Comparative Gene Finding

BMI/CS 776

www.biostat.wisc.edu/bmi776/

Spring 2009

Mark Craven

craven@biostat.wisc.edu

Why use comparative methods?

- genes are among the most conserved elements in the genome

  ⇒use conservation to help infer locations of genes

- some signals associated with genes are short and occur frequently

  ⇒use conservation to eliminate from consideration false candidate sites

TWINSCAN Overview

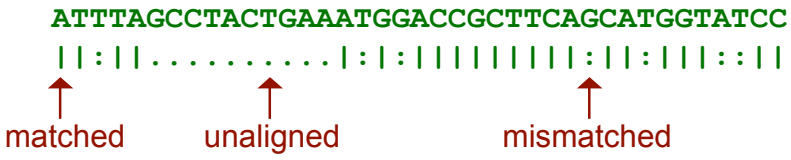
Korf et al., 2001

- prediction with TWINSCAN
  given: a sequence to be parsed, x
  using BLAST, construct a conservation sequence, c
  have HMM simultaneously parse (using Viterbi) x and c

- training with TWINSCAN
  given: set of training sequences X
  for each x in X
    construct a conservation sequence c for x
    infer emission parameters for both x and c

Conservation Sequences in TWINSCAN

- before processing a given sequence, TWINSCAN first computes a corresponding *conservation sequence*



Given: a sequence of length n, a set of aligned BLAST HSPs
ConSeq[1...n] = **`unaligned`**
sort BLAST HSPs by alignment score
for i = 1 to n
  for each BLAST HSP H (from best to worst)
    if H extends to position i
      if *ConSeq*[i] == **`unaligned`**
        ConSeq[i] = *H*[i]

Conservation Sequence Example

given sequence

```
ATTTAGCCTACTGAAATGGACCGCTTCAGCATGGTATCC
```

significant BLAST matches ordered from best to worst

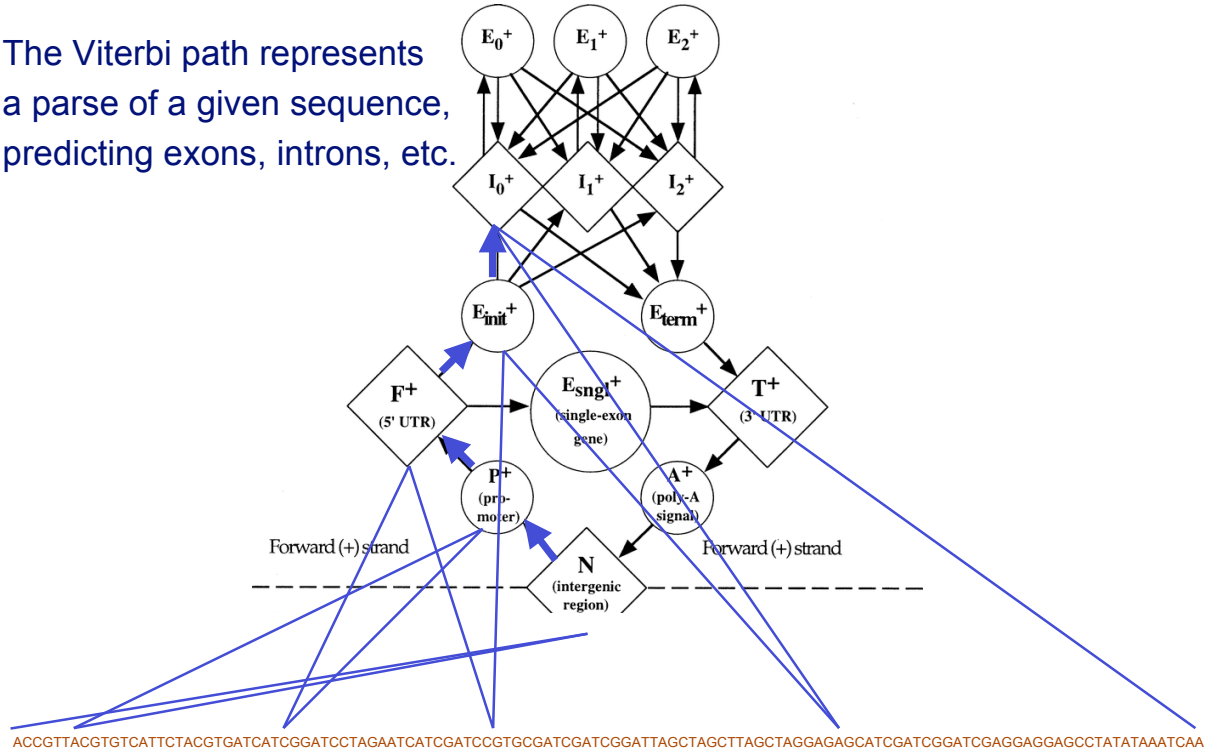
```
ATGGACCGCTTCAGC
|:|:|||||||||:|
ACGCACCGCTTCATC

                AGCATGGTATCC
                ||:|:|||::||
                AGAAGGGTCACC

ATTTA
||:||
ATCTA
```

resulting conservation sequence

```
ATTTAGCCTACTGAAATGGACCGCTTCAGCATGGTATCC
||:||..........|:|:|||||||||:||:|||::||
```

Parsing a DNA Sequence

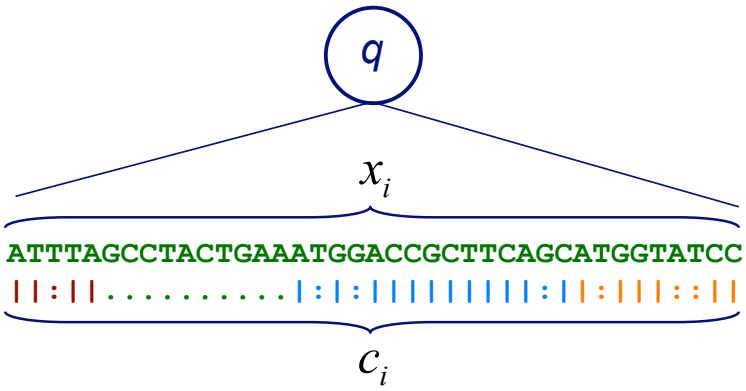
The Viterbi path represents a parse of a given sequence, predicting exons, introns, etc.



Modeling Sequences in TWINSCAN

- each state in "emits" two sequences
  - the given DNA sequence, x
  - the conservation sequence, c
- it treats them as conditionally independent given the state

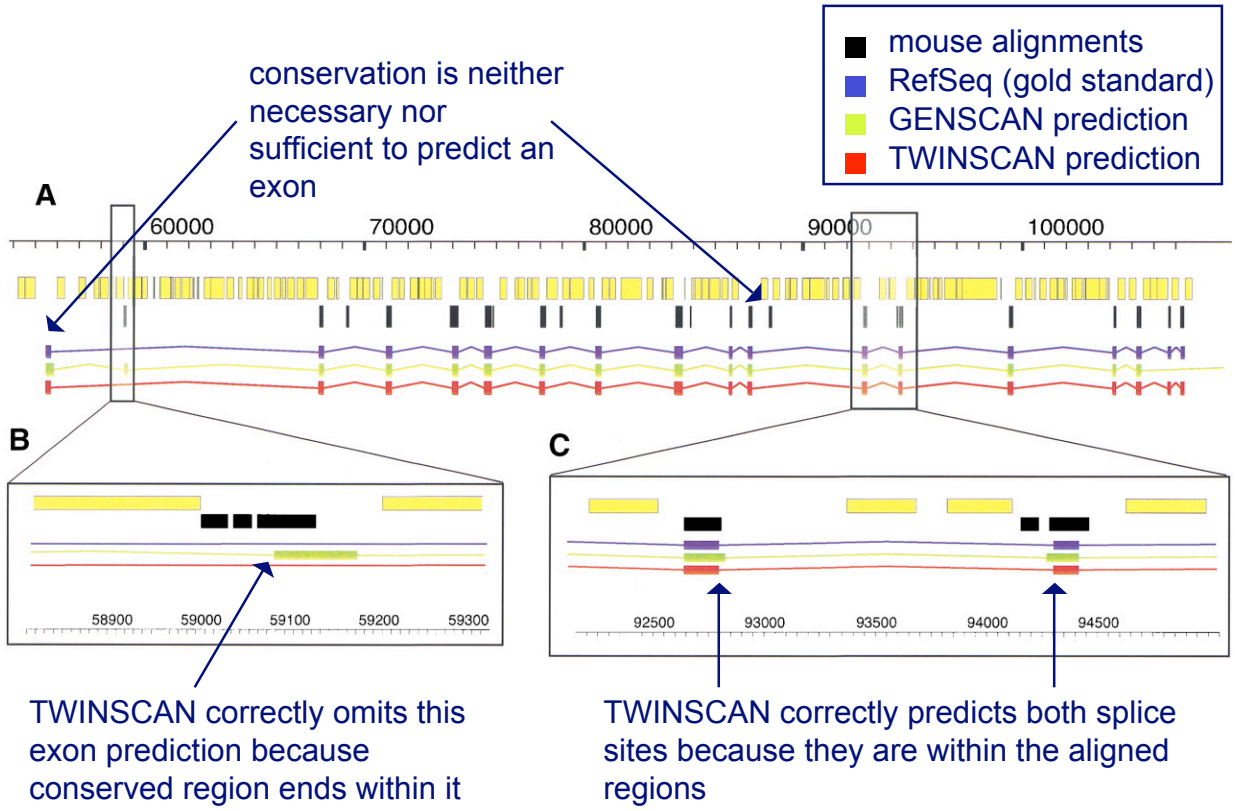
$$\Pr(x_i, c_i \mid q) = \Pr(d_i \mid q)\ \Pr(x_i \mid q, d_i)\ \Pr(c_i \mid q, d_i)$$



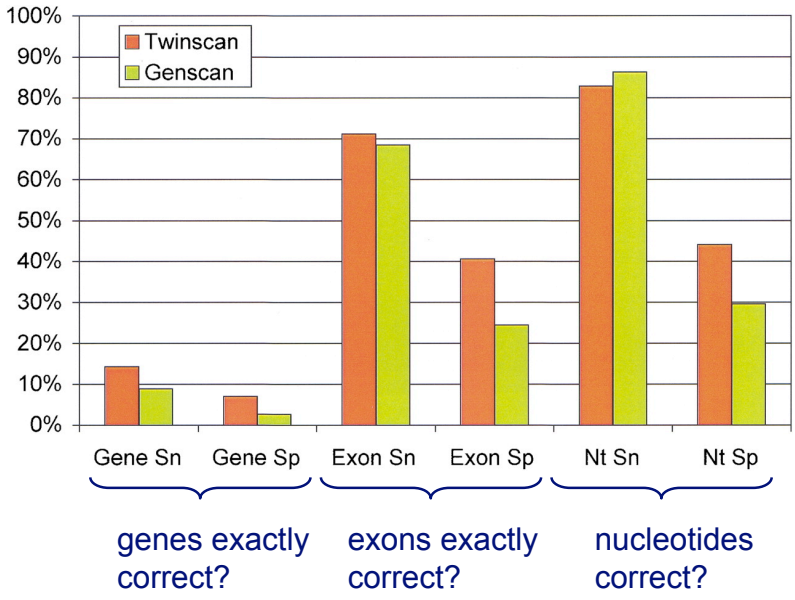
Modeling Sequences in TWINSCAN

- conservation sequence is treated just as a string over a 3-character alphabet (| , : , .)
- conservation sequence emissions modeled by
  - position-specific 2nd-order chains for splice sites
  - homogeneous 5th-order Markov chains for other states
- like GENSCAN, based on hidden semi-Markov models
- algorithms for learning, inference same as GENSCAN

TWINSCAN vs. GENSCAN



GENSCAN vs. TWINSCAN: Empirical Comparison



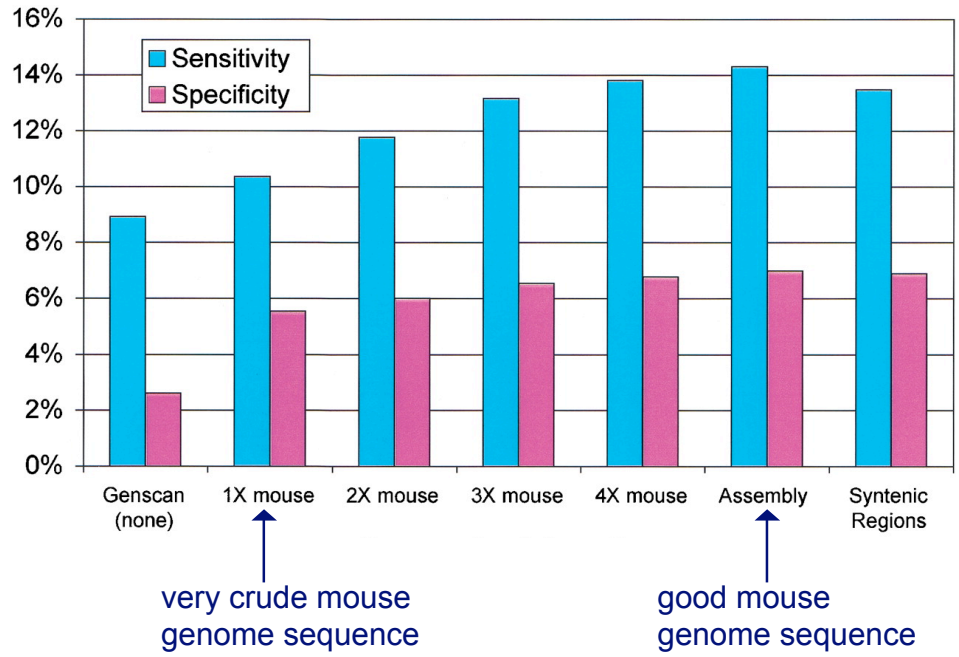
$$\text{sensitivity (Sn)} = \frac{TP}{TP + FN}$$

$$\text{specificity (Sp)} = \frac{TP}{TP + FP}$$

note: the definition of *specificity* here is somewhat nonstandard; it's the same as *precision*

Figure from Flicek et al., *Genome Research*, 2003

Accuracy of TWINSCAN as a Function of Sequence Coverage



SLAM Overview

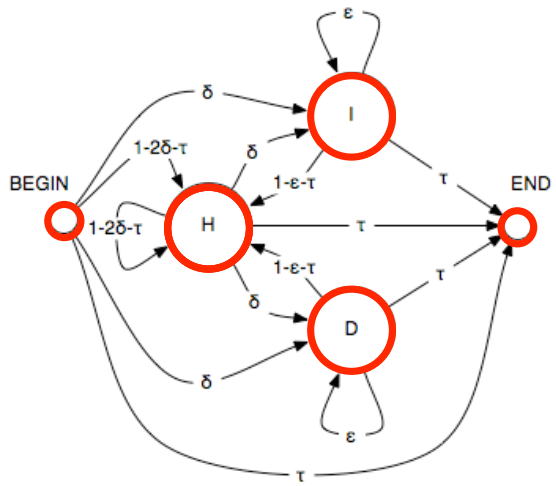
Pachter et al., 2001

- prediction with SLAM
  given: a pair of sequences to be parsed, x and y
  find approximate alignment of x and y
  run constrained Viterbi to have HMM simultaneously parse and align x and y

- training with SLAM
  given: a set of aligned pairs of training sequences X
  for each x, y in X
  infer emission/alignment parameters for both x and y

Pair Hidden Markov Models

- each non-silent state emits one or a pair of characters



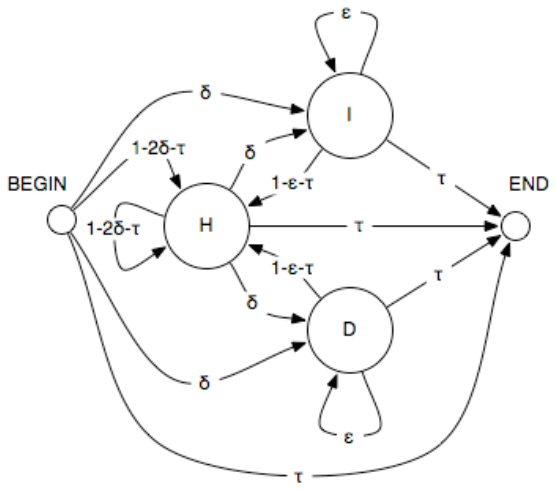
hidden: B H H I I H D H E

observed: A A G C G C
A T G T C

sequence 1: AAGCGC
sequence 2: ATGTC

Transition Probabilities

- probabilities of moving between states at each step



state i+1

state i	B	H	I	D	E
B		1-2δ-τ	δ	δ	τ
H		1-2δ-τ	δ	δ	τ
I		1-ϵ-τ	ϵ		τ
D		1-ϵ-τ		ϵ	τ
E					

Emission Probabilities

- Begin (B), and End (E) states silent
- possible emission probabilities for H, I, D:

Deletion (D)

A	0.3
C	0.2
G	0.3
T	0.2

single character

Insertion (I)

A	0.1
C	0.4
G	0.4
T	0.1

single character

Homology (H)

	A	C	G	T
A	0.13	0.03	0.06	0.03
C	0.03	0.13	0.03	0.06
G	0.06	0.03	0.13	0.03
T	0.03	0.06	0.03	0.13

pairs of characters

PHMM Paths = Alignments

Observed sequences

x: AAGCGC

y: ATGTC

Possible path

B H H I I H D H E

A A G C G - C

A T - - G T C

PHMM Viterbi

- probability of most likely sequence of hidden states generating length i prefix of x and length j prefix of y, with the last state being:

H
$$v^H(i,j) = e_H(x_i, y_j) \max \begin{cases} v^H(i-1,j-1)t_{HH}, \\ v^I(i-1,j-1)t_{IH}, \\ v^D(i-1,j-1)t_{DH} \end{cases}$$

I
$$v^I(i,j) = e_I(y_j) \max \begin{cases} v^H(i,j-1)t_{HI}, \\ v^I(i,j-1)t_{II}, \\ v^D(i,j-1)t_{DI} \end{cases}$$

D
$$v^D(i,j) = e_D(x_i) \max \begin{cases} v^H(i-1,j)t_{HD}, \\ v^I(i-1,j)t_{ID}, \\ v^D(i-1,j)t_{DD} \end{cases}$$

- note that the recurrence relations here allow $I \rightarrow D$ and $D \rightarrow I$ transitions

PHMM Alignment

- calculate probability of most likely alignment

$$v^E(m,n) = max(v^M(m,n)t_{HE}, v^I(m,n)t_{IE}, v^D(m,n)t_{DE})$$

- traceback, as in Needleman-Wunsch, to obtain sequence of state states giving highest probability

HIDHHDDIIHH...

Correspondence with NW

- NW values ≈ logarithms of PHMM Viterbi values

$$\log v^H(i,j) = \log e_H(x_i, y_j) + \max \begin{cases} \log v^H(i-1,j-1) + \log t_{HH}, \\ \log v^I(i-1,j-1) + \log t_{IH}, \\ \log v^D(i-1,j-1) + \log t_{DH} \end{cases}$$

$$\log v^I(i,j) = \log e_I(y_j) + \max \begin{cases} \log v^H(i,j-1) + \log t_{HI}, \\ \log v^I(i,j-1) + \log t_{II}, \\ \log v^D(i,j-1) + \log t_{DI} \end{cases}$$

$$\log v^D(i,j) = \log e_D(x_i) + \max \begin{cases} \log v^H(i-1,j) + \log t_{HD}, \\ \log v^I(i-1,j) + \log t_{ID}, \\ \log v^D(i-1,j) + \log t_{DD} \end{cases}$$

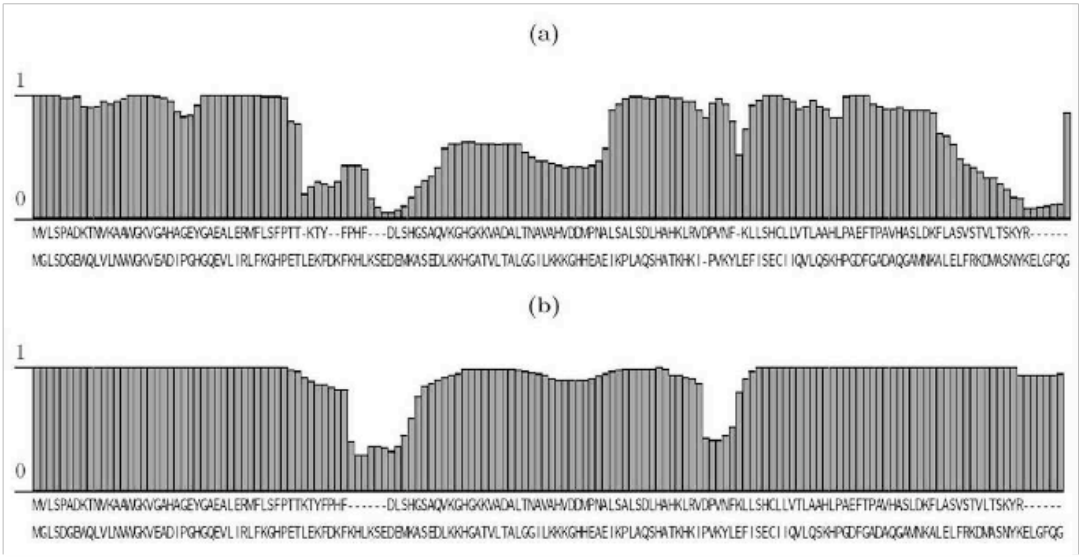
Posterior Probabilities

- there are similar recurrences for the *Forward* and *Backward* values
- from the *forward* and *backward* values, we can calculate the posterior probability of the event that the path passes through a certain state S, after generating length i and j prefixes

Uses for Posterior Probabilities

- suboptimal sampling of alignments
- posterior probability of pairs of residues being homologous (aligned to each other)
- posterior probability of a residue being gapped
- training model parameters (EM)

Posterior Probabilities



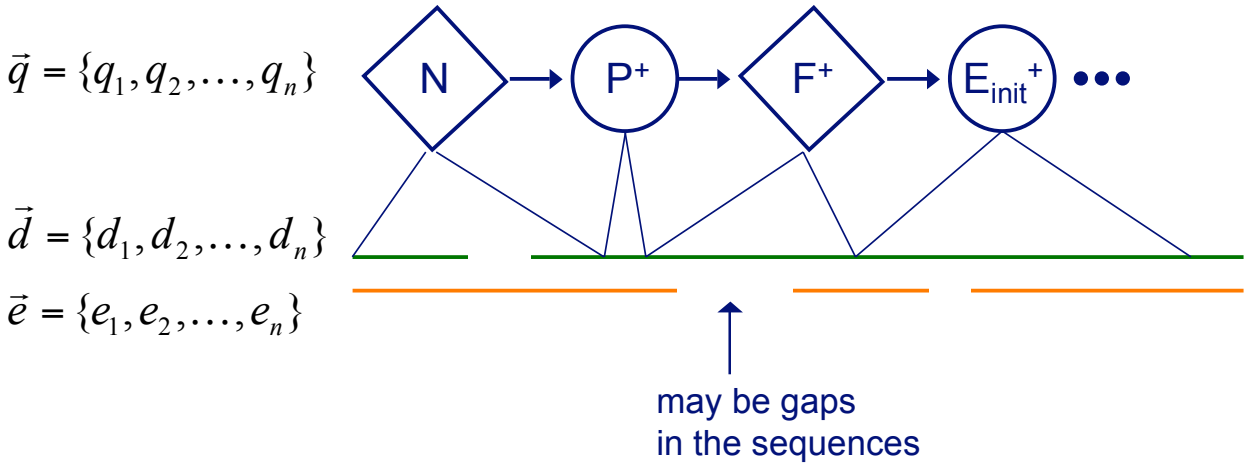
Plot posterior probability of each alignment column

Parameter Training

- supervised training
  - given: sequences and correct alignments
  - do: calculate parameter values that maximize joint likelihood of sequences and alignments
- unsupervised training
  - given: sequence pairs, but *no* alignments
  - do: calculate parameter values that maximize marginal likelihood of sequences (sum over all possible alignments)

Generalized Pair HMMs

- represent a parse π, as a sequence of states and a sequence of associated lengths for <u>each</u> input sequence



Generalized Pair HMMs

- representing a parse π, as a sequence of states and associated lengths (durations)

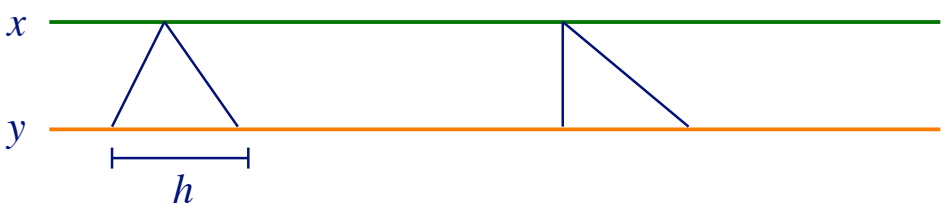
$$\vec{q} = \{q_1, q_2, \ldots, q_n\}$$
$$\vec{d} = \{d_1, d_2, \ldots, d_n\} \qquad \vec{e} = \{e_1, e_2, \ldots, e_n\}$$

- the joint probability of generating parse π and sequences x and y

$$P(x, y, \pi) = a_{start,1} P(d_1, e_1 \mid q_1) P(x_1, y_1 \mid q_1, d_1, e_1) \times \prod_{k=2}^{n} a_{k-1,k} P(d_k, e_k \mid q_k) P(x_k, y_k \mid q_k, d_k, e_k)$$

Prediction in SLAM

- could find alignment and gene predictions by running Viterbi
- to make it more efficient
  - find an approximate alignment (using a fast anchor-based approach)
  - each base in x constrained to align to a window of size h in y



- analogous to banded alignment methods

GENSCAN, TWINSCAN, & SLAM

	Nucleotide level			Exon level				
Test set	SN	SP	AC	SN	SP	(SN+SP)/2	ME	WE
The ROSETTA set								
ROSETTA	0.935	0.978	0.949	0.833	0.829	0.831	0.048	0.047
SGP-1	0.940	0.960	0.940	0.700	0.760	0.730	0.120	0.040
SLAM	0.951	0.981	0.960	0.783	0.755	0.769	0.038	0.057
TWINSCAN.p	0.960	0.941	0.940	0.855	0.824	0.840	0.045	0.081
TWINSCAN	0.984	0.889	0.923	0.839	0.767	0.803	0.034	0.118
GENSCAN	0.975	0.908	0.929	0.817	0.770	0.793	0.057	0.107
HoxA								
SLAM	0.852	0.896	0.864	0.727	0.533	0.630	0.000	0.333
TWINSCAN.p	0.976	0.829	0.896	0.773	0.531	0.652	0.000	0.312
TWINSCAN	0.949	0.511	0.704	0.591	0.173	0.382	0.000	0.707
SGP-2	0.640	0.637	0.619	0.409	0.173	0.291	0.091	0.596
GENSCAN	0.932	0.687	0.796	0.545	0.235	0.390	0.000	0.569
Elastin								
SLAM	0.876	0.981	0.926	0.802	0.859	0.831	0.121	0.059
TWINSCAN.p	0.942	0.950	0.945	0.879	0.889	0.884	0.066	0.056
TWINSCAN	0.933	0.877	0.903	0.835	0.826	0.831	0.110	0.120
SGP-2	0.755	0.998	0.873	0.593	0.900	0.291	0.352	0.017
GENSCAN	0.947	0.766	0.852	0.835	0.731	0.783	0.121	0.231

The measures of sensitivity SN = TP/TP + FN and specificity SP = TP/TP + FP (where TP = true positives, TN = true negatives, FP = false positives and FN = false negatives) are shown at both the nucleotide and exon level. ME is entirely missed exons, WE is wrong exons, and the approximate correlation AC = 1/2 (TP/TP + FN + TP/TP + FP + TN/TN + FP + TN/TN + FN) − 1 summarizes the overall nucleotide sensitivity and specificity by one number. Within each of the three data sets the methods are divided into three classes: those operating on a syntenic DNA pair, those operating on a human sequence using as evidence matches against a database of mouse sequences, and a single-organism gene finder (GENSCAN).

TWINSCAN vs. SLAM

- both use multiple genomes to predict genes
- both use generalized HMMs
- TWINSCAN
  - takes as an input a genomic sequence, and a conservation sequence computed from an informant genome
  - models probability of both sequences; assumes they're conditionally independent given the state
  - predicts genes only in the genomic sequence
- SLAM
  - takes as input two genomic sequences
  - models joint probability of pairs of aligned sequences
  - can simultaneously predict genes and compute alignments

Probabilistic Sequence Models: Key Technical Ideas We've Covered

- problems with hidden state: EM and Gibbs sampling
- constraining hidden variables to account for different assumptions (e.g. OOPS vs. ZOOPS vs. MCM)
- tying parameters
- using background knowledge to bias model topology, parameters
- varying the order of a model: interpolated and back-off models
- representing arbitrary dependencies among positions: the MDD approach
- duration modeling
- semi-Markov (a.k.a generalized) models
- models that represent multiple sequences: conditionally independent outputs and pair HMMs