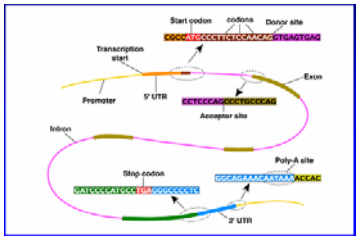
Computational gene finding

- Gene finding in eukaryotic DNA



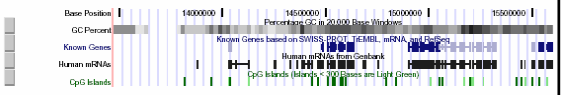
Ab initio methods

- Use information embedded in the genomic sequence *exclusively* to predict the gene structure.
- Find structure G representing gene boundaries + internal gene structure which maximizes the probability P(G|genomic sequence).
- Hidden Markov models are the predominant generative method for modeling the problem.

Ab-initio methods

- Advantages
  - Intuitive, natural modeling
  - Prediction of 'novel' genes, *i.e.*, with no a priori known cDNA or protein evidence
- Caveats
  - Not effective in detecting alternatively spliced forms, interleaved or overlapping genes
  - Difficulties with gene boundary identification
  - Potentially large number of false positives with over-fitting

A simple example: CpG Islands

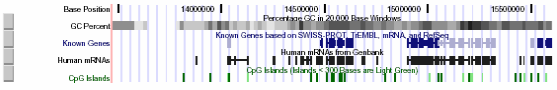


CpG nucleotides in the genome are frequently methylated. (Write CpG not to confuse with CG base pair)

C → methyl-C → T

Methylation often suppressed around genes, promoters→ CpG islands

Example: CpG Islands



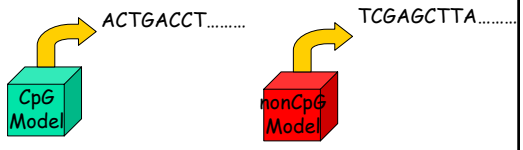
In CpG islands,

CG is more frequent than in the rest of the genome

Two problems

- Given a short DNA sequence, does it come from a CpG island or not?
  - Is this part of a CpG island or not?
- How to find the CpG islands in a long sequence?

Generative models



Models generate sequences of strings in the A,T,C,G alphabet. Model parameters are tuned to reflect characteristics of CpG and non CpG islands.

Markov processes: a quick intro

- We are interested in predicting weather, which can be either be sunny (s) or rainy (r).
- The weather on a given day depends only on the weather on the previous day.

$$P(w_t \mid w_{t-1},\ldots,w_1) = P(w_t \mid w_{t-1})$$

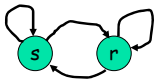
This is the Markov property.

Markov process example

- We have knowledge of the transition probabilities between sunny and rainy days.

Rows of the transition matrix sum to 1.

$$\begin{array}{c} \\ s \\ r \end{array}\begin{array}{c} \begin{array}{cc} s & r \end{array} \\ \begin{bmatrix} 0.9 & 0.1 \\ 0.5 & 0.5 \end{bmatrix} \end{array}$$



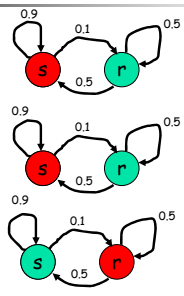
- We know the initial probabilities of s and r.

Generating weather sequences

- Let the probabilities of weather on the first day be [0.5 0.5]. Lets say we start with a sunny day.
- Now we consult our transition matrix and find that P(w|s) = [0.9 0.1]. It is more likely that the next day will be sunny too.
- We repeat this process, flipping coins biased by the probability $P(w_t|w_{t-1})$ to get a sequence representing weather for a consecutive set of days.

Generating sequences (Take 2)

$$\begin{array}{c} \\ s \\ r \end{array}\begin{array}{c} \begin{array}{cc} s & r \end{array} \\ \begin{bmatrix} 0.9 & 0.1 \\ 0.5 & 0.5 \end{bmatrix} \end{array}$$



sequence

s

s

r

Prediction

- Suppose day is rainy . We will represent this as a vector of probabilities over the two values.

$$\pi(1) = [0\ 1];$$

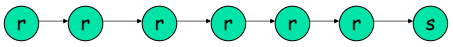
- How do we predict weather on day 2 given pi(1) and the transition probabilities P?
- From P, we can see that the probability of day 2 being sunny is .5, and for being rainy is 0.5

$$\pi(1) * P = [0.5\ 0.5];$$

Probability of a sequence

- What is the probability of observing the sequence "rrrrrrs"?

$$P(X = rrrrrrs) = \pi(r)P(r|r)P(r|r)P(r|r)P(r|r)P(r|r)P(s|r)$$
$$= \pi(r)\prod_{t=2..7} P(x_t|x_{t-1}) = (0.5)^7$$



Which weather pattern is more likely?

- Given a transition model

	s	r
s	0.9	0.1
r	0.5	0.5

- And an initial state distribution: [0.5 0.5]
- And two sequences: rrrrrrs and ssssssr
  Which is more likely, given the model?

Comparing likelihoods

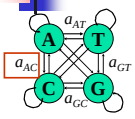
$$P(X = rrrrrrs \mid Model) = \pi(r)[P(r|r)]^5 P(s|r) = (0.5)^7$$
$$P(X = ssssssr \mid Model) = \pi(s)[P(s|s)]^5 P(r|s) = 0.5*(0.9)^5*0.1$$

Markov models (summary)

- States: S = $\{s_1,\ldots,s_N\}$, N states
- Transition probability:
  - $a_{ij} = P(X_{t+1}=s_j|X_t=s_i)$, i,j in [1..N]
- Initial state probability
  - $pi_i = P(X_1=s_i)$, i in [1..N]

Model generates sequences of states from S, and we can compute how likely a sequence is given the model.

Markov models for CpG islands



A state for each of the four letters A,C, G, and T in the DNA alphabet

$$a^+_{st} = \frac{c^+_{st}}{\sum_{t'} c^+_{st'}}$$

From a set of known CpG islands, and non CpG islands, estimate the transition probabilities

+	A	C	G	T
A	.180	.274	.426	.120
C	.171	.368	.274	.188
G	.161	.339	.375	.125
T	.079	.355	.384	.182

-	A	C	G	T
A	.300	.205	.285	.210
C	.322	.298	.078	.302
G	.248	.246	.298	.208
T	.177	.239	.292	.292

Using the model

- To use the model for classification of a given sequence, calculate the log-odds ratio.
- Is the sequence more likely to come from a CpG island or a non-CpG region?

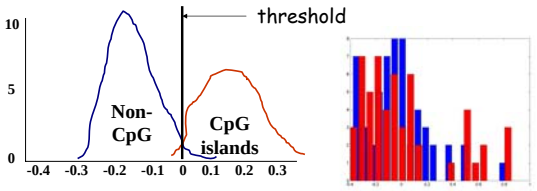
$$P(x|CpG) > P(x|nonCpG)$$
$$\frac{P(x|CpG)}{P(x|nonCpG)} > 1$$
$$\log\frac{P(x|CpG)}{P(x|nonCpG)} > 0$$

Log-odds ratio

The log-odds ratio

$$S(x) = \log \frac{P(x|CpG)}{P(x|nonCpG)} = \sum_{i=1}^{L} \log \frac{a^{+}_{x_{i-1}x_i}}{a^{-}_{x_{i-1}x_i}}$$

Histogram of log-odds scores



Given a short sequence *x*, does it come from CpG island (**Yes-No** question)?

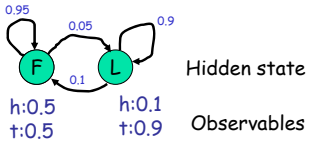
Decision rule: if S(x) > 0 then CpG else non-Cpg

How to locate CpG islands?

- Given a DNA sequence, find the CpG islands in it, if any.
- Approach: Calculate the log-odds score for a window of w nucleotides around every base in the sequence. Predict as CpG islands, those with a positive log-odds score.
- Problem: What should the size of the window w be? Predictions are sensitive to choice of w.

The occasionally dishonest casino

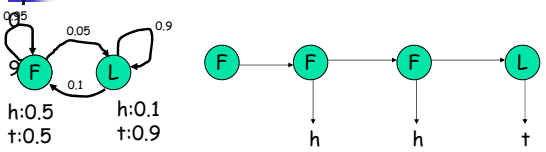
- A casino uses a fair coin most of the time, but occasionally they switch to a loaded coin. You can't see which coin they are using, just the results of the flips (heads and tails) are visible.



Generating coin flips

- Start in one of the states, F or L (i.e., pick a fair or loaded coin to start with) (initial probabilities).
- Move to the next state (F or L), based on the transition probabilities. Generate an h or t based on the emission probabilities of that state.
- Repeat above step.

Generating flips (take 2)



State sequence: FFFL (unobserved)
Obs sequence : htt (observed)

Hidden Markov Models

- S = $\{s_1,\ldots,s_N\}$, N states
- O = $\{o_1,\ldots,o_M\}$, M observation symbols
- $a_{ij} = P(S_{t+1}=s_j | S_t=s_i)$, i,j in [1..N]; transition probabilities
- $b_i(k)=P(E_t=o_k | S_t=s_i)$, k in [1..M],i in [1..N]; emission probabilities
- $pi_i = P(S_1=s_i)$, i in [1..N]; initial state probabilities

$\lambda = (A, B, \pi)$ specifies the HMM model

Dishonest casino as an HMM

- N = 2, S={F,L}
- M=2, O = {h,t}
- A =

	F	L
F	0.95	0.05
L	0.10	0.90

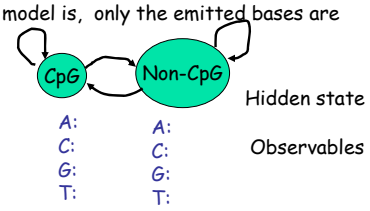
- B=

	h	t
F	0.5	0.5
L	0.1	0.9

- $\pi = [1\ 0]$

A generative model for CpG islands

- There are two hidden states: CpG and non-CpG. Each state is characterized by emission probabilities of the 4 bases. You can't see which state the model is, only the emitted bases are visible.



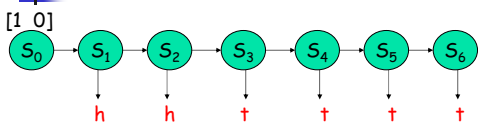
Filtering or the forward computation

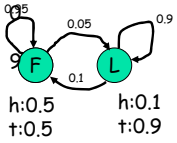
- Given an HMM model (A,B,pi), and an observation sequence $o_1 \ldots o_t$, can we find the most likely hidden state at time t, S_t?
  - $P(S_t | o_1 \ldots o_t)$: filtering

Observation sequence: h h t t t t

What is the hidden state here (F or L)?

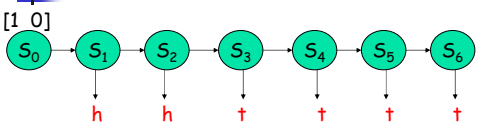
Filtering (contd.)

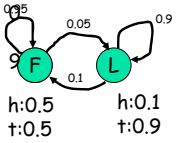




What is the distribution of S_1? Since, s_0=F, we can say that $P(S_1|S_0)$=[0.95 0.05], based on the transition probabilities alone. But is that all we know?

More filtering





We have also observed h at time 1. How can we fold it in into the assessment of the distribution of S_1?

Filtering (contd.)

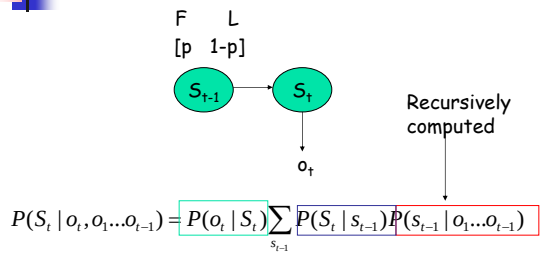
$$P(S_1 \mid o_1) = \frac{P(o_1 \mid S_1)P(S_1)}{P(o_1)}$$

$$P(S_1 = F \mid o_1 = h) = \alpha P(h \mid F)0.95 = \alpha(0.5)(0.95)$$
$$P(S_1 = L \mid o_1 = h) = \alpha P(h \mid L)0.05 = \alpha(0.1)(0.05)$$

$$\alpha(0.5)(0.95) + \alpha(0.1)(0.05) = 1$$

Therefore, $P(S_1)$=[0.99 0.01]

Filtering computation



$$P(S_t \mid o_t, o_1 \ldots o_{t-1}) = P(o_t \mid S_t) \sum_{S_{t-1}} P(S_t \mid s_{t-1}) P(s_{t-1} \mid o_1 \ldots o_{t-1})$$

Summary: filtering

Find $P(S_t \mid o_1,\ldots,o_t) = cP(S_t, o_1,\ldots,o_t)$.

Define $\alpha_t(i) = P(o_1,\ldots,o_t, S_t = s_i)$.

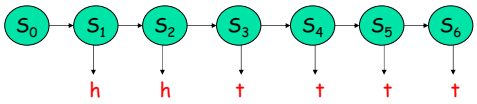
Initialize: $\alpha_0(i) = \pi_i, \quad 1 \le i \le n$

Recursion: $\alpha_{t+1}(j) = b_j(o_{t+1}) \sum_{i=1}^{n} \alpha_t(i) a_{ij}, \quad 0 \le j \le n, 1 \le t \le T-1$

Termination: $\alpha_T(i), \quad 1 \le i \le n$

Time complexity $O(n^2T)$

Smoothing/posterior decoding



Question: can we re-estimate the distribution at S_k where k < t, using information about the observed sequence upto time t?

That is, what is $P(S_k \mid o_1 \ldots o_t)$?

Backward computation

Backward computation

$$P(S_k \mid o_1,\ldots,o_t) = cP(o_{k+1},\ldots,o_t \mid S_k) P(S_k \mid o_1,\ldots,o_k)$$

Forward computation

Define $\beta_k(i) = P(o_{k+1},\ldots,o_t \mid S_k = s_i)$.

Initialize: $\beta_T(i) = 1, \quad 1 \le i \le N$.

Recursion: $\beta_k(i) = c \sum_{j=1}^{N} a_{ij} b_j(e_{k+1}) \beta_{k+1}(j), \quad 1 \le i \le N, T-1 \le k \le 1$

Time complexity: $O(n^2T)$

Posterior decoding

$$P(S_k = i \mid o_1,\ldots,o_t) = c\beta_k(i)\alpha_k(i)$$

Full Decoding

- Given HMM model (A,B,pi), and an observation sequence $o_1 \ldots o_t$, can we find the most likely hidden state sequence $s_1 \ldots s_t$?
  - argmax_{$s_1 \ldots s_t$} P($s_1 \ldots s_t$| $o_1 \ldots o_t$)

The Viterbi algorithm

$$\delta_t(i) = \max_{x_1,\ldots,x_{t-1}} P(s_1,\ldots,s_{t-1}, S_t = i, o_1,\ldots,o_t)$$

$$\text{Initialize}: \delta_0(i) = \pi_i, 1 \le i \le n$$

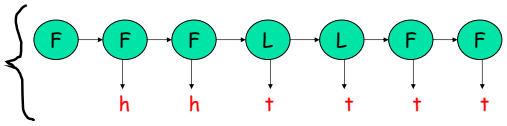
$$\text{Recursion}: \delta_{t+1}(j) = \max_i \delta_t(i) a_{ij} b_j(e_{t+1}),$$

$$1 \le t \le T-1, 1 \le j \le n$$

Computational complexity = $O(Tn^2)$

Learning an HMM: case 1

- Given observation sequences, and the corresponding hidden state sequences, can we find the most likely model (A,B,pi) which generated it?



Training data

Parameter estimation

- Initial state distribution
  - Fraction of times state i is state 1 in training data
- Transition probabilities
  - a_{ij} = (number of transitions from i to j)/(number of transitions from i)
- Emission probabilities
  - $b_k(i)$ = (number of times k is emitted in state i)/(number of times state i occurs)

Learning an HMM: case 2

- Given just the observation sequences, can we find the most likely model λ = (A,B,pi) which generated it?

$$\underset{\lambda}{\text{argmax}}\, P(o_1 \ldots o_t \mid \lambda)$$

Annotated training data is difficult to get; so we would like to derive model parameters from observable sequences.

The EM algorithm

1. Guess a model λ
2. Use observation sequence to estimate transition probabilities, emission probabilities, and initial state probabilities.
3. Update model
4. Repeat 2 and 3 till no change in model

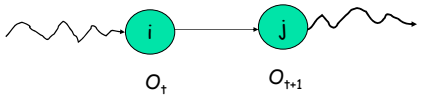
Re-estimating paraameters

- What is the probability of being in state i at time t and moving to state j, given the current model and the observation sequence O?

$$\xi_t(i,j) = P(S_t = i, S_{t+1} = j \mid O, \lambda)$$

Using forward and backward computation

$$\xi_t(i,j) = \frac{\alpha_t(i) a_{ij} b_j(o_{t+1}) \beta_{t+1}(j)}{\sum_{i=1}^{n}\sum_{j=1}^{n} \alpha_t(i) a_{ij} b_j(o_{t+1}) \beta_{t+1}(j)}$$



Re-estimating a_{ij}

- The transition probabilities a_{ij} can be re-estimated as follows

$$\hat{a}_{ij} = \frac{\sum_{t=1}^{T-1} \xi_t(i,j)}{\sum_{t=1}^{T-1}\sum_{j'=1}^{n} \xi_t(i,j')}$$

Initial state probabilities

$$\gamma_t(i) = \sum_{j=1}^{N} \xi_t(i,j)$$

Expected number of times in state i

Initial state probabilities are simply $\gamma_1(i)$

Emission probabilities

$$\hat{b}_i(k) = \frac{\text{expected number of times in state i and observe symbol k}}{\text{expected number of times in state i}}$$

$$\hat{b}_i(k) = \frac{\sum_{\substack{t=1 \\ o_t = k}}^{T} \gamma_t(i)}{\sum_{t=1}^{T} \gamma_t(i)}$$

The EM algorithm

1. Guess a model $\lambda = (a, b, \pi)$
2. Use observation sequence to estimate

$$\xi_t(i,j) \text{ and } \gamma_t(i)$$

3. Use these estimates to recalculate

$$\lambda' = (a', b', \pi')$$

4. Repeat 2 and 3 till no change in model

How to use the CpG island HMM

- Given a DNA region x, the Viterbi algorithm predicts locations of CpG islands on it.
- Given a nucleotide x_i the Viterbi parse tells whether x_i is in a CpG island in the most likely sequence.
- Posterior Decoding can assign locally optimal predictions of CpG islands.
- A fully annotated training data set can be used to estimate the generating HMM.
- Even without annotations, we can use the EM procedure to derive model parameters.