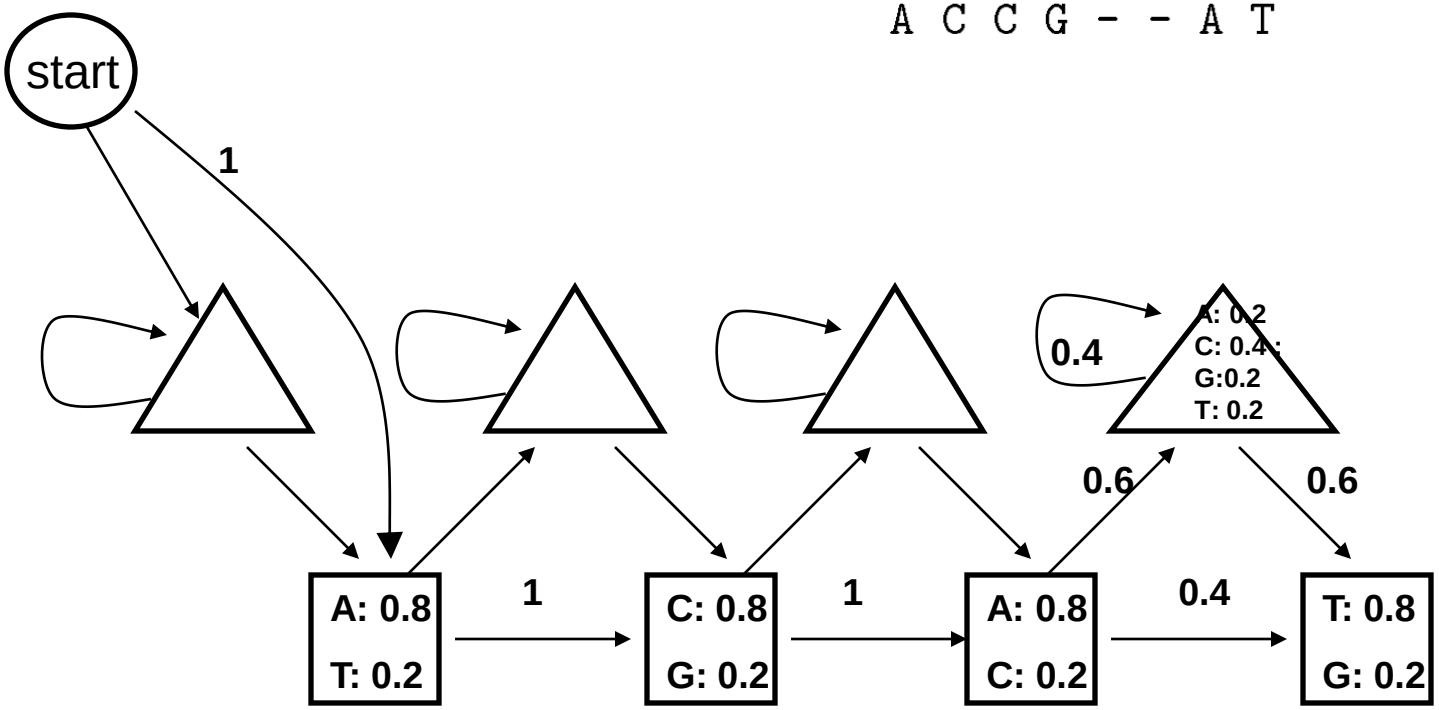
```
A C A - - - A T
T C A A C T A T
A C A C - - A G
A G A - - - A T
A C C G - - A T
```



Designing HMMs: Insertions

We next add states to allow insertions

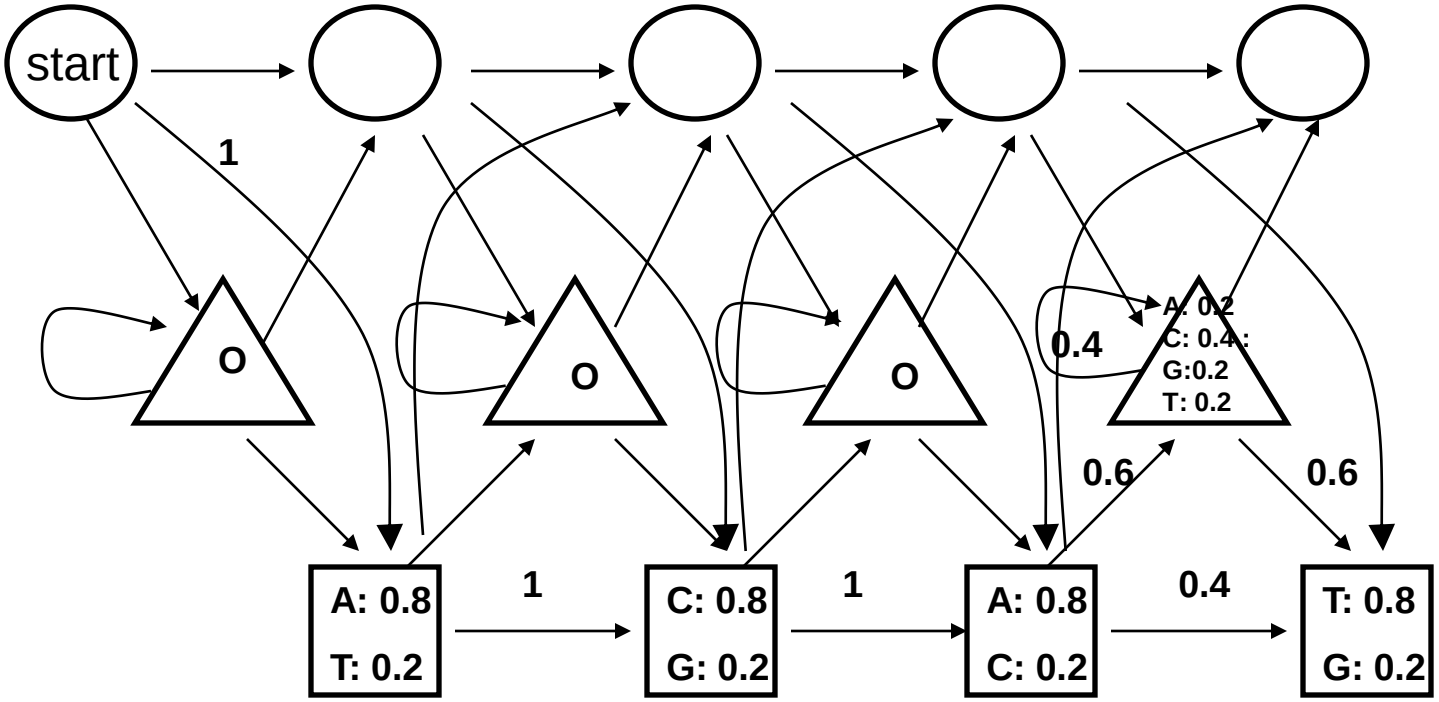
```
A C A - - - A T
T C A A C T A T
A C A C - - A G
A G A - - - A T
A C C G - - A T
```



Designing HMMs: Deletions

Finally we add states with **no** output to allow for deletions

```
A C A - - - A T
T C A A C T A T
A C A C - - A G
A G A - - - A T
A C C G - - A T
```



Training from unaligned continued

- Advantages:
  - You take full advantage of the expressiveness of your HMM.
  - You might not have a multiple alignment on hand.
- Disadvantages:
  - HMM training methods are local optimizers, you may not get the best alignment or the best model unless you're very careful.
  - Can be alleviated by starting from a logical model instead of a random one.

Summary

- Initial methods for sequence alignment relied on combinatorial and dynamic programming methods.
- These methods do not generalize well for multiple sequence alignment and for searching large databases.
- State of the art methods rely on AI techniques, primarily variants of HMMs to overcome this problem.