# Computational gene finding

Devika Subramanian
Comp 470

## Outline (3 lectures)

Lec 1
- The biological context
- Markov models and Hidden Markov models

Lec 2
- Ab-initio methods for gene finding
- Comparative methods for gene finding

Lec 3
- Evaluating gene finding programs



## The biological context

- Introduction to the human genome and genes
- The central dogma: transcription and translation



## Facts about the human genome

- The human genome contains 3 billion chemical nucleotide bases (A, C, T, and G).
- About 30,000 genes are estimated to be in the human genome. Chromosome 1 (the largest human chromosome) has the most genes (2968), and the Y chromosome has the fewest (231).



## More facts

- The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.



## More facts

- Genes appear to be concentrated in random areas along the genome, with vast expanses of non-coding DNA between.
- About 2% of the genome encodes instructions for the synthesis of proteins.
- We do not know the function of more than 50% of the discovered genes.

## More facts

- The human genome sequence is almost (99.9%) exactly the same in all people. There are about 3 million locations where single-base DNA differences occur in humans (Single Nucleotide Polymorphisms or SNPs).
- Over 40% of the predicted human proteins share similarity with fruit-fly or worm proteins.



## A great site to learn more

http://www.dnai.org/index.htm



## Genome sizes

| Organism | Genome Size (Bases) | Estimated Genes |
|---|---|---|
| Human (*Homo sapiens*) | 3 billion | 30,000 |
| Laboratory mouse (*M. musculus*) | 2.6 billion | 30,000 |
| Mustard weed (*A. thaliana*) | 100 million | 25,000 |
| Roundworm (*C. elegans*) | 97 million | 19,000 |
| Fruit fly (*D. melanogaster*) | 137 million | 13,000 |
| Yeast (*S. cerevisiae*) | 12.1 million | 6,000 |
| Bacterium (*E. coli*) | 4.6 million | 3,200 |
| Human immunodeficiency virus (HIV) | 9700 | 9 |

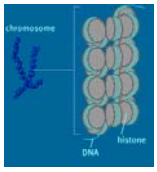


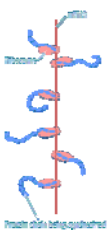
## Codons

- 3 consecutive DNA bases code for an amino acid. There are 64 possible codons, but only 20 amino acids (some amino acids have multiple codon representations).
- Four special codons: start codon (ATG) and three stop codons (TAG, TGA, TAA). They indicate the start and end of translation regions.



## The central dogma



mRNA produced by transciption

DNA → mRNA → proteins



## Transcription

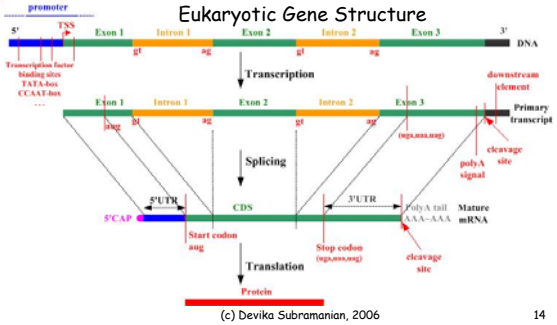
- When a gene is "expressed" the sequence of nucleotides in the DNA is used to determine the sequence of amino acids in a protein in a two step process.
- First, the enzyme RNA polymerase uses one strand of the DNA as a template to synthesize a complementary strand of messenger RNA (mRNA) in a process called transcription. RNA is identical to DNA except that in RNA T is replaced with U (for uracil). Also, unlike DNA, RNA usually exists as a single stranded molecule.

## Splicing and Translation

- In eukaryotes, after a gene is transcribed the introns are removed from the mRNA and the adjacent exons are spliced together in the nucleus prior to translation outside the nucleus.
- After the mRNA for a particular gene is made it is used as a template with which ribosomes synthesize the protein in a process called translation.

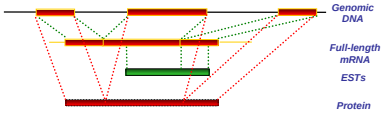


## The Biological Model





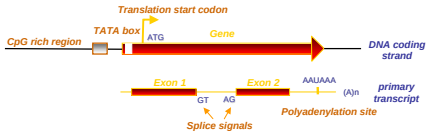

## How genes are validated




- cDNA - single-stranded DNA complementary to an RNA, synthesized from it by reverse transcription
- full-length mRNAs (GenBank *RefSeq*, ~16000 human sequences)
- ESTs – Expressed Sequence Tags
  - relatively short, 500 bp long on average
  - span one or more exons
  - large data sets required (GenBank *dbEST*– 4.3 M human sequences)



## Signals




- Upstream regulatory signals (TATA boxes)
- Translation start codon (ATG)
- Translation stop codon (*e.g.*, TAA)
- Polyadenylation signal (~AATAAA)
- Splice recognition signals (*e.g.*, GT-AG, branch point)



## Computational gene finding

- Gene finding in prokaryotes
- Gene finding in eukaryotes
  - Ab initio
  - Comparative



## Finding genes in prokaryotes

- Prokaryotes are single-celled organisms without a nucleus (e.g., bacteria).
- Few introns in prokayotic cells. Over 70% of H. influenzae genome codes for proteins.
- No introns in coding region.

gene1 gene2 gene3

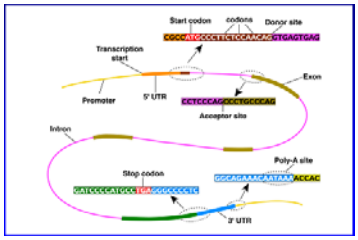
## Finding genes in prokaryotes

- Main idea: if bases were drawn uniformly at random, then a stop codon is expected once every 64/3 (about 21) bases. Since coding regions are terminated by stop codons, a simple technique to find genes is to look for long stretches of bases without a stop codon. Once a stop codon is found, we work backward to find the start codon corresponding to the gene.
- Main problems: misses short genes, overlapping ORFs.



## 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.

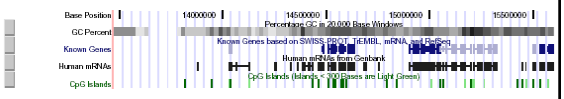


## Ab-initio methods

- 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



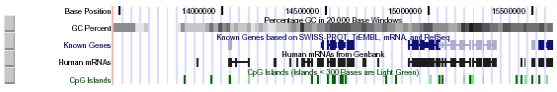

CpG nucleotides in the genome are frequently methylated. (Write CpG not to confuse with CG base pair)

$C \rightarrow$ methyl-$C \rightarrow T$

Methylation often suppressed around genes, promoters $\rightarrow$ CpG islands



## Example: CpG Islands




In CpG islands,

CG is more frequent than in the rest of the genome

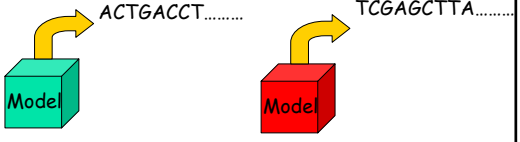
## Two problems

- 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 sunny or rainy.
- The weather on a given day is dependent 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.

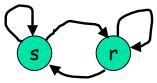


## Markov process example

- We have knowledge of the transition probabilities between the various states of the weather: P(s,s').

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 day 1 be [0.5 0.5]. We flip a fair coin, and get heads, and obtain sunny to be our weather for day 1.
- Now we consult our transition matrix and find that P(w|s) = [0.9 0.1]. So we flip a biased coin and obtain heads again, so weather on day 2 is also summy.
- We repeat this process, flipping coins biased by the probability $P(w_t|w_{t-1})$ to get a sequence drawn from the s,r alphabet.



## Prediction

- Suppose day 1 is rainy. We will represent this as a vector of probabilities over the three values.

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

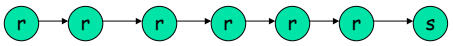
- How do we predict the weather for day 2 given pi(1) and the transition probabilities P?
- From P, we can see that the probability of day 2 being sunny is .5, and for being rainy is 0.5

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

## 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

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

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



## Comparing likelihoods

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



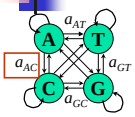
## Markov models

- 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 |

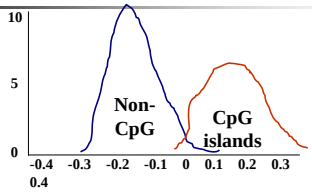


## Using the model

- To use these models for discrimination, calculate the log-odds ratio.

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

# Histogram of log-odds scores




Q1: Given a short sequence *x*, does it come from CpG island (**Yes-No** question)?

- *S(x)*

Q2: Given a long sequence *x*, how do we find CpG islands in it (**Where** question)?

- Calculate the log-odds score for a window of, say, 100 nucleotides around every nucleotide, plot it, and predict CpG islands as ones w/ positive values
- Drawbacks: Window size