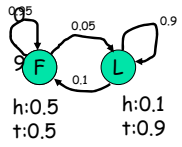
How to design an HMM for a new problem

- Architecture/topology design:
  - What are the states, observation symbols, and the topology of the state transition graph?
- Learning/Training:
  - Fully annotated or partially annotated training datasets
  - Parameter estimation by maximum likelihood or by EM
- Validation/Testing:
  - Fully annotated testing datasets
  - Performance evaluation (accuracy, specificity and sensitivity)

HMM model structure

- Duration modeling



What is the probability of staying with the fair coin for T time steps?

Duration modeling

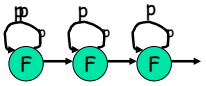
- The duration in state F follows an exponentially decaying distribution called a geometric distribution.

$$P(X = F^T) = (0.95)^{T-1}(0.05)$$

- This may be inappropriate for some applications.

Duration modeling

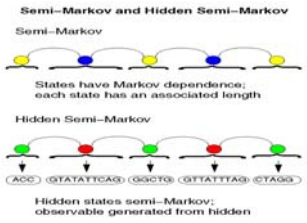
- To obtain non-geometric length distributions, we use an array of n F states, as follows:



$$P(|X| = L) = \binom{L-1}{n-1} p^{L-n}(1-p)^n$$

- Generated length distribution is a negative binomial.

Semi-Markov HMMs



Hidden Semi-Markov models

- Each state is associated with an explicit duration model of the form: P(|X|=L), where |X| is the length of the hidden state sequence in state X.

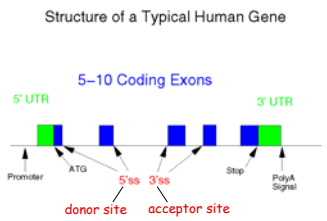
Genscan

- The Genscan HMM model
- Training Genscan
- Validating Genscan

Structure of a human gene

Exon-intron structure

Gene structure assumed by Genscan

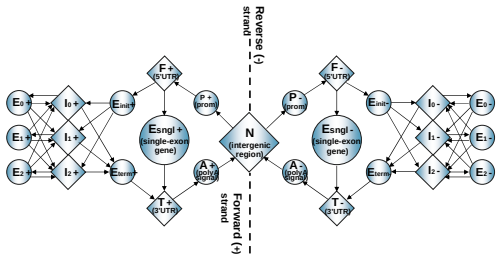


Genscan's architecture (1)

- HMM states for exons and introns in three different phases, single exon, 5' and 3' UTRs, promoter region, polyA site and intergenic region.
- Explicit length modeling of introns and exons.

("*Prediction of complete gene structures in human genomic DNA*"(1997) Burge and Karlin, *JMB* **268**, p. 86)

Genscan HMM



Genscan model components

- Vector of initial probabilities π
- State Transition probability Matrix T
- Set of length distributions $f_{Q(i)}$ conditional on state
- Sequence generating model P(s|q,d) conditional on state and length.

Isochore groups

Group	I	II	III	IV
C + G% range	<43	43-51	51-57	>57
Number of genes	65	115	99	101
Est. proportion single-exon genes	0.16	0.19	0.23	0.16
Codelen: single-exon genes (bp)	1130	1251	1304	1137
Codelen: multi-exon genes (bp)	902	908	1118	1165
Introns per multi-exon gene	5.1	4.9	5.5	5.6
Mean intron length (bp)	2069	1086	801	518
Est. mean integenic length (bp)	83000	36000	5400	2600

Initial probabilities

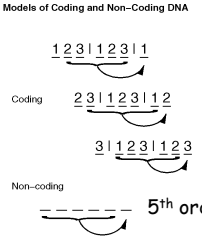
	I	II	III	IV
Intergenic (N)	0.892	0.867	0.54	0.418
Intron (I0+,I1+,I2+,I0-,I1-,I2-)	0.095	0.103	0.338	0.388
5' Untranslated region (F+, F-)	0.008	0.018	0.077	0.122
3' Untranslated region (T+, T-)	0.005	0.011	0.045	0.072

All other probabilities set to zero.

Transition probabilities

- Sure transitions are assigned probability 1.
- The others are set according to maximum likelihood values in training data.

Exon and intron models



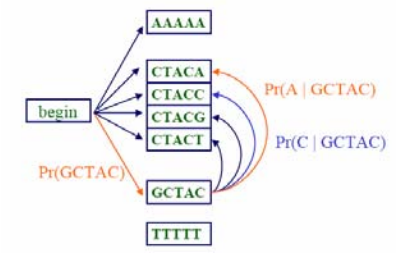
Phases of the exons

5th order inhomogeneous Markov model

5th order homogeneous Markov model :

$$P(o_t \mid o_{t-1}o_{t-2}o_{t-3}o_{t-4}o_{t-5})$$

A Fifth Order Markov Chain

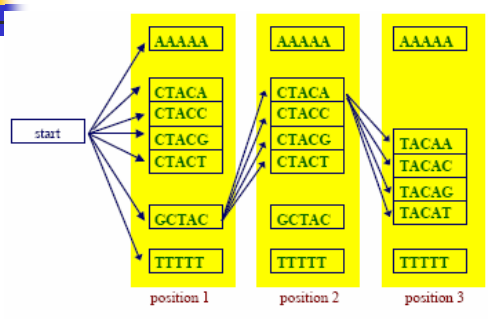


Inhomogenous Markov Chains

- In the Markov chain models we have considered so far, the probabilities do not depend on where we are in a given sequence
- In an *inhomogeneous* Markov model, we have different distributions at different positions in the sequence.

$$a^1_{x_1x_2}\, a^2_{x_2x_3}\, a^3_{x_3x_4}\, a^1_{x_4x_5}\, a^2_{x_5x_6}$$

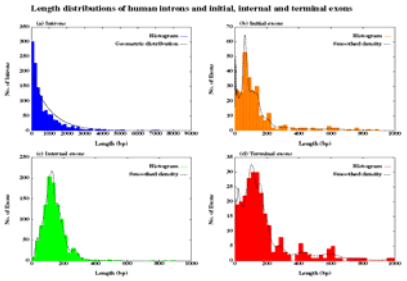
A Fifth Order Inhomogenous Markov Chain



Exon/intron/UTR model

- **Exons** -- inhomogeneous 3-periodic fifth order Markov model.
- **Introns** and **intergenic regions** - homogeneous 5th order Markov model
- **5'** and **3' UTRs** - homogeneous 5th order Markov model

Length distributions



Length distribution for introns

- No introns < 65bp. After that geometric (exponential) distribution.
- Substantial difference between different C+G groups.
- So, intron length is modeled as geometric distribution with different parameters of different C+G groups.

Exon length distribution model

- Exons are very important to model.
- Substantial differences in length distribution between initial, internal and terminal exons.
- No substantial difference between different C+G compositional groups.
- Exon length means considered between 50 and 300 bps.
- Account for phase (3*codons + phase)

Other length distributions

- 5' UTR -> Geometric with mean 769bp
- 3' UTR -> Geometric with mean 457bp

Genscan architecture (2)

- Weighted matrix and weighed arrays for acceptor splice site, polyA site and promoter region.
- Decision tree (maximal dependence decomposition) for donor sites.
- Different model parameters for regions with different GC content.

Signal models

- WMM (Weight Matrix Method)
  - $p_j(i)$ is probability of nucleotide j at position i.
  - Multiplicative.
- WAM (Weight Array Model)
  - Markov chains. $p_{j,k}(i-1,i)$ is probability of nucleotide k at position i conditional on nucleotide j at position i-1.
- MDD (Maximal Dependence Decomposition)

Weighted matrix

- Computed by measuring the frequency of every element of every position of the site (weight)

TACGAT
TATAAT
TATAAT
GATACT
TATGAT
TATGTT

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

- Score for any putative site is the sum of the matrix values (converted in probabilities) for that sequence (log-likelihood score)

Transcriptional and Translational Signals

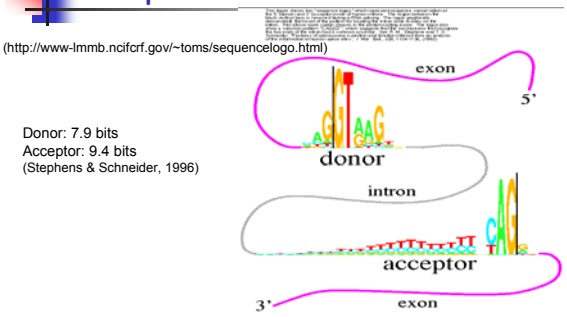
- PolyA signal
  - 6 base pairs WMM (AATAAA)
- Translation Initiation signal
  - 12 base pairs WMM (6 base pairs prior to start codon)
- Translation termination signal
  - 1 of 3 stop codons according to observed frequency
  - Next 3 nucleotides using WMM

Promotor model

- Promoters
  - 30% of them lack apparent TATA signal
  - So, split model:
    TATA containing promoter
    - Generated with probability 0.7
    - 15 bp TATA-box WMM and 8 bp cap site WMM
  - TATA-less
    - Generated with probability 0.3
    - Modeled as intergenic-null regions of 40bp

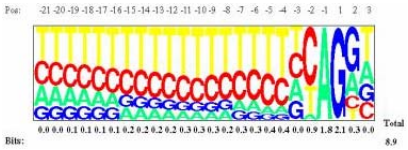
2. Splice Site Detection

(http://www-lmmb.ncifcrf.gov/~toms/sequencelogo.html)



Donor: 7.9 bits
Acceptor: 9.4 bits
(Stephens & Schneider, 1996)

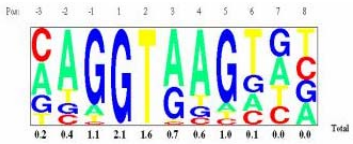
Acceptor splice site



Acceptor splice site model

- Consensus region from -20 to +3
- Windowed second-order WAM model (WWAM)

Donor splice site



Donor splice site model

- Consensus region -3 to +6 (3 on exon, 6 on intron)
- WMM or WAM not sufficient to model because of dependencies on non-adjacent nucleotides.

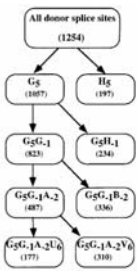
MDD algorithm

Absence of nucleotide G at position +5 implies a great consensus matching at position -1.

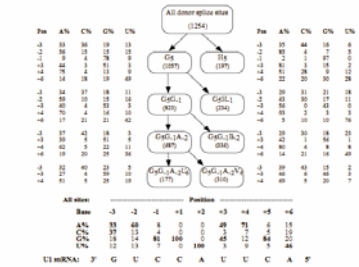
H = A/C/U

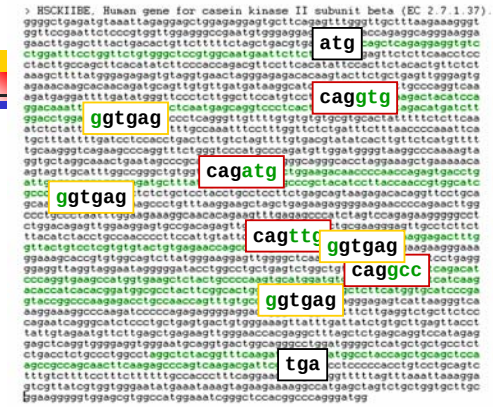
B=C/G/U

V=A/C/G



MDD algorithm





105

Exon emission models

- Inhomogeneous 3-periodic fifth order Markov model.
- Different model for C+G group I.
- Maintain phase.

Non-coding emission models

- For UTR, intergenic and intron regions,
  - Homogeneous fifth-order Markov model

Using Genscan for gene finding

- Model's goal is to generate "Optimal Parse"
- Parse (X) consists of
  - Ordered set of states = $\{s_1,s_2,\ldots,s_n\}$ where $s_i \in \{S_j / j=1 \text{ to } 27\}$
  - Associated lengths (durations) $(d) = \{d_1,d_2,\ldots,d_n\}$
  - It generates DNA sequence O of length $L = \Sigma_{i=1 \text{ to } n} d_i$.

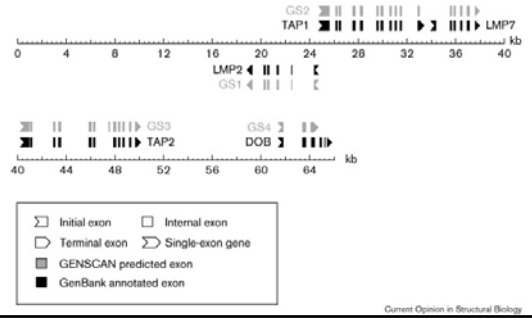
Running the model

- An initial state s_1 is chosen according to an initial distribution π on the states, i.e. $\pi_i = P(s_1=S_i)$
- A length distribution d_1 is generated conditional on $s_{1,i.e.}$ $f_{s1}(d_1)$
- A sequence segment s_1 of length d_1 is generated conditional of s_1 and d_1 i.e. $P(s_i|s_1,d_1)$
- Subsequent state s_2 is generated, conditional on s_1. First order Markov. $a_{ij} = P(s_{k+1}= S_j \ |s_k=S_i\}$

Using model

- Optimal parse can be computed by Viterbi algorithm (see Rabiner's extension in section 4D, pages 269-270).

Genscan output



Genscan

- The Genscan HMM model
- Training Genscan
- Validating Genscan

Evaluating gene finders

- Calculating accuracy of programs' predictions
- Several evaluation studies:
  - Burset and Guigó, 1996 (vertebrate sequences)
  - Pavy *et al.*, 1999 (*Arabidopsis thaliana*)
  - Rogic *et al.*, 2001 (mammalian sequences)

Accuracy Metrics

		actual class: positive	actual class: negative
predicted	positive	true positives (TP)	false positives (FP)
predicted	negative	false negatives (FN)	true negatives (TN)

$$\text{sensitivity} = \frac{TP}{\text{all pos}} = \frac{TP}{TP+FN}$$

$$\text{specificity} = \frac{TP}{\text{predicted pos}} = \frac{TP}{TP+FP}$$

Measures of Prediction Accuracy

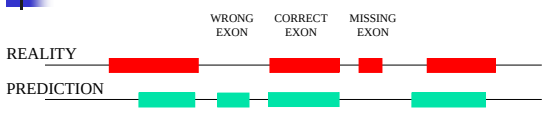
Nucleotide level accuracy

Sensitivity $Sn = \frac{TP}{TP+FN}$ — number of correct exons / number of actual exons

Specificity $Sp = \frac{TP}{TP+FP}$ — number of correct exons / number of predicted exons

Measures of Prediction Accuracy

Exon level accuracy



$$ESn = \frac{TE}{AE} \qquad ESp = \frac{TE}{PE}$$

$$AC = \frac{1}{2}\left(\frac{TP}{TP+FN} + \frac{TP}{TP+FP} + \frac{TN}{TN+FP} + \frac{TN}{TN+FN}\right) - 1$$

$$CC = \frac{(TP * TN) - (FN * FP)}{((TP+FN) * (TN+FP) * (TP+FP) * (TN+FN))^{\frac{1}{2}}}$$

Evaluation Results

Programs	# of sequences	Nucleotide accuracy				Exon accuracy							
		Sn	*Sp*	*AC*	*CC*	*ESn*	*ESp*	*(ESn+ESp)/2*	*ME*	*WE*	*PCn*	*PCp*	*OL*
FGENES	195 (5)	0.86	0.88	0.84 ± 0.19	0.83	0.67	0.67	0.67 ± 0.32	0.12	0.09	0.20	0.17	0.02
GeneMachines	195 (0)	0.87	0.89	0.84 ± 0.18	0.83	0.53	0.54	0.54 ± 0.36	0.13	0.11	0.29	0.27	0.09
Genie	195 (15)	0.91	0.90	0.89 ± 0.16	0.88	0.71	0.70	0.71 ± 0.30	0.19	0.11	0.15	0.15	0.02
Genscan	195 (3)	0.95	0.90	0.91 ± 0.12	0.91	0.70	0.70	0.70 ± 0.32	0.08	0.09	0.21	0.19	0.02
HMMgene	195 (5)	0.93	0.93	0.91 ± 0.13	0.91	0.76	0.77	0.76 ± 0.30	0.12	0.07	0.14	0.14	0.02
Morgan	127 (0)	0.75	0.74	0.70 ± 0.21	0.69	0.46	0.41	0.43 ± 0.26	0.20	0.28	0.28	0.25	0.07
MZEF	119 (8)	0.70	0.73	0.68 ± 0.21	0.66	0.58	0.59	0.59 ± 0.28	0.32	0.23	0.08	0.16	0.01

Genscan and Chromosome 22

- I. Dunham, Nature 402:489-95, 1999
- Chromosome 22
  - Annotated genes: 94% predicted partially
  - Annotated exons: 84% predicted partially
  - Predicted exons: 30% more than annotated exons. How many of them are real exons?